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New Three-Step Syntheses of Racemic and Optically Active Ipsdienol from Myrcene

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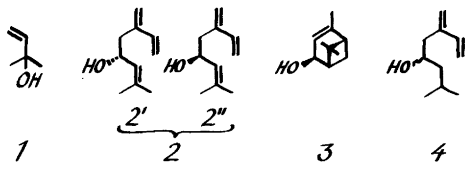
2-Methyl-6-methylene-3,7-octatriene-2-ol (*6*), which is readily available from photooxidation of myrcene (*5*), was transformed into racemic and optically active ipsdienol (*2*). Treatment of the trienol *6* with perchloric acid in acetic acid yielded ipsdienyl acetate which on hydrolysis gave racemic ipsdienol (*2*) in 83% overall yield. Oxidation of the trienol *6* with pyridinium chlorochromate in the presence of pyridine hydrochloride furnished 2-methyl-6-methylene-2,7-octadien-4-one (*8*) in 43% yield. Reduction of this ketone with lithium aluminium hydride partially decomposed by one molar eq. each of ethanol and either (*R*)-(+)- or (*S*)-(−)-2,2′-dihydroxy-1,1′-binaphthyl gave (*R*)-(−)- or (*S*)-(+)-ipsdienol (*2′* or *2″*, respectively) in 70% yield and 60–65% ee.

The bark beetles of the *Ips* genus are major pests in conifer forests. *Ips typographus* (L.) is responsible for mass attacks on Norway spruce *Picea abies* (L.) Karst. 2-Methyl-3-buten-2-ol (*1*),[1,2] ipsdienol (*2*),[2–4] (*S*)-*cis*-verbenol (*3*)[1–3] and ipsenol (*4*)[3] are some of the known pheromone components of this insect. Compounds *2*, *3* and *4* are also present in the pheromones of many other *Ips* spp. A lure of compounds *1*, *2* and *3* is successfully used in Sweden and Norway for trapping *Ips typographus* on a large scale.[5] The racemate of ipsdienol (*2*) is used in this lure. It is known, however, that in some *Ips* spp the activity of one enantiomer of a phermone component (*e.g.* ipsdienol) can be inhibited by the opposite one.[6,7] It is not clear whether this is true for *Ips typographus*. In fact, except for the attractive power of the lure, the details of the behavioral responses elicited by the compounds *1*–*4* are not known. A thorough study of the biological effects of pheromone components is dependent on their availability. In cases of chirality, knowledge about their optical purities is of importance.



1 *2* *3* *4*

Although racemic ipsdienol (*2*) has been prepared *via* many[8–15] routes and a few attempts have been made to synthesize the pure enantiomers*,[16–18] *2′* and *2″* (38–91% ee) starting from optically active natural products, there is still a need for simple and inexpensive procedures leading to these compounds.

We now report simple methods for the syntheses of racemic ipsdienol (*2*) and mixtures considerably enriched in either optical isomer *2′* or *2″ via* the key intermediate (*E*)-2-methyl-6-methylene-3,7-octadien-2-ol (*6*).** According to the synthetic plan, allylic rearrangements of the trienol *6* would furnish either ipsdienol (*2*) or, with simultaneous oxidation, the ketone *8*. A suitable chiral reducing agent would transform the latter into predominantly one enantiomer of ipsdienol (*2′* or *2″*).

The tertiary trienol *6* can be readily prepared from inexpensive myrcene (*5*) either by epoxidation, addition of PhSeH, oxidation and elimination[20] or more simply by sensitized photooxidation. The latter method was used here employing the one-pot procedure we recently published.[21] In this reaction

*Ipsdienol isolated from *Ips paraconfusus* has been estimated to be of 75% ee.[16]

**Trienol *6* has been identified as a pheromone component of *Ips amitinus*[4] and as a constituent of the frass of *Ips paraconfusus*.[19]

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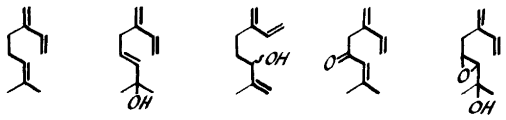


5 6 7 8 9

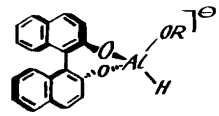
the two isomeric alcohols 6 and 7 are formed in equal amounts as the major products. The secondary allylic alcohol 7 has shown interesting biological properties in relation to *Ips typographus*. It gives a distinct response in electroantennographic (EAG) measurements[22] and preliminary field tests show that it has attracting properties similar to that of ipsdienol itself.[23]

The tertiary trienol 6 has previously been transformed into racemic ipsdienol (2) *via* a five-step procedure involving chlorination and allylic rearrangement.[10] An earlier attempt to perform a direct acid catalyzed allylic rearrangement of 6 was not successful[11] but Lewis acid treatment of the target molecule 2 to form the tertiary alcohol 6 has been reported.[4]

The main obstacle which prevents efficient conversion of 6 to 2 is hydrocarbon formation by loss of water. However, we found that when dissolved in acetic acid and treated with catalytic amounts of perchloric acid for 120 sec at room temperature the tertiary alcohol 6 smoothly gave ipsdienyl acetate (2, OH replaced by OAc) in 85% yield containing less than 5% of hydrocarbon. If a mixture of the two isomeric alcohols 6 and 7, resulting from photooxidation of myrcene, was treated in the same way, only the tertiary alcohol 6 was affected leaving the secondary alcohol 7 intact, which greatly simplified separation. Hydrolysis of ipsdienyl acetate with KOH in methanol gave a quantitative yield of ipsdienol (2).

Oxidative rearrangements of tertiary allylic alcohols leading to ketones have been performed with different chromium(VI) reagents.[24-26] The best reported results have been achieved using pyridinium chlorochromate (PCC) in dichloromethane which works well for cyclic systems but less so for the acyclic counterparts.[26] Unfortunately when this method was applied to the trienol 6 the yield of the desired ketone 8 was only 10%. However, the yield was substantially improved by adding pyridine hydrochloride (PHCl) as a proton source. Oxidation with an equimolar amount of PCC in the presence of four molar equivalents of PHCl in refluxing dichloromethane gave a 33% yield of the myrcenone 8 and a 34% yield of recovered starting material which again was subjected to oxidation. Thus the overall yield was increased to 43%. Not unexpectedly (*cf.* Ref. 27) an epoxide to which the structure 9 was assigned was also isolated, in a low yield. Using an excess of PCC in this reaction substantially reduced the amount of recovered starting material without improving the yield of the ketone.

The myrcenone 8 could also be prepared, in a similar yield, from ipsdienol (2) using the PCC, PHCl method. Various other chromium(VI) reagents and MnO_2 gave inferior results.



10′: R = C_2H_5-
11′: R = CH_3-
12′: R = 

10″–12″: mirror images of 10′–12′

The asymmetric reduction of the myrcenone 8 was accomplished using the recently described[28] chiral complexes *10′* and *10″*. These are reported to give good chemical and excellent optical yields of chiral alcohols from various unsaturated ketones.[28,29] The reagent *10′* reduced acetophenone as described to (+)-phenylmethylcarbinol (98% ee. Lit.[27] 96% ee). Similar reduction of the myrcenone 8 with the reagent *10′* [from (*R*)-(+)-2,2-dihydroxy-1,1′-binaphthyl[30]] at −80 °C furnished (*R*)-(−)-ipsdienol in 70% chemical yield and 63% ee, as determined by NMR-measurements of the diastereomeric mixture obtained on esterification with (−)-α-methoxy-α-trifluoromethylphenylacetic acid [(−)-MTPA]. Similarly, (*S*)-(+)-ipsdienol was obtained using the reagent *10″*. Modified reducing agents such as *11′* and *12′* gave lower optical yields of chiral ipsdienol.

Thus, the procedures reported here provide three-step syntheses of racemic and optically active ipsdienol from readily available myrcene.

EXPERIMENTAL

The NMR-spectra were recorded on a Varian EM 360 (60 MHz) or a Bruker WP200 (200 MHz) spectrometer using deuteriochloroform containing

tetramethylsilane as solvent. Silica gel chromatography was performed with Merck Kieselgel 60, 230 – 400 mesh. GLC: capillary column, 25 m, coated with carbowax 20M, 70 – 180 °C. TLC: silica gel, 15 % ethyl acetate in hexane, developed with vanillin – sulfuric acid.

2-Methyl-6-methylene-2,7-octadien-4-ol, racemic ipsdienol (2). Perchloric acid (90 %, one drop) was added to glacial acetic acid (10 ml) and this solution was added (30 sec) to a stirred solution of (*E*)-2-methyl-6-methylene-3,7-octadien-2-ol (*6*, 1.28 g, 8.42 mmol) in glacial acetic acid (10 ml). No discoloration occurred. After additional 120 sec the reaction mixture was poured into brine (50 ml). Extraction with three portions of pentane followed by washing of the combined extracts once with $NaHCO_3$ solution, drying ($MgSO_4$) and solvent removal gave ipsdienyl acetate (1.42 g, 7.32 mmol, 87 %) containing < 5 % hydrocarbon (GLC). The product was treated with KOH solution (10 % in MeOH, 15 ml) at room temperature for 3 h, poured into brine (50 ml) and extracted with three portions of pentane. Drying ($MgSO_4$) and solvent removal gave essentially pure (GLC, TLC) *2* (1.06 g, 6.96 mmol, 95 %) in 83 % overall yield. Column chromatography on silica gel led to partial decomposition of the acetate. Hydrolysis of the collected acetate gave a quantitative yield of ipsdienol (*2*) but the overall yield was lowered to 60 – 75 %.

Treatment of a mixture of the alcohols *6* and *7* in an identical manner provides ipsdienyl acetate and unchanged secondary alcohol *7*. Due to the large difference in R_f values, these compounds were easily separated by column chromatography in contrast to the two alcohols *6* and *7*.[21]

2-Methyl-6-methylene-2,7-octadien-4-one (8). (*E*)-2-Methyl-6-methylene-3,7-octadien-2-ol (*6*, 3.04 g, 20 mmol) in methylene chloride (160 ml) was refluxed under nitrogen. A solution of pyridinium chlorochromate (4.50 g, 20.9 mmol) and pyridine hydrochloride (4.50 g, 39 mmol) in methylene chloride (150 ml) was added *via* a syringe pump during 3 h. After refluxing for an additional hour the mixture was diluted with pentane washed with dilute hydrochloric acid, water, sat. sodium bicarbonate solution, brine and finally dried ($MgSO_4$). The solvent was evaporated leaving a mixture (2.1 g) which was subjected to silica gel chromatography. Elution with pentane containing increasing amounts of ethyl acetate (0 – 15 %) gave first the desired ketone (1.00 g) followed by the slightly impure starting alcohol *6* (1.03 g). This was oxidized with PCC (1.75 g) and PHCl (2.0 g) as described above. An additional amount (0.30 g) of the ketone *8* was obtained giving a total of 1.30 g (43 %) in addition to starting alcohol *6* (0.31 g, 10 %) and a product A (0.17 g, 6 %, *vide infra*). The NMR-spectrum of the myrcenone *8* obtained was identical to that described.[8] Oxidation of ipsdienol with Cr(VI) reagents gave the best results with PCC and PHCl which furnished the ketone *8* in 30 – 40 % yield. A minor side product in these oxidations was a product B (*vide infra*).

3,4-Epoxy-2-methyl-6-methylene-7-octen-2-ol (*9*, product A, described above) NMR (60 MHz): δ 1.20, 1.27 [6H, 2s, $>(CH_3)_2$], 2.15 (1H, bs, O*H*), 2.35 – 2.55(2H, bd, $-CH_2-$, *J* 6 Hz), 2.70 [1H, d, $-CH(O)-$, *J* 3 Hz], 3.15[1H, dt, $-CH(O)-$, *J* 3 and 6 Hz], 4.95 – 5.30 (4H, m, $=CH_2$ and $=CH_2$), 6.33 (1H, dd, $-CH=$, *J* 11 and 18 Hz).

2,3-Epoxy-2-methyl-6-methylene-7-octen-4-ol (product B, mentioned above) NMR (60 MHz): δ 1.23, 1.30 [6H, 2s, $>C(CH_3)_2$], 2.3 – 2.8 [2H, m, $-CH_2-$], 2.72 [1H, d, $-CH(O)-$, *J* 8 Hz], 3.4 – 3.8 [1H, m, $-CH(OH)-$], 5.0 – 5.25 (4H, m, $=CH_2$ and $=CH_2$), 6.33 (1H, dd, $-CH=$, *J* 11 and 18 Hz).

Preparation of the chiral aluminiumhydride reagents 10′ – 12′. Lithium aluminium hydride (LAH, 25 g) was refluxed in dry THF (100 ml) for 2 h. The solution was cooled and filtered in a nitrogen atomsphere through a pad of celite (dried at 200 °C overnight). The resulting clear solution (0.250 ml) was titrated under nitrogen with a standard solution (0.01 M) of dried distilled pentanol in dry THF containing a small amount of 2-(2,4-dihydroxyphenylnaphthoquinone. The orange-red quinone [31] is reduced by LAH to the colorless anion of the hydroquinone. When all the hydride is consumed the bright blue anion [31] of the quinone is formed on further addition of the titration solution.

The LAH solution (4 mmol) was added to dry THF (10 ml) and stirred at 0 °C under N_2. Ethanol, methanol or 2,6-di-*t*-butylphenol (4.2 mmol) in THF (5 ml) was added followed by (*R*)-(+)-2,2′-dihydroxy-1,1′-binaphthyl [30] {1.20 g, 4.2 mmol, $[\alpha]_D^{20}$ +37.0° (MeOH)} in THF (10 ml). The milky solutions of the reagents *10′ – 12′* were stirred for 1 h at room temperature prior to use. We found that the reagent *10′* gave (*R*)-(+)-phenylmethyl carbinol in 98 % ee (Lit.[28] 96 % ee) from acetophenone.

(R)-(−)-Ipsdienol (2′). The chiral reducing agent *10′* was cooled to −100 °C and stirred under N_2. 2-Methyl-6-methylene-2.7-octadien-4-one (*8*, 152 mg, 1 mmol) in dry THF (5 ml) was added. The reaction was followed by TLC. After 72 h the reaction was quenched by addition of moist ether. After warming, the solution was exhaustively extracted with sodium hydroxide solution (2M, to remove the dihydroxybinaphthyl). The ether phase was washed with hydrochloric acid (2 M), sat. sodium bicarbonate solution and brine. After drying ($MgSO_4$) the solvent was evaporated to give an oil, which was subjected to silica gel chromatography. This gave unconverted ketone *8* (20 mg, 13 %) followed by ipsdienol (105 mg, 70 %), $[\alpha]_D^{20}$ −7.0° ±1.5° (c 3, MeOH) (Lit.[16] −13.2° – −13.6°

estimated for pure (*R*)-(−)-ipsdienol). Reduction of the ketone with the reagent *10″* prepared from (*S*)-(−)-2,2′-dihydroxyl-1,1′-binaphthyl,[30] $[\alpha]_D^{20}$ −35.8° (MeOH) furnished (*S*)-(+)-ipsdienol in similar chemical and optical yields. The optical yields were determined more accurately by an NMR-method *vide infra.*

Ester of (R)-(−)-ipsdienol with (−)-α-methoxy-α-trifluoromethyl-α-phenylacetic acid (MTPA) (*R*)-(−)-ipsdienol {10 mg, $[\alpha]_D^{20}$ −7.0° (MeOH)} in chloroform (0.1 ml) was stirred at room temperature. The chloride (30 mg) from (−)-MTPA was added, followed by pyridine (0.1 ml). The mixture was heated for 90 min at 70 °C, cooled and poured into water. After stirring for 15 min the mixture was extracted with ether. The ether solution was washed with hydrochloric acid (1 M), sat. sodium bicarbonate solution and water. After drying the solvent was evaporated and the residue purified by chromatography (pentane as eluent) to give the MTPA ester (23 mg, 95%). NMR (200 MHz): δ 1.73 (6 H, m, $(CH_3)_2C=$), 2.53 (2 H, 12 line m, $C-CH_2-C$), 3.55 (3H, m, OCH_3), 4.85−5.35 (4H, m, 4 vinylic H), 5.71 (1H, m, $=CH-CH-$(OMTPA)$-CH_2-$), 6.35 (1H, 6 line m, $-C(=CH_2)-CH=CH_2$), 7.30−7.65 (5H, m, C_6H_5). Irradiation at δ 5.71 gave a six line signal at δ 2.52. This signal represents the sum of two pairs of doublets $(=C^4H(OMTPA)-C^5H_AH_B-C^6(=CH_2)-)$ arising from the diastereotopic protons on carbon 5 of the ipsdienyl moiety. The H_A and H_B protons of the (*R*)-(−)-ipsdienyl moiety give rise to the two doublets at δ 2.45 (J 13.8 Hz) and δ 2.60 (J 13.8 Hz). The H_A and H_B protons of the (*S*)-ipsdienyl moiety give rise to the two doublets at δ 2.38 (J 13.8 Hz) and δ 2.62 (J 13.8 Hz). The ratio of the integral of the signals from the (*R*)-form to that of the (*S*)-form was 4.33/1. The six line signal at δ 6.35 can similarly be analyzed to be composed of the sum of one doublet of doublets (J 8.3 and 13.1 Hz) centered at δ 6.33 (integral 1) and another at δ 6.37 (J 8.3 and 13.1 Hz, integral 4.65). The ee of the ipsdienyl moiety is thus 62−65%.

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Optimum Conditions for Enamine Synthesis by an Improved Titanium Tetrachloride Procedure

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A modified procedure for enamine synthesis which results in shorter reaction times and increased yield has been developed. Optimum conditions for the synthesis have been obtained from a series of eight different acyclic ketones with three different amines. The optimum conditions were determined either by response surface methodology or by simplex technique. In view of the results some consideration of the mechanism is briefly discussed.

In 1967 White and Weingarten presented a new enamine synthesis.[1] In their method, titanium tetrachloride was used as a catalyst and also as a water scavenger. This made it possible to synthesize enamines from acyclic ketones, such as methyl ketones, which had previously been difficult with conventional methods.[2] An optimization of the White and Weingarten procedure has already been reported from this laboratory.[3] Though this procedure seems to be generally applicable to enamine synthesis, we found that it fails in the case of several branched acyclic ketones. As part of our studies of regiocontrol in acyclic ketones, we needed a series of enamines with increasing sterical hindrance. Some of these were not available by White and Weingarten's method. We report here a modified procedure and the optimum conditions for enamine formation from acyclic ketones. This method permits the synthesis of enamines from sterically congested ketones, for which the original procedure has failed. In this modified procedure the ketone is added to a preformed complex between the amine and titanium tetrachloride. The procedure is optimized with regard to yield and reaction time and the following experimental variables are considered: relative amount of titanium tetrachloride, relative amount of amine and reaction temperature.

It is obvious that optimum conditions may change with different substrates. Thus, it is necessary to investigate optimum conditions for each individual substrate. It is also obvious that many important conclusions about the scope and limitation of a synthetic procedure should be drawn from investigations performed under optimum conditions.

The common practice for investigating scope and limitation is to use standardized conditions and to record the variation in yield in such experiments.[4] Results from such investigations can be misleading, however, if optimum conditions are susceptible to variation when the substrate is changed. To our knowledge, the present investigation is the first where the optimum conditions have been determined for all the various substrates considered.

METHODS

Optimization. A common approach to optimization is to change one variable at a time. However, we note that this approach is bound to fail to attain a true optimum when the variables are not independent.[5] Chemical phenomena, such as yield, rarely depend on a single variable and the only reasonable way to optimize yields is to use multivariate methods for analyzing the simultaneous influence of several variables and the interaction among them. We have used the following multivariate strategies: the simplex technique[6] and response surface methodology.[5b,7] For a general discussion, see Ref. 3.

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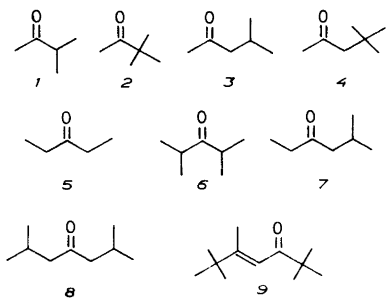

Fig. 1. Ketones *1* – *8* have been investigated. Ketone *9* is a condensation product of *2*, formed during morpholine enamine synthesis of methyl *tert*-butyl ketone.

Yield determination. Yields were determined by gas liquid chromatography (GLC) using the internal standard technique.

RESULTS

The following ketones were investigated: 3-methyl-2-butanone, *1*; 3,3-dimethyl-2-butanone, *2*; 4-methyl-2-pentanone, *3*; 4,4-dimethyl-2-pentanone, *4*; 3-pentanone, *5*; 2,4-dimethyl-3-pentanone, *6*; 5-methyl-3-hexanone, *7*; and 2,6-dimethyl-4-heptanone, *8* (Fig. 1). Optimum conditions for the synthesis of 24 enamines are shown in Tables 1 – 3. We measured the reaction times when no further increase in yield could be observed. These times are given in Tables 1 – 3. For each amine component, optimum conditions for one of the ketones *1* – *8* were determined either by response surface methodology or by the simplex technique. The remaining ketones were investigated under the established conditions. If a yield less than 90 % was found, the conditions were further investigated. The low temperature used for dimethylamine enamine

Table 1. Optimum conditions for morpholine enamine synthesis.

Ketone	Yield %	TiCl eq.	Morpholine eq.	Temp. °C	Reaction time min	Method of optimization[a]
1	93	0.9	5.7	70	15	
2	88	0.92	9.2	110	240	B
3[c]	98	0.9	5.7	70	15	B
4	89[b]	0.92	9.2	70	180	
5	98	0.9	5.7	70	15	
6	67	1.28	11	110	240	A,B
7	79[b]	0.9	6.0	110	90	
	98	1.0	8.0	70	15	A
	100	1.1	7.0	70	120	
8	94	1.33	7.8	70	120	A,B
	88[b]	1.33	7.8	70	120	

[a] A = Simplex, see Fig. 2 for an example. B = Response surface, see Fig. 3 for an example. [b] Isolated yield of distilled product. [c] Model Substrate for first optimization.

Table 2. Optimum conditions for pyrrolidine enamine synthesis.

Ketone	Yield %	$TiCl_4$ eq.	Pyrrolidine eq.	Temp. °C	Reaction time min	Method of optimization[a]
1	98	0.9	6.0	0	5	
2	96	0.9	6.0	rt[d]	15	
3[c]	100	0.9	6.0	0	5	A
4	93	1.0	7.0	rt[d]	15	
5	98	0.9	6.0	0	5	
6	93	1.2	7.5	70	240	A
7	82[b]	0.9	6.0	70	15	
8	99	0.9	7.0	70	120	

[a] A = Simplex, see Fig. 2 for an example. B = Response surface, see Fig. 3 for an example. [b] Isolated yield of distilled product. Could not be analyzed by GLC. [c] Model substrate for first optimization. [d] rt = room temperature, 20 – 25 °C.

Table 3. Optimum conditions for dimethylamine enamine synthesis.

Ketone	Yield %	$TiCl_4$ eq.	Dimethyl-amine eq.	Temp. °C	Reaction time min	Method of optimization[a]
1[c]	98	1.1	7.3	−36	60	A,B
2	79	0.9	7.7	−30	120	A
	74[b]	1.0	7.0	rt[d]	120	
3	92	0.9	7.7	−30	30	
4	97	1.0	6.0	rt[d]	5	A
5	97	0.9	7.7	−30	30	
6	64	1.0	10	35	360	A
7	92	1.1	7.0	rt[d]	15	
8	99	1.0	10	rt[d]	60	A

[a] A = Simplex, see Fig. 2. for an example. B = Response surface, see Fig. 3 for an example. [b] Isolated yield of distilled product. [c] Model substrate for first optimization. [d] rt = room temperature, 20−25 °C.

synthesis was necessary in view of the volatility of dimethylamine. However, some of the ketones showed very slow reaction rates at low temperature, and an increase in temperature was necessary to give conveniently short reaction times. The temperatures given for the pyrrolidine and morpholine enamine syntheses (0 °C, room temperature or reflux) were chosen for reasons of convenience, and optimization of the amounts of titanium tetrachloride and amine was performed subject to this practical constraint. The synthesis of the morpholine enamine from 3,3-dimethyl-2-butanone, *2*, was attended by complications owing to considerable self-condensation of *2* to give 2,2,3,6,6-pentamethyl-3-hepten-5-one, *9*, (Fig. 1). Similar conditions produced high yields of enamine from the other ketones. The enamine and *9* were difficult to separate by simple distillation, and tedious fractionation on an efficient column was necessary to obtain pure enamine. This illustrated a general problem in synthesis, namely the yield of the desired product is decreased by a parasitic reaction. To overcome these difficulties, a response surface study was undertaken, and the yields of both enamine and *9* were described as second degree polynomials in the experimental variables. A central composite experimental design[5b,8] with 18 experiments was used to establish the response surface models. Fig. 4 shows the features of these response surfaces. With the use of these response surface models we could determine the experimental conditions which give less than 5 % of *9* and which maximize the yield of enamine. These conditions are shown in Table 1. It is seen from the Tables 1 − 3 that the sterically hindered ketones, *i.e.*, those with α-branching, require more drastic reaction conditions and/or prolonged reaction time.

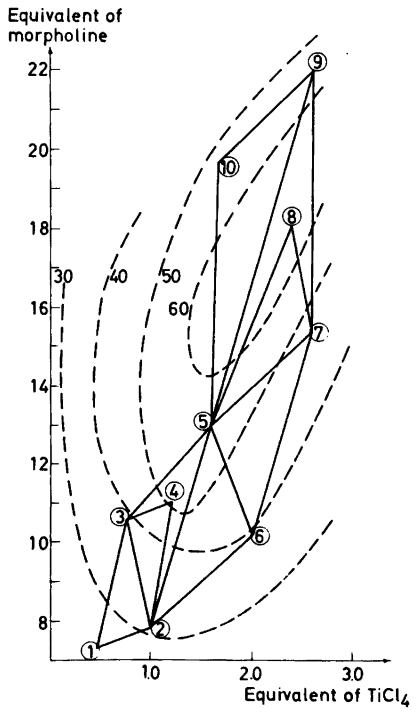

Fig. 2. Simplex optimization of the morpholine enamine synthesis of diisopropyl ketone. Encircled numbers refer to the experiments. After experiment 10, the simplex shrinks and becomes almost stationary around experiment 8.

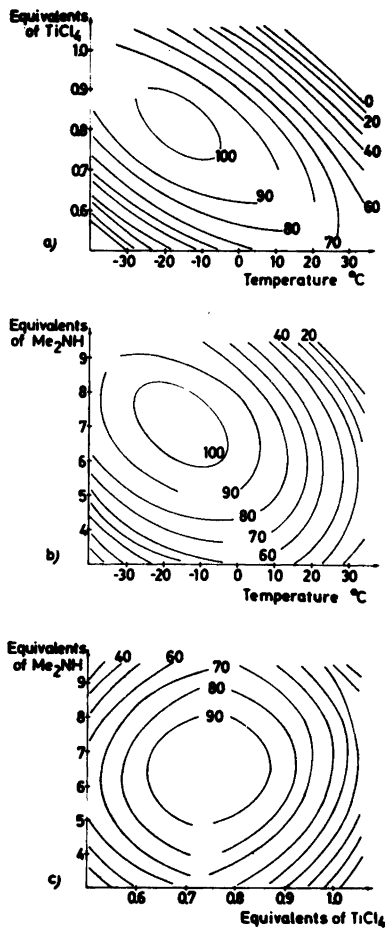

Fig. 3. Response surface obtained in optimization of the dimethylamine enamine synthesis from methyl isopropyl ketone. The numbers at the isoresponse contour lines show the yield (%). The projections are (*a*) amount of dimethylamine 6.3 equivalents, (*b*) amount of titanium tetrachloride 0.78 equivalent, (*c*) temperature −2 °C.

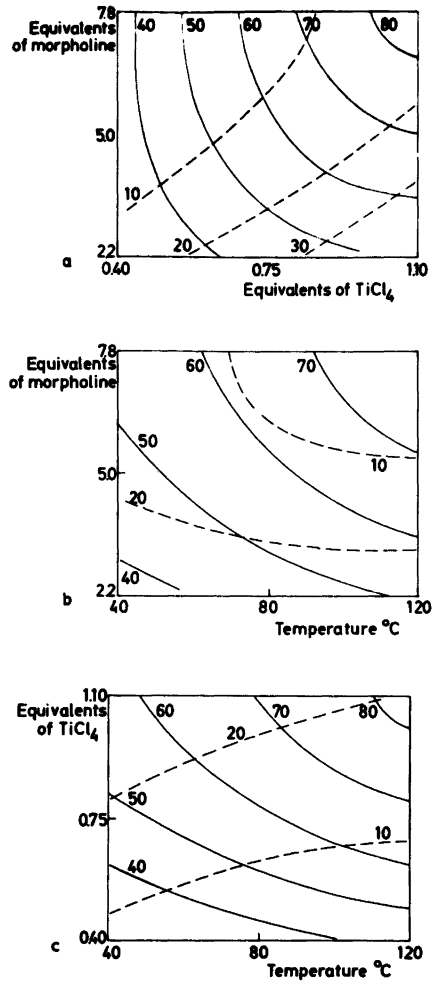

Fig. 4. Response surface obtained in optimization of the morpholine enamine from 3,3-dimethyl-2-butanone. The numbers in the figures refer to the yield (%). The solid isoresponse contour lines show the yield of enamine, the dashed isoresponse contour lines show the yield of condensation product *9*. The projections are: (*a*) temperature 80 °C, (*b*) amount of titanium tetrachloride 0.75 equivalent, (*c*) amount of morpholine 5.0 equivalents.

DISCUSSION

Our experiments were designed to ascertain the optimum conditions for enamine synthesis and not to reveal mechanistic information. However, our finding that addition of ketone to a preformed complex between amine and titanium tetrachloride gives considerably shorter reaction times as compared with the White and Weingarten procedure, suggests that the reactive species is not titanium tetrachloride *per se* but a complex between titanium tetrachloride and amine. The stoichiometry of this complex cannot be stated with any certainty but the results in Tables 1 – 3 show that at least a fivefold excess of amine over titanium tetrachloride is essential for optimum conditions. The observation that too large an excess of amine

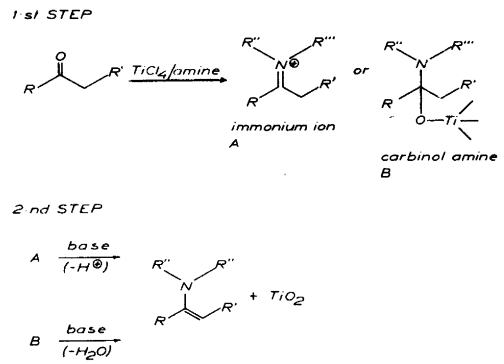

Fig. 5. The enamine formation.

leads to a decrease in yield with several ketones (see, for instance, Fig. 3) suggests that the enamine formation is at least a two-step reaction (Fig. 5). The first step is probably a reaction between a reactive amine – titanium tetrachloride complex and ketone to yield either an immonium ion or a titanium-coordinated carbinol amine. The second step involves either a deprotonation of the immonium ion or base-catalyzed elimination of the elements of water from the carbinol amine. Support for these mechanisms is furnished by some observations from the synthesis of dimethylamine enamine from diisopropyl ketone, *6*. When this ketone was treated with a dimethylamine – titanium tetrachloride complex in a 6:1 ratio, rapid consumption of the ketone was observed but no enamine was formed. A larger excess of dimethylamine resulted in enamine formation. Rapid formation of the dimethylamine enamine occurred when pyrrolidine was added to the reaction mixture (6:1 dimethylamine – titanium tetrachloride) after *ca.* 50 % of the ketone had been consumed. If a suspension in pentane of the isolated immonium trifluoroacetate from the dimethylamine enamine of *6* was treated with dimethylamine at room temperature, no deprotonation resulting in enamine could be observed (GLC). This was also found to be the case upon treatment with triethylamine, quinuclidine and *tert*-butylamine. When the isolated immonium salt was treated with pyrrolidine, rapid formation of the *pyrrolidine enamine* occurred. Previous studies on the deprotonation of immonium salts from morpholine enamines[9] have shown that the counter ion does not influence the course of the deprotonation reaction. If this also holds for dimethylamine-derived immonium salts, the observations stated above seem to rule out the involvement of a free immonium salt as an intermediate. An intermediate in the enamine-forming reaction sequence might be a titanium-coordinated immonium salt or a titanium-coordinated carbinol amine. The dependence of optimum conditions on the joint influence of the amount of amine and the amount of titanium tetrachloride can thus be rationalized; for a reactive titanium tetrachloride – amine complex of correct stoichiometry, the amine – titanium tetrachloride ratio is critical. Too high or too low a ratio shifts the complexation equilibria and reduces the overall rate of the first step. For a high rate of the base-catalyzed second step, it is essential to have a sufficiently high concentration of free amine. These results show that the mechanism of enamine formation is more complex than that suggested by White and Weingarten.[1]

EXPERIMENTAL

An ABC 80 Z-80 bit microcomputer (Scandia Metric) was used for computation and calculations for response surface modelling and for simplex optimization. The microcomputer programs were written in BASIC and are available from this laboratory.[10]

GLC analyses. PYE M 64 Gas Chromatography with FID were used with 5 % Peg 20M + 0.5 % KOH (1.5 m, 4 mm ID) and 12 % QF 1 (2.1 m, 2 mm IR) on Chromosorb W AW DMCS (100 – 120 mesh) glass columns. Phenylcyclohexane *puriss* and *t*-butylbenzene *purum* were used as internal standards. Integrated peak areas were used for quantification, and a Spectra Physics Minigrator^{R} was used to measure the peak areas.

NMR. Spectra were recorded on a JEOL C-60 HL or on a Bruker WM-250. Chemical shifts were measured at 26 °C using TMS as internal standard.

Chemicals. Ketones and amines were commercial *puriss* or *p.a.* products; titanium tetrachloride was technical grade and was used without purification.

Solvents. Pentane, hexane or ligroin (b.p. 36, 69, 110 °C) of technical grade were dried over $CaCl_2$ prior to use. *Note:* The solvents used in the enamine synthesis from pyrrolidine are preferably dried over sodium wire prior to use since these enamines are very sensitive to moisture.

A typical procedure for optimization experiments. A 250 ml three-necked flask equipped with a dropping funnel, reflux condenser and stirrer (Hershberg) was purged with dry nitrogen prior to use and protected from moisture. Using the amounts of titanium tetrachloride and amines given in Tables 1 – 3 the reactions were performed as follows: 4 g internal

standard and amine were dissolved in 100 ml of solvent. After cooling to 0 °C, the given amount of $TiCl_4$ dissolved in 10 ml of solvent was added dropwise with vigorous stirring. After addition was complete, 0.05 mol of ketone in 20 ml of solvent was added in one portion. When addition was complete, the reaction was allowed to proceed at the temperature and for the time according to Tables 1 – 3. Samples were withdrawn at regular intervals, filtered, diluted with hexane and analyzed by GLC.

Synthesis of 4-(2,6-dimethyl-3-hepten-4-yl)morpholine. To a vigorously stirred, cold (0 °C) solution of 680 g (7.8 mol) of morpholine in 1500 ml of hexane was added 146 ml (1.33 mol) of titanium tetrachloride in 300 ml of hexane. After addition was complete 142.2 g (1.0 mol) of diisobutyl ketone was added in one portion. The cooling bath was removed and the reaction allowed to proceed under reflux for two hours. After cooling, the mixture was filtered through a sintered glass filter and the solvent removed under reduced pressure, giving a yellow oil which was distilled under reduced pressure. Yield 171.4 g (87.7%), b.p. 106 – 108 °C/10 mmHg.

Note. A similar procedure was used for the other enamines. However, pyrrolidine enamines are prone to severe foaming upon distillation, which can be somewhat relieved if, after evaporation of the solvent, the crude product is filtered a second time through a sintered glass filter of pore size 4.

Physical properties of the enamines. The ^1H NMR spectra are all in accordance with the expected spectra. For brevity, only the chemical shifts for the vinylic protons are given below.

Morpholine enamines from ketone.

1, b.p. 69 – 71 °C/7 mmHg (lit.[1] 100 °C/35 mmHg).
2, b.p. 99 – 101 °C/35 mmHg (lit.[11] 89 – 90 °C/15 mmHg).
3, b.p. 84 – 85 °C/10 mmHg (lit.[12] 76 – 77 °C/3 mmHg).
4, b.p. 99 – 101 °C/10 mmHg, NMR (benzene) δ (s) 3.97 (2 H).
5, b.p. 74 – 75 °C/10 mmHg (lit.[13] 77 – 78 °C/9 mmHg).
6, b.p. 87 °C/10 mmHg (lit.[13] 102 – 103 °C/12 mmHg).
7, b.p. 104 – 105 °C/10 mmHg, NMR($CDCl_3$) mixture of regio isomers, δ (q) 4.52, $J = 6.6$ Hz (1 H), δ (d) 4.17, $J = 9.6$ Hz (1 H).
8, b.p. 106 – 108 °C/10 mmHg, NMR($CDCl_3$) δ (d) 4.26, $J = 8.8$ Hz.

Pyrrolidine enamines from ketone.

1, b.p. 76 – 79 °C/35 mmHg, NMR (benzene) δ (s) 3.52, 3.33 (1:1 H).
2, b.p. 62 – 63 °C/10 mmHg, NMR (benzene) δ (s) 4.4, 4.05 (1:1 H).
3, b.p. 70 – 72 °C/10 mmHg, NMR (benzene) δ (s) 3.84, 3.75 (1:1 H).
4, b.p. 78 °C/12 mmHg, NMR (benzene) (s) 3.83 (1 H.
5, b.p. 62 – 63 °C/10 mmHg (lit.[13] 62 – 67 °C/8 mmHg).
6, b.p. 69 – 70 °C/9 mmHg (lit.[14] NMR data).
7, b.p. 82 – 83 °C/ – 10 mmHg, NMR (benzene) mixture of regio isomers, δ (q) 4.4, $J = 7.5$ Hz (1 H), δ (d) 4.25, $J = 9$ Hz (1 H).
8, b.p. 93 – 95 °C/10 mmHg (lit.[15] 96 – 100 °C/10 mmHg).

Dimethylamine enamine from ketone.

1, b.p. 118 – 120 °C/754 mmHg (lit.[1] 56 °C/83 mmHg).
2, b.p. 58 – 59 °C/83 mmHg (lit.[1] 57 °C/71 mmHg).
3, b.p. 76 – 78 °C/115 mmHg, NMR (benzene) δ (s) 3.95, 3.85 (1:1 H).
4, b.p. 81 – 82 °C/90 mmHg, NMR (benzene) δ (s) 3.98 (2 H).
5, b.p. 68 – 70 °C/79 mmHg (lit.[16] NMR data).
6, b.p. 73 – 75 °C/80 mmHg (lit.[1] 82 °C/82 mmHg).
7, b.p. 103 °C/48 mmHg, NMR (benzene) mixture of regio isomers, δ (q) 4.5, $J = 7.5$ Hz (1 H), δ (d) 4.18, $J = 9$ Hz (1 H).
8, b.p. 93 – 96 °C/37 mmHg, NMR (benzene) (s) 4.4, 4.23 (1:1 H).

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Reaction of Grignard Reagents with Benzaldehyde, 2,2-Dimethylpropanal and Cinnamates of Chiral Alcohols in Chiral Solvents

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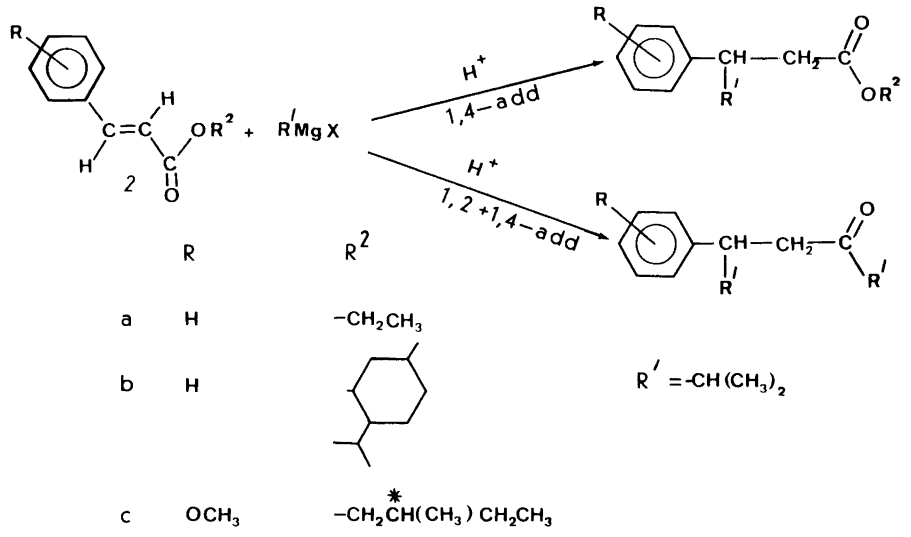
The asymmetric induction in a Grignard reaction carried out in (−)-1-isopropyl-2-methoxy-4-methylcyclohexane was higher than in the reaction carried out in (+)-1-methoxy-2-methylbutane. The chirality of the solvent contributed more than that of the reagent to the stereodifferentiation in the reaction of (+)-2-methylbutylmagnesium bromide with benzaldehyde in (+)-1-methoxy-2-methylbutane.

The asymmetric addition of benzylmagnesium chloride to chiral cinnamates has been examined by Kawana and Emoto.[1] They found that the addition of cuprous chloride to the reactions did not significantly change the stereoselectivity. However, the addition of cuprous chloride to the reactions of phenylmagnesium bromide with chiral crotonates caused a significant increase in stereoselectivity, even reversing the sign of rotation of the product.[1,2] Other asymmetric Grignard reactions[3,4,5] as well as reactions with lithium diorganocuprates[5,6,7,8] have later been extensively investigated. Partial stereoselective syntheses have also been accomplished with the Simmons-Smith reagent and chiral cinnamates.[9]

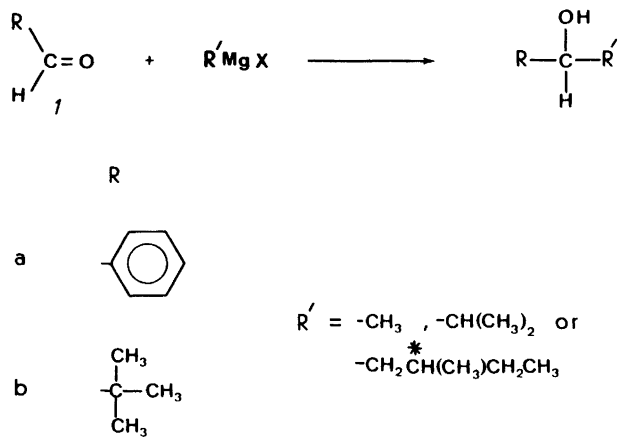
The purpose of the present study was to examine the contribution of chiral substrates, reagents and solvents to the stereoselectivity in the reactions of some Grignard reagents with cinnamates (Scheme 1) and two aldehydes (Scheme 2).

(+)-1-Methoxy-2-methylbutane and (−)-1-isopropyl-2-methoxy-4-methylcyclohexane were



Scheme 1.

0302-4369/83 $2.50



Scheme 2.

used as chiral solvents. The asymmetric induction was generally somewhat higher in the latter solvent (reactions 3, 5, 9 and 10; Table 1) but the total yield was lower. The efficiency of the asymmetric induction in the reactions of benzaldehyde increased as the steric bulk of the Grignard reagents was increased (reactions 1, 2, 3 and 5). The results of reactions 3 and 4 show that the chirality of the reagent does not contribute to the stereodifferentiation in these reactions. The d.e. in the reaction of the chiral reagent in (+)-1-methoxy-2-methylbutane and (−)-methyl menthyl ether was 5.28 and 5.4% respectively. The asymmetric induction, according to GLC was reversed in (−)-methyl menthyl ether compared with that in (+)-1-methoxy-2-methylbutane. The diastereomers were almost completely separated on a 50 m SP-1000 capillary GLC column. Although the capillary GLC analyses did not provide information of the sign of optical rotation, GLC appeared to be an excellent method for determining the diastereomeric excess of these reaction products in small quantities and without isolating the diastereomers.

Table 1. Addition of R^1MgX to some aldehydes and alkyl 3-phenylpropenoates according to Scheme 1 and 2, (d) in (+)-1-methoxy-2-methylbutane, (e) in (±)-1-methoxy-2-methylbutane and (f) in (−)-1-isopropanol-2-methoxy-4-methylcyclohexane. R* = (+)-2-methylbutyl.

Reaction No.	Substrate	Reagent	Solvent	1,4-add. ratio/%	1,2+1,4-add. ratio/%	Total GLC yield/%	$[\alpha]_D^{20}$ (°)	c g ml^{-1} (benzene)	e.e./%
1	*1a*	MeMgI	d			92	+0.06	0.085	0.11
2	*1a*	i-PrMgBr	d			72	+0.11	0.22	0.24
3	*1a*	R*MgBr	d			67			5.28[a]
4	*1a*	R*MgBr	e			69			—
5	*1a*	R*MgBr	f			58			5.40[a]
6	*1b*	PhMgBr	f			78	−5.04	0.17	19.4
7	*2a*	i-PrMgBr	d	67		69	+0.008[b]	0.25	0.026
					33		−0.09	0.088	
8	*2c*	i-PrMgBr	d	72		72	−0.24[c]	0.49	0.72
					28		+0.023	0.13	
9	*2b*	i-PrMgBr	d	99		72	+0.72[c]	0.55	2.15
10	*2b*	i-PrMgBr	f	100		65	+1.19[c]	0.043	3.56

[a] Determined by GLC. [b] Measured as ethyl ester. [c] Measured as methyl ester.

The highest asymmetric induction (e.e. 19.4%) was achieved in the reaction of 2,2-dimethylpropanal with phenylmagnesium bromide in chiral methyl menthyl ether. An enantiomeric excess of 11% has earlier been achieved in the same reaction carried out in (+)-2-methyltetrahydrofuran.[10]

The reaction of ethyl and (+)-2-methylbutyl cinnamate with isopropylmagnesium bromide gave besides the 1,4-addition product a ketone (*10*) resulting from diaddition (1,2+1,4-addition). The diaddition was almost completely suppressed when the alcohol components above were replaced by the bulkier menthyl or *tert*-butyl[11] group. The asymmetric induction was somewhat higher in chiral methyl menthyl ether than in (+)-1-methoxy-2-methylbutane. But the chiral solvent alone exerted a poor asymmetric induction. The highest stereodifferentiation on the cinnamates was achieved with (−)-menthyl cinnamate in chiral methyl menthyl ether (3.56%).

EXPERIMENTAL

A Varian 2400 gas chromatograph equipped with a 30 m × 0.2 mm glass capillary column (stationary phase SP 1000) was used for GLC analyses. Mass spectra were recorded at 70 eV on an LKB 9000 instrument equipped with the same capillary column GLC system. ^{1}H NMR spectra were obtained on a Perkin-Elmer R 12 A spectrometer at 60 MHz. The Grignard reactions were carried out under purified nitrogen. The chiral solvents were distilled from sodium prior to use. The concentration of Grignard reagents were determined by standard titration. The magnesium turnings were synthetical grade.

Preparation of chiral substances

(+)-1-Methoxy-2-methylbutane (d) was prepared from (−)-2-methyl-1-butanol and methyl iodide in the presence of sodium by the method of Williamsson.[12] Distillation of the crude product over sodium gave 63% of *d* b.p. 85−90 °C, $[\alpha]_D^{20}$ +0.37°, Lit.[13] $[\alpha]_D^{20}$ +0.34°.

(+)-1-methoxy-2-methylbutane (e) was prepared in the same way from (±)-2-methyl-1-butanol.

(+)-1-Bromo-2-methylbutane[14] *(4)* was synthesized from (−)-2-methyl-1-butanol (0.5 mol), concentrated hydrobromic acid (0.625 mol) and concentrated sulfuric acid (0.25 mol). Distillation at 120 °C gave 55% of *4* $[\alpha]_D^{20}$ +3.2°. Lit.[14] $[\alpha]_D^{20}$ +3.68°, which corresponds to 87% optical purity.

(±)-1-Bromo-2-methylbutane was synthesized in the same way from (±)-2-methyl-1-butanol.

(+)-2-Methylbutyl 3-(2-methoxyphenyl)propenoate (2c) was prepared by refluxing 3-(2-methoxyphenyl)propenoic acid (0.25 mol) and (−)-2-methyl-1-butanol (0.3 mol) in the presence of a cation-exchange resin (Dowex 50 W × 8, 5.55 g) and benzene (0.75 mol) for 48 h. Distillation at 189−191 °C/1.2 kPa gave 55% of *2c* $[\alpha]_D^{20}$ +8.

(±)-2-Methylbutyl 3-(2-methoxyphenyl)propenoate was prepared in the same way from 3-(methoxyphenyl)propenoic acid and (±)-2-methyl-1-butanol.

(−)-Menthyl 3-phenylpropenoate[15] *(2b)* was prepared by the reaction of 3-phenylpropenoyl chloride with one equivalent (−)-menthol at 140 °C. The yield of the product boiling at 229−232 °C/3.6 kPa was 54% and $[\alpha]_D^{20}$ −77°, lit.[15] $[\alpha]_D^{20}$ −77°.

(−)-1-Isopropyl-2-methoxy-4-methylcyclohexane (f). (−)-Menthol (1 mol) was reacted with sodium (2 mol) for 56 h and then with methyl iodide. Distillation of the crude product from sodium gave 59% of *f*, b.p. 60 °C/2.7 kPa, $[\alpha]_D^{20}$ −95.2°, lit.[16] $[\alpha]_D^{20}$ −95.7°.

2,2-Dimethylpropanal[10] *(8).* *t*-BuMgCl (0.75 mol) in diethyl ether was reacted with *N,N*-dimethylformamide (1.5 mol) at 0 °C. After reflux for 16 h and normal work-up, *8* was isolated as a bisulphite addition complex. Distillation at 75 °C gave 31% of *8*.

General procedure for the Grignard reactions in chiral solvents. The Grignard reagents were prepared from Mg turnings (0.03 mol) and the appropriate alkyl or aryl halide (0.025 mol) in 15 ml of the chiral ether. The Grignard reactions were carried out by adding dropwise the substrates to the Grignard reagents in the proportion one to three at 0 °C. The reactions were followed by GLC until complete conversion. The reaction mixtures were decomposed with 5 M cold hydrochloric acid, extracted with diethyl ether, neutralized with $NaHCO_3$ and dried with Na_2SO_4.

Benzaldehyde and methylmagnesium iodide in (+)-1-methoxy-2-methylbutane. The main reaction product was (+)-1-phenylethanol b.p. 85 °C/1.9 kPa. The specific rotation was $[\alpha]_D^{20}$ +0.006° (*c* 0.085 benzene) and e.e. = 0.11%.

Benzaldehyde and isopropylmagnesium bromide in (+)-1-methoxy-2-methylbutane. The main reaction product was 2-methyl-1-phenylpropanol, b.p. 92−93 °C/1.3 kPa. The specific rotation was $[\alpha]_D^{20}$ +0.11° (*c* 0.22, benzene) and the optical purity 0.24%. Small amounts (< 1%) of phenylmethanol and 2-methyl-1-phenyl-1-propenone were also formed according to GLC-MS analyses.

Benzaldehyde and (+)-2-methyl-1-butylmagnesium

bromide in (+)-1-methoxy-2-methylbutane. The diastereomers of 3-methyl-1-phenylpentan-1-ol were formed almost exclusively according to GLC-MS. The same reaction was also carried out with (a) the chiral reagent in racemic ether and (b) with the racemic reagent in racemic ether in order to check the influence of the chiral reagent on the asymmetric induction. The separation of the diasteromers was almost complete on a 30 m capillary column (SP-1000). The proportions of the diastereomers in reactions a and b were the same, which shows that the chiral reagent does not have a discernable asymmetric induction in this reaction. The integrated areas of the slightly overlapping peaks gave the same diastereomer proportion *i.e.* 50.48 to 49.52%. The correction factor Δ because of overlapping peaks is then 0.96%.

The reaction with the chiral reagent and the chiral ether gave the diastereomers in the proportion 53.12 to 46.88%. The e.e. after correction is then 5.28%. The results show that the asymmetric induction (measurably by GLC) in this reaction is only dependent on the chiral ether.

Ethyl 3-phenylpropenoate and isopropylmagnesium bromide in (+)-1-methoxy-2-methylbutane. The reaction was performed with isopropylmagnesium bromide (0.1 mol) and ethyl 3-phenylpropenoate (0.03 mol) in 30 cm^3 of the chiral ether. The reaction time was 10 h at 20 °C. After ordinary work-up, ethyl 4-methyl-3-phenylpentanoate (*9*) and 2,6-dimethyl-5-phenylheptane-3-one (*10*) were identified by GLC-MS. The ketone (*10*) was separated by alkaline hydrolysis and distillation at 120 −125 °C/1.3 kPa. The specific rotation $[\alpha]_D^{20}$ was −0.09° (*c* 0.088, benzene). Anal. $C_{15}H_{22}O$:C, H. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.7 (3H, d, *J* 6.5 Hz), 0.8 (3H, d, *J* 6.5 Hz), 0.9 (6H, d, *J* 6.5 Hz), 1.8 (1H, m), 2.3 (1H, sep. *J* 6.5 Hz), a multiplet with the highest signal at 2.7 (3H, m), 7.0 (5H, s).

MS [IP 70 eV; *m/e* (% rel. int.)]: 218 (2, M), 175 [7,M−$CH(CH_3)_2$], 133 (90), 132 (98), 105 (25), 104 (23), 91 (91), 71 (73), 43 (100).

Ethyl 4-methyl-3-phenylpentanoate (9) was distilled at 94−96 °C/0.5 kPa, $[\alpha]_D^{20}$ +0.008° (*c* 0.25, benzene). Anal. $C_{14}H_{20}O_2$: C, H. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.7 (3H, d, *J* 6.5 Hz), 0.9 (3H, d, *J* 6.5 Hz), 0.95 (3H, t, *J* 7.2 Hz), 1.8 (1H, m), a multiplet with the highest signal at 2.7 (3H, m), 3.8 (2H, q, *J* 7.2 Hz), 7.0 (5H, s).

MS [IP 70 eV; *m/e* (% rel. int.)]: 220 (18, M), 175 (7, M−OC_2H_5), 135 (100, $PhCHOC_2H_5$) 133 [68, $PhCHCH(CH_3)_2$], 132 (67), 105 (77), 104 (50), 91 (77).

(+)-2-Methylbutyl 3-(2-methoxyphenyl)propenoate and isopropylmagnesium bromide in (+)-1-methoxy-2-methylbutane. The reaction gave 1,4- and 1,2+1,4-addition products in the ratio 3 to 1. The ester (*11*) was separated from the ketone (*12*) by alkaline hydrolysis. After acidification and methylation with diazomethane *methyl 4-methyl-3-(2-methoxyphenyl)pentanoate (11)* was distilled at 160 °C/2.7 kPa, $[\alpha]_D^{20}$ −0.24° (*c* 0.49, benzene).

^{1}H NMR (60 MHz, $CDCl_3$): δ 0.7 (3H, d, *J* 6.5 Hz), 0.9 (3H, d, *J* 6.5 Hz), 1.8 (1H, m), a multiplet with the highest signal at 2.6 (3H, m), 3.4 (3H, s), 3.7 (3H, s), 6.5−7.1 (4H, aromatic).

MS [IP 70 eV; *m/e* (% rel. int.)]: 236 (29, M), 193 [49, M−$CH(CH_3)_2$], 163 (17), 151 (100, $CH_3OPhCHOCH_3$), 121 (90, CH_3OPhCH_2), 91 (28).

2,6-Dimethyl-5-(2-methoxyphenyl)-heptan-3-one (12) was distilled at 126−128 °C/2.7 kPa, $[\alpha]_D^{20}$ +0.023° (*c* 0.13, benzene).

^{1}H NMR (60 MHz, $CDCl_3$): δ 0.7 (3H, d, *J* 7.0 Hz), 0.85 (3H, d, *J* 7.0 Hz), 9.0 (6H, d, *J* 7.0 Hz), 1.8 (1H, m), 2.3 (1H, m), a multiplet with the highest signal at 2.8 (3H, m), 3.8 (3H, s), 6.4−7.0 (4H, aromatic).

MS [IP 70 eV; *m/e* (% rel. int.)]: 248 (32, M), 205 (27, M−C_3H_7), 164 (70), 163 (78), 121 (77, CH_3OPhCH_2), 91 (70), 71 (36), 43 (100).

(−)-Menthyl 3-phenylpropenoate and isopropylmagnesium bromide in (+)-1-methoxy-2-methylbutane. The ratio of 1,4- and 1,2+1,4-addition products was 99 to 1%. The ester was submitted to alkaline hydrolysis and the acid was methylated with diazomethane. Methyl 4-methyl-3-phenylpentanoate (*13*) was distilled at 133 −135 °C/2.6 kPa, $[\alpha]_D^{20}$ +0.72° (*c* 0.55, benzene).

^{1}H NMR (60 MHz, $CDCl_3$): δ 0.7 (3H, d, *J* 6.5 Hz), 0.9 (3H, d, *J* 6.5 Hz), 1.8 (1H, m), a multiplet with the highest signal at 2.6 (3H, m), 3.3 (3H, s), 7.0 (5H, s).

MS [IP 70 eV; *m/e* (% rel. int.)]: 206 (18, M), 164 (10), 163 (9, M−C_3H_7), 132 (35), 121 (100, $PhCHOCH_3$), 104 (48, $PhCHCH_2$), 91 (28), 77 (10), 59 (10).

Benzaldehyde and (+)-2-methyl-1-butylmagnesium bromide in (−)-1-isopropyl-2-methoxy-4-methylcyclohexane. The diastereomers of 3-methyl-1-phenylpentane-1-ol was formed in the ratio 52.7 to 47.3% as measured by capillary GLC. The e.e. is then 5.4%. The asymmetric induction is the opposite to the one observed when the reaction was carried out in (+)-1-methoxy-2-methylbutane.

(−)-1-Methyl 3-phenylpropenoate and isopropylmagnesium bromide in (−)-1-isopropyl-2-methoxy-4-methylcyclohexane. The only reaction product formed was the 1,4-addition product, which was isolated as *methyl 4-methyl-3-phenylpropenoate* at 133−135 °C/2.6 kPa, $[\alpha]_D^{20}$ +1.19° (*c* 0.43, benzene).

2,2-Dimethylpropanal and phenylmagnesium bromide in (−)-1-isopropyl-2-methoxy-4-methylcyclohexane. The main reaction product was 2,2-

dimethyl-1-phenylpropanol which was isolated by preparative GLC (6 m × 1.2 cm metal column, SE-30 15 %). $[\alpha]_D^{20}$ −5.04° (*c* 0.17, benzene) e.e. 19.4 %.

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The Michael Reaction. Mechanism, Stereochemistry and a Synthetically Useful Modification

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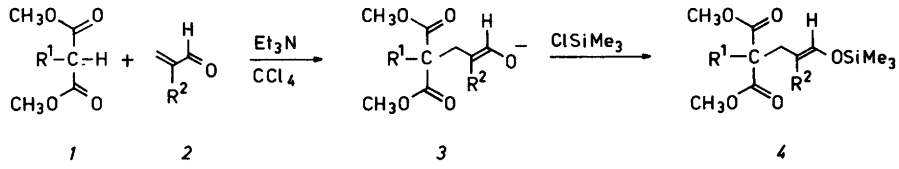
Dimethyl malonate anions react with α,β-unsaturated aldehydes giving products with *trans* stereochemistry, Scheme 1. The enolate anions may be trapped with trimethylchlorosilane to give the enol ethers *4* with better than 95% stereoselectivity. Triethylamine, trimethylchlorosilane and acrolein react similarly to give the enol ether *7* with the opposite, *i.e. cis*, stereochemistry. Dipole minimization in the transition states (Schemes 2 and 3) seems an obvious rationalization of the observed stereochemistry. The triethylamine catalyzed addition of dimethyl nitromalonate to atropaldehyde produces a stereoisomeric mixture of the enols *Z-5* and *E-5* as the initial product.

The mechanism of the Michael reaction is generally assumed to involve the formation of stereochemically non-specified enolate anions, subsequently protonated and tautomerizing to give the final adducts.[1–4] We present evidence substantiating this general scheme, but extended to provide information about the stereochemical course of the addition. Thus, the addition of a malonic ester anion to α,β-unsaturated aldehydes proceeds in a *trans* fashion with a stereoselectivity exceeding 95%. "*trans*" here refers to the position of the methylene carbon atom relative to the enolic oxygen atom in the adducts *3* (Schemes 1 and 2).

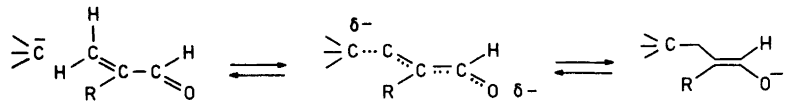
The proposed configuration of the primary enolates *3* rests on trapping the enolate anions with trimethylchlorosilane (Scheme 1) and comparing the coupling constants of the resulting enol ethers *4* with literature values[5,6] (see Experimental). The reactions *1*→*3*→*4* (Scheme 1) are kinetically controlled. We conclude from these experiments that the stereochemistry of the C–C bonding step of the Michael reaction is nearly 100% stereoselective and determined by stereoelectronic requirements of the transition state, rather than by steric effects. This is illustrated in Scheme 2, where the transition state is formulated such as to minimize the dipole moment. The stereoselectivities of the reactions *1a* and *1b* to *4a* and *4b*, respectively, are higher than those of the corresponding reactions of *1c* (*ca.* 95%) and *1d*. This may be attributed to the better localized charge on anions of the unsubstituted (*1a*) or monoalkylated (*1b*) malonic esters, as compared to the anions of the more acidic esters *1c* and *1d*.



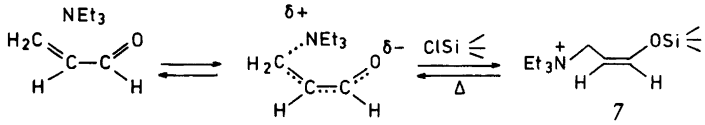
1 *2* *3* *4*

Scheme 1. Stereoselective formation of enolates and of silyl enol ethers.
a: $R^1 = R^2 = H$
b: $R^1 = E\text{-}CH_2CH{=}CH{-}OSi(CH_3)_3$, $R^2 = H$
c: $R^1 = NO_2$, $R^2 = C_6H_5$
d: $R^1 = CN$, $R^2 = C_6H_5$

0302-4369/83 $2.50



Scheme 2. Illustrates transoid transition state.



Scheme 3. Illustrates cisoid transition state.

The trimethyl silyl enol ether *Z-4d*, Scheme 1, is the thermodynamically least stable isomer. Upon heating for four hours at 150 °C it stereomutates to give a *ca.* 40:60 ratio of the *Z-4d* and *E-4d* isomers. Higher temperatures employed in a GLC analysis of the mixture resulted in still more advanced isomerization (see Experimental).

Addition of triethylamine to a mixture of acrolein and trimethylchlorosilane in petroleum ether gave the enol ether 7 with a stereochemistry opposite to that of the enol ethers formed by addition of the anionic nucleophiles. This observation supports the suggested mechanism, as illustrated in Scheme 3, where dipole minimization again predicts the observed stereochemistry. The polarity of the solvent is decisive: in light petroleum, the content of the *E*-isomer in the crude product was 3%, in carbon tetrachloride 20% and in deuteriochloroform about 50%. The salt 7 hydrolyzes rapidly to acrolein and reacts with C-nucleophiles, such as the anions of *1a* and *1b*, to products of type *4*, demonstrating the reversibility of the reaction in Scheme 3.

Further details regarding the mechanism of the Michael reaction were obtained by following (^{1}H NMR) the triethylamine catalyzed reaction of dimethyl nitromalonate *1c* with atropaldehyde in carbon tetrachloride and benzene, see Fig. 1. The Michael adduct *6* was *not* the first to appear. A considerable (*ca.* 30%) amount of enol *Z-5* was

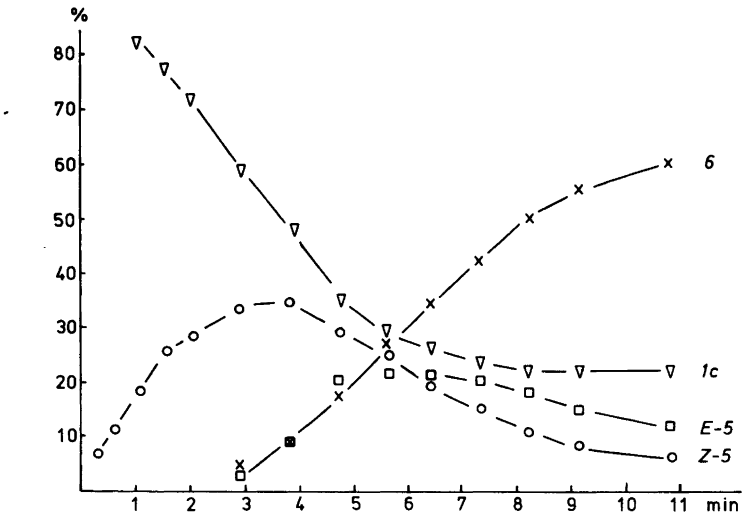
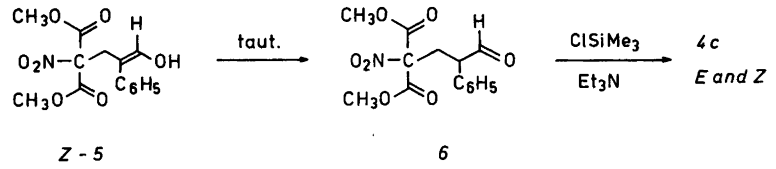


Fig. 1. Time course for the reaction of dimethyl nitromalonate (▽) with atropaldehyde to enols *Z-5* (○) and *E-5* (□), and finally to Michael adduct *6* (×). Percentages total 100%.



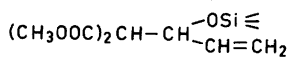
Z - 5 6

Scheme 4. Tautomerization of *Z-5* (*3c* protonated) to Michael adduct *6*, see Fig. 1.

formed before the concentrations of the isomeric enol *E-5* and the tautomer *6* could be observed. The delayed formation of the two latter products suggests that *Z-5* is the common precursor. Enol *E-5* could be detected for a longer time (*ca.* 5 min) than enol *Z-5*, suggesting that the tautomerization of the latter is fastest. Identification of the postulated enols *Z-5* and *E-5* relies on the observed resonances of the ester groups and the simultaneous appearances and disappearances of resonances at δ-values matching those of the corresponding silyl ethers *Z-4c* and *E-4c*, respectively, see Experimental.

The modification of the Michael reaction described here has synthetic advantages as compared to the original and commonly employed version. It is experimentally simple and affords a protected aldehyde function, permitting further functionalization under basic conditions as illustrated in Scheme 1 (reaction *1b*→*4b*). Trimethylsilyl enol ethers are useful and versatile starting materials.[7-10] The scope of the reaction has not been studied in detail. Steric hindrance and/or low acidity may render the reaction synthetically useless: compare, for instance, the reaction conditions for *1a*→*4a* with those for *4a* (=*1b*)→*4b*.

A recent communication describes a Michael reaction sterically controlled by repulsion of negative charges on oxygen.[11]



8

EXPERIMENTAL

Mass spectra were recorded on a VG Micromass 7070 F instrument, IP 70 eV. NMR spectra were obtained on Bruker WH-90, HXL-90, and HX-270 instruments. Solvent: $CDCl_3$. Acrolein (Ferak, "rein", stabilized) was kept at 0 °C. Trimethylchlorosilane (Fluka *purum*) contained *ca.* 10% hexamethylsiloxane (NMR). Triethylamine was kept over potassium hydroxide.

General procedure. To a mixture of the malonic ester derivative (100 mmol), acrolein (7.2 ml), and trimethylchlorosilane (24 ml) in carbon tetrachloride (100 ml), triethylamine (30 ml) was added at room temperature. The mixture was protected from moisture and refluxed (see below), cooled to −20 °C, and stirred with ice for 5 min. The water phase was washed twice with carbon tetrachloride, the combined organic phases dried (Na_2SO_4), concentrated *in vacuo* and distilled.

Methyl 2-carbomethoxy-5-trimeth-ylsilyloxy-4E-pentenoate, E-4a. Reaction time: 3 h at 77 °C (reflux). Slow distillation (20 cm by 2 cm Vigreux column) gave a forerun (see below) b.p. 38−84 °C/0.15 mmHg, and *4a*. Yield 11.1 g (43%), b.p. 84 °C −103 °C/0.15−0.3 mmHg. Redistillation gave b.p. 90 °C/0.25 mmHg. Anal. $C_{11}H_{20}O_5Si$: C, H. ^{1}H NMR: δ 0.17 (9H, s), 2.47 (2H, dt, *J* 1.1 and 7.5 Hz), 3.36 (1H, t, *J* 7.5 Hz), 3.71 (6H, s), 4.89 (1H, dt, *J* 12 and 7.5 Hz), 6.24 (dt, *J* 12 and 1.1 Hz). ^{13}C NMR [22.63 MHz]: δ −0.8 (q, CH_3Si), 27.0 (t, C3), 52.0 (q, CH_3O), 52.4 (d, C2), 106.5 (d, C4), 142.0 (d, C5), 168.9 (C=O).

Isomerization of 4a. *E-4a* (325 mg) in ether (0.8 ml) was irradiated for 22 h with UV light (quartz apparatus, Hanovia lamp 679A-36) at 25 °C. ^{1}H NMR showed a *ca.* 60% content of *Z-4a*: δ 0.18 (s), 2.67 (dt, *J* 1.5 and 7.5 Hz), 3.42 (t, *J* 7 Hz), 3.71 (s), 4.44 (dt, *J* 5.8 and 7.1 Hz), 6.17 (dt, *J* 5.8 and 1.5 Hz). None of this isomer was detected in *E-4a* prepared above.

The forerun (above) contained (NMR) dimethyl malonate (*ca.* 21 mmol), the enol silyl ether *4a* (*ca.* 13 mmol), and the silylated 1,2-adduct *8* (*ca.* 12 mmol). Fractionation of several foreruns gave *8*, b.p. 65−67 °C/0.2 mmHg. Anal. $C_{11}H_{20}O_5Si$: C, H. ^{1}H NMR: δ 0.10 (9H, s), 3.49 (d, *J* 9.2 Hz), 3.68 (3 H, s), 3.73 (3 H, s), 4.13 (1 H, dddd, *J* 9.2, 6.4, *ca.* 1, and *ca.* 1 Hz), 5.11 (1 H, ddd, *J* 10,0, *ca.* 2, and 1 Hz), 5.26 (1 H, ddd, *J* 17.0, *ca.* 2, and *ca.* 1 Hz), 5.84 (1 H, ddd, *J* 17.0, 10.0 and 6.4 Hz). ^{13}C NMR [22.63 MHz]: δ −0.2 (silyl), 52.0 (methoxy), 59.4 (C2), 72.6 (C3), 116.6 (C5), 137.5 (C4), 166.7 and 167.3 (diastereotopic C=O).

4,4-Dicarbomethoxy-1,7-di(trimethysiloxy)-1E-

6E-*heptadiene* 4b. Enol ether *E-4a* (11.1 g, above) was refluxed (general procedure, above), for 22 h. Yield 6.62 g recovered *4a* (b.p. 80–123 °C/0.15 mmHg) and *4b*. Yield 5.90 g (38%; 95% based on consumed *4a*), b.p. 123–143 °C/0.15 mmHg, mainly 133 °C/0.15 mmHg. Anal. $C_{17}H_{32}O_6Si_2$: C, H. ^{1}H NMR: δ 0.17 (18 H, s), 2.46 (4 H, dd, *J* 8.0 and 1.2 Hz), 3.67 (6 H, s), 4.74 (2 H, dt, *J* 12 and 8.0 Hz), 6.18 (2 H, dt, *J* 12 and *ca.* 1 Hz). ^{13}C NMR [22.63 MHz]: δ −0.7 (silyl), 30.7 (C3 and C5), 52.0 (methoxy), 58.5 (C4), 104.7 (C2 and C6), 142.4 (C1 and C7), 171.1 (C=O).

Methyl 2-carbomethoxy-2-nitro-4-phenyl-5-trimethylsilyloxy-4Z-pentenoate, Z-4c. Dimethyl nitromalonate[12] (1.5 ml), atropaldehyde[13] (1.0 g), and trimethylchlorosilane (1.4 ml) in carbon tetrachloride (5 ml) were cooled to 2 °C and triethylamine (2.0 ml) was added. The mixture was kept at 20 °C for a further 10 min. Work-up as above. The crude product could not be distilled (0.2 mmHg) without extensive decomposition. Crystallization from toluene–ligroin (6 ml, 1:1) at −80 °C gave 1.9 g, m.p. 56–58 °C. Two recrystallizations from ether (10 ml) gave m.p. 61–62 °C. Anal. $C_{17}H_{23}NO_7Si$: C, H, N. ^{1}H NMR: δ 0.15 (9 H, s), 3.42 (6 H, s), 3.51 (2 H, broadened d, *J* 0.9 Hz), 6.38, (1 H, only partly resolved t, *J* < 1 Hz), 7.11–7.31 (5 H, m). A resonance at δ 0.19 suggests a maximum content of 4% *E-4c* in a sample of crude *4c*. ^{13}C NMR [67.89 MHz]: δ 37.6 (C3), 53.3 (methoxy), 96.0 (C2), 112.3 (C4), 126.3, 127.5, 128.7, and 135.7 (phenyl), 141.0 (C5), 162.1 (C1), *J* (C3,H3) 134 Hz, *J* (C3,H5) 4 Hz, *J* (C5,H5) 177 Hz, *J* (C5,H3) 7 Hz. The 4 Hz coupling constant is consistent with a C3 *cis* to H5 configuration (1.7 × 4 = 6.8 Hz, *cf. J* (*cis*-protons) 5.8 Hz in *Z-4a*).[6] MS [*m/z* (% rel. int.)]: 381 (6, M), 336 (2), 303 (3), 217 (15), 207 (21), 205 (9), 199 (11), 179 (8), 173 (8), 171 (12), 115 (9), 113 (29), 105 (10), 75 (9), 73 (100, $(CH_3)_3Si$).

A mixture of *E-4c* and *Z-4c* was prepared for comparison. Dimethyl nitromalonate (1.53 g), atropaldehyde (1.0 g), ether (2.5 ml) and triethylamine (0.1 ml) were refluxed for 20 min, cooled, washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. Yield 2.18 g of yellow oil, *6*. The product decomposed upon attempted distillation at 0.2 mmHg. ^{1}H NMR: δ 2.66 (dd, *J* 5.5 and 15 Hz), 3.60 (dd, *J* 5.5 and 15 Hz), 3.69 (s), 4.06 (broad t, *J* 5.5 Hz), 7.22–7.44 (m), 9.50 (d, *J* 0.5 Hz). The main impurity was triethylamine. MS [isobutane C.I.]: 310 (M + 1). The crude aldehyde (*6*, above, 0.30 g), trimethylchlorosilane (0.25 ml), carbon tetrachloride (3 ml), and triethylamine (0.3 ml) was stirred for 40 min at 20 °C. The mixture was diluted with light petroleum, washed with water, dried (Na_2SO_4) and concentrated *in vacuo*. ^{1}H NMR on the crude product showed *ca.* 30% *Z-4c* (as above) and 70% of the *E* isomer. δ 0.19 (s), 3.42 (s), 3.75 (s), 6.44 (s), 7.16 (s). ^{13}C NMR [67.89 MHz]: *J* (C3,H5) 6 Hz, *cf.* 4 Hz, above, suggesting C3 *trans* to H5.[6]

2,2-Dicarbomethoxy-4-phenyl-5-trimethylsilyloxy-4-Z-pentenonitrile Z-4d. Methyl cyanomalonate[14] (1.6 g), atropaldehyde[13] (1.3 g), trimethylchlorosilane (1.4 ml), and triethylamine (1.7 ml) were stirred at 40–47 °C for 40 min. Work-up as above gave 3.51 g of crude *4d*. ^{1}H NMR: δ 0.20 (9 H, s), 3.20 (2 H, partly resolved d, *J* < 1 Hz), 3.47 (6 H, s), 6.53 (1H, partly resolved t, *J* < 1 Hz) 7.00–7.42 (5H, m). A signal at δ 0.24 shows the presence of *ca.* 5% of the *E* isomer. Distillation of the crude product (b.p. 155–172 °C/0.2 mmHg) resulted in *ca.* 15% isomerization. The distillate was kept in an ampoule *in vacuo* at 148–152 °C for 4 h. ^{1}H NMR singlets at δ 0.24, 3.47, 3.51, 6.58, and 7.20 appeared and were assigned to the *E* isomer of *4d*. No splitting of the ester methyls was observed. Integration gave a 58:42 *E*–*Z* ratio. A GLC/MS analysis (1.5 m by 2 mm 5% OV-101, 170 °C isothermal, injection temperature *ca.* 200 °C) showed an isomerization to an approximately 2:1 ratio of *E*–*Z 4d*. MS [ip 70 eV; *m/z* (% rel. int. of *Z*; % rel. int. of *E*)]: 361 (M, 12; 14), 346 (M − 15, 3; 0), 205 (loss of cyanomalone ester, 86; 98), 73 ($Si(CH_3)_3$, 100).

N-*3(Trimethylsilyloxy)allyl*-N,N,N-*triethylammonium chloride* Z-7. Acrolein (0.63 ml), trimethylchlorosilane (1.4 ml), triethylamine (1.7 ml), and light petroleum (10 ml) were stirred for 30 min at 20 °C. The colorless crystals were washed with light petroleum and dried *in vacuo* to give crude salt 7. ^{1}H NMR: δ 0.24 (9 H, s), 1.42 (9 H, t, *J* 7.2 Hz), 3.40 (6 H, q, *J* 7.2 Hz), 3.98 (2 H, d, *J* 8.0 Hz), 4.60 (1 H, dt, *J* 5.7 and 8.0 Hz), 6.64 (1 H, d, *J* 5.7 Hz). Resonances at δ 1.40 (t, *J* 7.3 Hz) and 3.13 (dq, *J* 4.7 and 7.3 Hz) show the presence of triethylammonium chloride (*ca.* 30%), and at δ 6.94 (d, *J* 11.3 Hz) show the presence of the *E* isomer of 7 (*ca.* 3%). The experiment was repeated (1:20 scale, in deuteriochloroform). ^{1}H NMR (after 2 min at 20 °C) showed the presence of 7 and of the *E* isomer of 7 (ratio 1:1). ^{1}H NMR: δ0.24 (s), 1.39 (t, broadened), 3.39 (q, *J* 7 Hz), 4.09 (d, *J* 8.0 Hz), 4.84 (dt, *J* 11.3 and 8.0 Hz), 6.94 (d, *J* 11.3 Hz). Unreacted acrolein and some hexamethyldisiloxane was also present. Salt 7 hydrolyzes readily to acrolein, hexamethyldisiloxane, and triethylammonium chloride.

Methyl 2-carbomethoxy-2-nitro-4-phenyl-5-hydroxy-4-pentenoate. Enols *Z-5* and *E-5*, Fig. 1 and Scheme 4. Atropaldehyde (85 mg), diethyl nitromalonate (*1c*, 140 mg), carbon tetrachloride (0.2 ml), and deuteriobenzene (0.1 ml) were cooled to *ca.* −80 °C in an NMR tube. Triethylamine (10 µl) was added, the contents mixed rapidly and ^{1}H NMR spectra were recorded at intervals as

indicated in Fig. 1. Resonances at δ 3.62, 3.49, 3.33 and 3.30 were assigned the methoxy groups of dimethyl nitromalonate, the Michael adduct *6*, and the anols *E-5* and *Z-5*, respectively. Resonance heights normalized to 100% gave the product distributions plotted in Fig. 1. The accuracy of the measurements is low. The resonances of *E-5* and of *Z-5* are only partly resolved. Resonances at δ 3.44 and at 6.50[15] (broad and variable) increased and declined synchroneously with that of δ 3.30 and were assigned to the methylene and the vinylic protons of *Z-5*, respectively, *cf.* δ 3.51 and 6.38 for the corresponding protons of *Z-4c*. A resonance at δ6.72 (broad and variable) was assigned to the vinylic proton of *E-5*, *cf.* 6.44 for *E-4c*.[15] The variability of the vinylic protons may be due to exchange of enolic protons as the acidity of the medium changes.

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Synthesis of *N*-Phosphorylated Dimethylsulfoximides

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N-Phosphorylated-*S*,*S*-dimethylsulfoximides were prepared in good yields from the reaction between *N*-unsubstituted-*S*,*S*-dimethylsulfoximide and dialkyl (diaryl) phosphites, phosphorochloridates or phosphorochloridothionates. The same products could be obtained from trialkyl phosphites using *N*-bromo-*S*,*S*-dimethylsulfoximide and from the reaction between *N*-silylated dimethylsulfoximide and dialkyl phosphorochloridate. The new compounds obtained were characterized by their IR and ^{1}H NMR data.

The widespread interest in the chemistry of sulfoximides in recent years is evidently due to the versatility of their application in stereochemical studies[1–4] and as reagent in organic synthesis.[5] Also their biological activity is of interest[6] and for some *N*-sulfonylated sulfoximides slight antimalarial effects have been found.[7]

N-Substituted sulfoximides have been synthesized by nucleophilic substitution of *N*-unsubstituted sulfoximides with alkyl halides, acid halides, acid anhydrides and sulfonyl halides.[8,9] No reports on phosphorylated or thiophosphorylated sulfoximides are found in the literature, although such compounds seem interesting for stereochemical studies regarding both the S and the P atoms. Also the biological effect of this new structure may be of interest.

This paper describes the syntheses of simple model compounds containing the >P(O)N=S(O)< and the >P(S)N=S(O)< groupings.

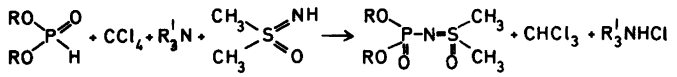
We have found that *N*-unsubstituted sulfoximide can be *N*-phosphorylated with satisfactory results by the modified Atherton-Todd procedure.[10,11] This involves the application of dialkyl phosphite–tetrachloromethane system, in the presence of tertiary amine, as a source of dialkyl phosphorochloridate as the reactive phosphorylating agent, (method 1, Scheme 1).

This slightly exothermic reaction proceeds in $CHCl_3$ solution under mild conditions giving good yields and often analytically pure crude products.

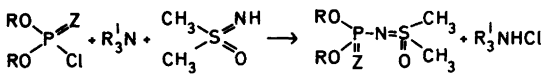
Nucleophilic substitution of preformed phosphorochloridates or phosphorochloridothionates with *S*,*S*-dimethylsulfoximide offers an alternative route to *N*-phosphorylated dimethylsulfoximides, (method 2, Scheme 2).

Methods 1 and 2 are versatile and can be used complementarily depending on the availability of the respective phosphites or phosphorochloridates.

N-Phosphorylated sulfoximides are also accessible from trialkyl phosphites by reaction with *N*-bromo-*S*,*S*-dimethylsulfoximide in an Arbusov-type rearrangement, (method 3, Scheme 3).



Scheme 1. (Compound,R): *1*,Me; *2*,Et; *3*,Pr; *4*,Pr^i; *5*,Bu; *7*,Bzl.



Scheme 2. (Compound,R,Z): *2*,Et,O; *5*,Bu,O; *6*,$(Me)_2C(CH_2)_2$,O; 8,Ph,O; *9*,Et,S; *10*,Pr,S; *11*,Bu,S.

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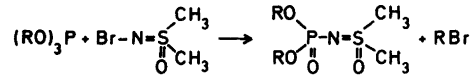


Scheme 3. (Compound,R): *2*,Et.

This reaction may be carried out without isolation of *N*-bromo-*S*,*S*-dimethylsulfoximide, which is prepared by direct bromination of dimethylsulfoximide.[12] Filtering off the sulfoximidium bromide from the bromination mixture gave a solution of *N*-bromo-*S*,*S*-dimethylsulfoximide, adequate for reaction with the trialkyl phosphite.

It is well known that *N*-silylation of amidic nitrogen enhances its reactivity towards electrophilic reagents. Thus presilylated dimethylsulfoximide[13] can be readily converted into *N*-phosphorylated-*S*,*S*-dimethylsulfoximide by reaction with dialkyl phosphorochloridate in CH_2Cl_2 solution, (method 4, Scheme 4).

The prepared *N*-phosphorylated dimethylsulfoximides are stable oily pale yellow liquids which can be purified by high vacuum distillation; or colourless solids which can be recrystallized from butanone. All compounds gave satisfactory microanalytical data and their IR and ^1H NMR spectral data (given in the experimental part) were in accordance with the proposed structure.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer model 225 grating spectrograph, ^1H NMR spectra on a JEOL JNM-MH-60/II instrument. Elemental analyses were carried out by the Microanalysis Department of Chemical Laboratory II, University of Copenhagen, The H. C. Ørsted Institute. Melting points (uncorrected) were determined on a Büchi melting point apparatus. Refractive indexes were determined on Zeiss' Abbe refractometer.

Starting materials. Dialkyl phosphites, trialkyl phosphites, dialkyl phosphorochloridates and phosphorochloridothionates were prepared by conventional methods or were commercial reagents, distilled prior to use.

Dimethylsulfoximide was prepared by action of hydrazoic acid on dimethyl sulfoxide.[13]

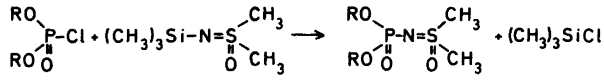
N-(Trimethylsilyl)-S,S-dimethylsulfoximide.[13] A solution of trimethylchlorosilan (0.1 mol) in dry benzene (15 ml) was added slowly into a refluxing solution of dimethylsulfoximide (0.1 mol) and triethylamine (0.1 mol) in dry benzene (100 ml). The reaction mixture was gently refluxed for a further 2 h, cooled and the precipitated triethylamine hydrochloride filtered off and washed with benzene. The filtrate was evaporated and the residue distilled *in vacuo* affording *N*-(trimethylsilyl)-*S*,*S*-dimethylsulfoximide as a colourless liquid. Yield 82 %, b.p. 45–46 °C/0.15 mmHg, n_D^{23} 1.4632. The compound crystallized on refrigeration, m.p. 34 °C.

N-Bromo-S,S-dimethylsulfoximide[12] was prepared by bromination of dimethylsulfoximide in tetrahydrofuran solution. The solution was used for further experiments (see below).

Method 1. Reaction of dialkyl phosphites with tetrachloromethane and dimethylsulfoximide. Dialkyl phosphite (0.1 mol) was added dropwise into a stirred mixture of dimethylsulfoximide (0.1 mol), triethylamine (0.1 mol) and CCl_4 (20 ml) dissolved in chloroform (50 ml). The temperature rose slowly from 20 to 70 °C. The reaction mixture was stirred at room temperature for 4 h, and evaporated to dryness (40 °C bath). Benzene (25 ml), was added and evaporated again to remove the traces of $CHCl_3$. Dry benzene (100 ml) was added to the residue and the precipitate of triethylamine hydrochloride was filtered off and washed with benzene (50 ml). From the combined filtrate and washings the solvent was evaporated. The crude *N*-(dialkoxyphosphinyl)-*S*,*S*-dimethylsulfoximides were oily, pale yellow liquids or colourless solids. They were purified by means of high vacuum distillation or recrystallization, respectively.

Method 2. Reaction of phosphorochloridates and phosphorochloridothionates with dimethylsulfoximide. A solution of dialkyl phosphorochloridate (or phosphorochloridothionate) (0.1 mol) in chloroform (15 ml) was added dropwise into a stirred solution of sulfoximide (0.1 mol) and triethylamine (0.1 mol) in chloroform (60 ml). A slightly exothermic reaction took place. After the addition the reaction mixture was refluxed (64 °C) for 15 min and then stirred for 2 h at room temperature. The reaction mixture was worked up analogous to method 1.

Method 3. Reaction of trialkyl phosphites with N-bromo-S,S-dimethylsulfoximide. A solution of bromine (0.1 mol) in dry benzene (50 ml) was added dropwise to a solution of *S*,*S*-dimethylsulfoximide



Scheme 4. (Compound,R): *2*,Et; *5*,Bu.

(0.2 mol) in a mixture of tetrahydrofuran (50 ml) and benzene (100 ml) at room temperature. No exothermic effect was observed. The bromine colour disappeared instantaneously, precipitate was formed, and the solution became yellow. The reaction mixture was stirred for 0.5 h at room temperature, and the yellowish precipitate of *S,S*-dimethylsulfoximidium bromide was filtered off and washed thoroughly with dry benzene. The combined filtrates were concentrated to the total volume of 50 ml (30 °C bath).

Triethyl phosphite (0.1 mol) diluted with benzene (15 ml) was added dropwise to this solution of *N*-bromo-*S,S*-dimethylsulfoximide. The temperature of the reaction was kept at 40 °C. After addition the reaction mixture was refluxed for 10 min and then stirred for 2 h at room temperature. Solvent was evaporated and the crude *N*-(diethoxyphosphinyl)-*S,S*-dimethylsulfoximide was distilled *in vacuo*.

Method 4. Reaction of dialkyl phosphorochloridates with N-*(trimethylsilyl)*-S,S-*dimethylsulfoximide.* A solution of dialkyl phosphorochloridate (0.1 mol) in dichloromethane (20 ml) was added dropwise at room temperature into a stirred solution of *N*-(trimethylsilyl)-*S,S*-dimethylsulfoximide (0.1 mol) in dichloromethane (70 ml). Slight exothermic effect was observed. The reaction mixture was then refluxed for 2 h, evaporated and the residue was distilled *in vacuo* affording *N*-(dialkoxyphosphinyl)-*S,S*-dimethylsulfoximide.

N-*(Dimethoxyphosphinyl)*-S,S-*dimethylsulfoximide.* 1 was prepared by method 1 in 34% yield, b.p. 118 °C/0.005 mmHg, n_D^{22} 1.4805. Anal. $C_4H_{12}NO_4PS$: C, H, N. ^1H NMR ($CDCl_3$): δ 3.33 (6 H, d, J_{HP} 1.2 Hz), 3.74 (6 H, d, J_{HP} 11.8 Hz). IR ($CHCl_3$, cm^{-1}): 3000 (s), 1250 (s), 1160 (s), 1030 (s), 820 (s).

N-*(Diethoxyphosphinyl)*-S,S-*dimethylsulfoximide* 2 was prepared by the methods 1, 2, 3, 4 in yields yields of 81 %, 77 %, 40 % and 60 %, respectively. B.p. 123 °C/0.001 mmHg, n_D^{20} 1.4670. Anal. $C_6H_{16}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 1.33 (6 H, dt, J_{HH} 7.1 Hz and J_{HP} 0.9 Hz), 3.32 (6 H, d, J_{HP} 1.0 Hz), 4.11 (4 H, dq, J_{HH} 7.1 Hz and J_{HP} 7.1 Hz). IR ($CHCl_3$, cm^{-1}): 2980 (s), 1250 (s), 1160 (s), 1030 (s) 950 (s), 810 (s).

N-*(Dipropoxyphosphinyl)*-S,S-*dimethylsulfoximide* 3 was prepared by method 1 in 83% yield, b.p. 127 °C/0.005 mmHg, n_D^{22} 1.4632. Anal. $C_8H_{20}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 0.78–1.13 (6 H, m), 1.38–2.05 (4 H, m), 3.30 (6 H, d, J_{HP} 1.0 Hz), 3.98 (4 H, q). IR ($CHCl_3$, cm^{-1}): 2960 (s), 1250 (s), 1160 (s), 1020 (s), 850 (s).

N-*(Diisopropoxyphosphinyl)*-S,S-*dimethylsulfoximide* 4 was prepared by method 1 in 79% yield, b.p. 115 °C/0.001 mmHg, m.p. 66–68 °C. Anal. $C_8H_{20}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 1.30 (6 H, s), 1.40 (6 H, s), 3.30 (6 H, s), 4.32–4.94 (2 H, m). IR (CCl_4, cm^{-1}): 2990 (s), 1250 (s), 1170 (s).

N-*(Dibutoxyphosphinyl)*-S,S-*dimethylsulfoximide* 5 was prepared by method 1 and 2 in 70 % yield and by method 4 in 48 %. B.p. 134 °C/0.015 mmHg. Anal. $C_{10}H_{24}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 0.73–1.90 (14 H, m), 3.29 (6 H, d, J_{HP} 1.0 Hz), 3.83–4.23 (4 H, m). IR ($CHCl_3$, cm^{-1}) 2960 (s), 1250 (s), 1160 (s), 1010 (s), 805 (s).

N-*(2-Oxo-5,5-dimethyl-1,3,2-dioxaphosphorinan-2-yl)*-S,S-*dimethylsulfoximide* 6 was prepared by method 2 in 68 % yield, m.p. 126 °C from butanone. Anal. $C_7H_{16}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 0.90 (3 H, s), 1.22 (3 H, s), 3.30 (6 H, s), 3.89–4.12 (4 H, m). IR ($CHCl_3$, cm^{-1}): 2960 (m), 1270 (s), 1170 (s), 1060 (s).

N-*(Dibenzyloxyphosphinyl)*-S,S-*dimethylsulfoximide* 7 was prepared by method 1 in 69% yield, m.p. 89–90 °C from butanone. Anal. $C_{16}H_{20}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 3.15 (6 H, s), 5.00 (2 H, d), 7.33 (10 H, s). IR ($CHCl_3$, cm^{-1}): 2970 (m), 1240 (s).

N-*(Diphenoxyphosphinyl)*-S,S-*dimethylsulfoximide* 8 was prepared by method 2 in 60% yield, m.p. 91–92 °C from butanone–hexane. Anal. $C_{14}H_{16}NO_4PS$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 3.16 (6 H, s), 7.31 (10 H, s). IR ($CHCl_3$, cm^{-1}): 1595 (m), 1500 (s), 1250 (s), 1160 (s).

N-*(Diethoxyphosphinothioyl)*-S,S-*dimethylsulfoximide* 9 was prepared by method 2 in 50 % yield, *b.p.* 118–119 °C/0.001 mmHg, n_D^{22} 1.5168. Anal. $C_6H_{16}NO_3PS_2$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 1.18–1.55 (6 H, m), 2.55–3.20 (1 H, m), 3.35 (6 H, d, J_{HP} 1.0 Hz), 3.83–4.40 (3 H, m). IR ($CHCl_3$, cm^{-1}): 3000 (s), 1260 (s), 1160 (s), 1020 (s).

N-*(Dipropoxyphosphinothioyl)*-S,S-*dimethylsulfoximide* 10 was prepared by method 2 in 55 % yield, b.p. 122 °C/0.005 mmHg, n_D^{22} 1.5060. Anal. $C_8H_{20}NO_3PS_2$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 0.74–1.14 (6 H, m), 1.36–1.97 (4 H, m), 2.48–3.05 (0.6 H, m), 3.32 (6 H, d, J_{HP} 1.0 Hz), 3.76–4.22 (3.4 H, m). IR ($CHCl_3$, cm^{-1}): 2980 (s), 1260 (s), 1160 (s), 990 (s).

N-*(Dibutoxyphosphinothioyl)*-S,S-*dimethylsulfoximide* 11 was prepared by method 2 in 54 % yield, b.p. 132 °C/0.005 mmHg, n_D^{22} 1.4998. Anal. $C_{10}H_{24}NO_3PS_2$: C, H, N, S. ^1H NMR ($CDCl_3$): δ 1.72–1.87 (14 H, m), 2.58–3.12 (1.1 H, m), 3.32 (6 H, d, J_{HP} 1.0 Hz), 3.78–4.24 (2.9 H, m). IR ($CHCl_3$, cm^{-1}): 2950 (s), 1260 (s), 1160 (s), 1010 (s).

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A ^{13}C and ^{1}H NMR Study of 1-Pyrazolino [4.3-*c*]cephalosporins and Their Oxidized Derivatives

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The presumed conformation of the title compounds (*1a–d*) is in accordance with the ^{1}H NMR data of other known cepham derivatives. Information gained from the ^{13}C NMR investigations shows that the effects of oxidation can reasonably be explained in conformity with examples of other types to be found in the literature.

The cycloaddition of diazomethane to the 4-nitrobenzyl ester of 7-phenoxyacetamido-3-methyl-3-cephem-4-carboxylate (*3a*) was earlier found to lead to the regio- and stereospecific formation of one product,[1] to which structure *1a* was assigned. We now report a ^{13}C and ^{1}H NMR study of *1a–d* in order to confirm the previous conclusions.

Only scattered information is available on the ^{13}C NMR spectroscopy of cephams.[2–4] More data can be found on various sulfoxides and sulfides, although systematic investigations have been carried out only on simple systems (for reviews, see Refs. 5b and 6).

EXPERIMENTAL

^{1}H NMR spectra of *1a–d* and *3a–d* were run on a JEOL MH 100 spectrometer, in DMSO-d_6 with TMS as internal standard. ^{13}C NMR spectra were measured at natural abudance on a JEOL FX 60 spectrometer in DMSO-d_6 and in pyridin-d_5 relative to TMS. Data were collected over a 4 KHz sweep width in 4 K of memory using a tip angle of 45° and 2–4 sec repetition times. Coupled spectra were obtained in gated I mode. ^{1}H noise off-resonance and SFORD decoupled spectra were measured for each compound.

DISCUSSION

Molecular models show that the tetrahydrothiazine ring in *1a–d* can adopt two conformations: A and B (Fig. 1). The triple condensed ring system means that only the S–C-2–C-3 moiety of the tetrahydro thiazine can assume a structure similar to that in cyclohexane. In the case of conformation A the $S(\beta)$ axial oxygen of the sulfoxide group is *syn-anti* to H-2_α, but the dihedral angle between the *syn*-clinal Me-3_α and H-2_α is already smaller than it would be in a "pure" *gauche* position. The steric relations between the oxygens and C-2 hydrogens are reversed in conformation B. $^{1}H-\{^{1}H\}$–NOE investigations indicate that the conformation of substituted cephams *2a–d*, a related case found in the literature,[3] is analogous to A.

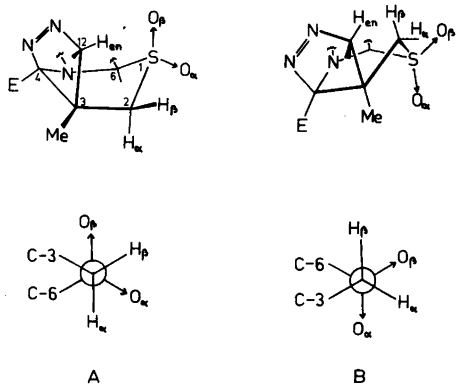

Fig. 1. The two possible conformations of *1a–d*. For clarity the β-lactam ring is omitted.

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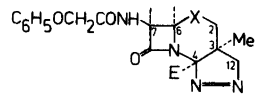
Table 1. ^{1}H NMR data of the cycloadducts *1a–d*.



1a-d E = -$COOCH_2C_6H_4$-pNO_2

X	H-2α(ax)	$\Delta\delta H_{2ae}$	H-2β(eq)	H-6	H-7	H-12en	$\Delta\delta H_{12ee}$	H-12exo	Me-3
1a S	3.04[a]		3.04	5.28	5.52	4.78	0.22	5.00	1.07
1b R(α)eq S→0	3.02 (0.02)[b]		3.02 (0.02)	4.75 (0.53)	5.61 (−0.09)	4.53 (0.25)	0.39	4.92 (0.08)	1.17 (−0.1)
1c S(β)ax S→0	2.98 (0.06)	0.94	3.92 (−0.88)	5.27 (0.01)	5.98 (−0.46)	5.54 (−0.76)	0.68	4.86 (0.16)	0.81 (0.26)
1d SO_2	3.57 (−0.56)	0.32	3.89 (−0.81)	5.38 (−0.1)	6.01 (−0.5)	5.09 (−0.31)	0.12	4.97 (0.03)	0.92 (0.15)

[a] ppm values in δ units relative to TMS in DMSO-d_6. [b] Chemical shift differences in parentheses from the same proton of the sulfide. A positive value denotes an upfield shift and *vice versa*.

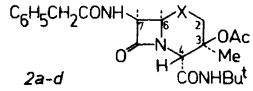
DISCUSSION OF ^{1}H NMR RESULTS

Chiral sulfoxides produce an orientation-dependent shielding or deshielding in their immediate environment. This diamagnetic anisotropy is said to possess an acetylene-type anisotropy although this concept has been criticized in view of the anomalies found so far.

In ^{1}H NMR spectra the C-2 protons of cephems usually appear as AB quartets, although this does not hold for *1a*. In contrast to the sulfoxides, the equatorial proton of cephalosporin-sulfides is assigned at higher field. On the other hand, if the sulfoxide group has an axial lone-pair of electrons, the shift difference $\Delta\delta H_{2ae}$* between the axial and

* The following abbrevations are used for chemical shift differences *e.g.*:
$\Delta\delta H_{2ae} = \delta H_{2ax} - \delta H_{2eq}$
$\Delta\delta H_{2ax} = (\delta H_{2ax})_{\text{axial SO}} - (\delta H_{2ax})_{\text{equatorial SO}}$

Table 2. ^{1}H NMR data of the cephams *2a–d*.

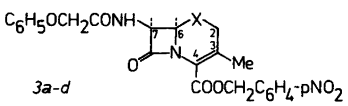


2a-d

X	H-2α(ax)	$\Delta\delta H_{2ae}$	H-2β(eq)	H-6	H-7	H-4	Me-3
2a S	3.75[a]		3.20	5.30	5.58	4.40	1.54
2b R(α)eq S→0	3.30 (0.45)[b]	0.5	3.80 (−0.6)	4.24 (1.46)	5.27 (0.31)	4.90 (−0.5)	1.65 (−0.11)
2c S(β)ax S→0	3.35 (0.4)	0.98	4.33 (−1.13)	4.97 (0.33)	6.00 (−0.42)	4.6 (−0.2)	1.58 (−0.04)
2d SO_2	4.07 (−0.32)	0.17	3.90 (−0.7)	5.14 (0.16)	6.00 (−0.42)	4.44 (−0.04)	1.57 (−0.03)

[a,b] See footnotes to Table 1. Data from Ref. 29.

Table 3. ^{1}H NMR data of the cephems *3a–d*.



3a-d

X	$H_{2\alpha}$(ax)	$\Delta\delta H_{2ae}$	$H_{2\beta}$(eq)
3a *S*	3.55[a]	0.3	3.25
3b *R*(α)eq S→0	3.69	0.54	4.23
	(−0.14)[b]		(−0.98)
3c *S*(β)ax S→0	3.67	0.31	3.98
	(−0.12)		(−0.73)
3d SO_2	4.06	0.34	3.72
	(−0.51)		(−0.47)

[a,b] See footnotes to Table 1.

equatorial protons is usually greater then in the opposite case.[6–8] It is true for cephem-sulfoxides (Table 3), but not for *2b* and *c* (Table 2) and the cycloadducts *1b* and *c* (Table 1). The axial→equatorial sulfoxide reversal usually causes a smaller effect at axial hydrogens ($\Delta\delta = \sim 0.2$ ppm);[6,8,9] a similar relation can be deduced from the chemical shift data on *3b–c*, *2b–c* and *1b–c* ($\Delta\delta H_{2ax}$ = 0.02, 0.05 and 0.04 ppm; $\Delta\delta H_{2eq}$ = 0.25, 0.53 and 0.9 ppm, respectively). The H-2_{eq} proton in the *R*(α)-equatorial cephem-sulfoxide *3b* is deshielded relative to the *S*(β)-axial sulfoxide, while in the case of cephams *2b–c* and cycloadduct sulfoxides *1b–c* shielding is seen. The reason for this is uncertain, because the difference in dihedral angle between the *S*(β)-sulfoxide oxygen and H-2_{eq} in derivatives of *1 vs. 2* and *3* is not great enough to confirm this phenomenon. As a whole, the above-mentioned ^{1}H resonance data on *1a–c* and *2a–c* show that the changes in resonance have parallel trends pointing to related (*i.e.* A) conformations.

$\Delta\delta_{2ae}$ for the cycloadduct sulfone *1d* is greatly reduced, similarly as for other sulfones.[7,10] Two possible interpretations could account for this: (a) The sulfone group possesses a diamagnetic anisotropy considerably different from that of the sulfoxide group (similar to that proposed for the nitro group);[11] (b) the lack of a lone-pair in the sulfone could theoretically bring about a similar effect (*cf.* an assumed $n \rightarrow \sigma^*$ interaction between the axial lone-pair and the *anti*-periplanar hydrogens in *N*-heterocycles[12]), the loss of this influence results in a lessening of $\Delta\delta H_{2ae}$, but these assumptions would lead to contradictions in sulfoxides of *1* and *2*.

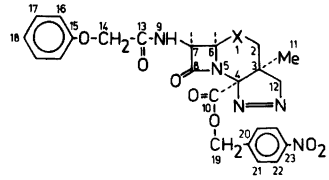
The condensed pyrazoline ring in compounds *1a–d* is less puckered than in a monocyclic 1-pyrazoline ring. It is known[13,14] that the pseudo-axial protons of 1-pyrazolines with an envelope conformation resonate at higher fields. The signal at 4.78 ppm in *1a* was tentatively assigned to the 12-*endo* hydrogen, in accordance with the fact that the dihedral angle between the *endo* C–H bond and the C-3–C-4 bond is about 20° smaller than the corresponding *exo* angle. If it is assumed that the chemical shift of the *exo* hydrogen is less influenced by changes in the oxydation state of the sulfur, and the chemical shift parameters are assigned as shown in Table 1, it is understandable that in the case of the *R*(α)-sulfoxide *1b* the *endo* proton is situated in the shielding cone of the sulfoxide bond ($\Delta\delta H_{en}$ = 0.25 ppm), while in the *S*(β)-sulfoxide *1c* it is strongly deshielded ($\Delta\delta H_{en} = -0.76$ ppm). A similar but smaller deshielding is also seen in *1d*. For a related example see Ref. 30. The spatial proximity of a lone-pair – if the geometry is fortunate – can also cause deshielding in certain cases,[16,17] but here, if any, it should then appear in *1a* and *b*.

The ^{1}H NMR signals of H-6 in *1a–d* are similar to those usually found in other β-lactam compounds, *i.e.* a higher shielding in the *R*(α)-sulfoxide and deshielding in the sulfone.[18]

^{13}C NMR RESULTS

The oxidation state of the sulfur also has a characteristic influence on the ^{13}C NMR resonance of C-2, C-3 and C-6 (see Table 4; Table 5 contains a concise summary of the more important shift parameters and differences). It is regarded of general validity that in 6-membered carbo- and heterocycles an axial substituent causes smaller downfield shifts at C-2 and C-6 (β-effect) and larger upfield shifts at C-3 and C-5 (γ-effect) than in the case of an equatorial substituent.[21] This is apparent from the data on C-2 and C-3 of the cycloadduct (Table 5), in line with those for other thiane derivatives, and can also be observed in penicillins and cephalosporins.[2] This phenomenon of shielding differences caused by the two sulfoxide isomers at C-2 was found by Buchanan and Durst[22] *via* electric field calculation to be about 9 ppm. In the cases of *1b* and *c* this difference 8 ppm agrees well. In the sulfone *1d* there is an additional −1.6 ppm down-

Table 4. ^{13}C-NMR data of the cycloadducts *1a–d*.



1a X = S
1b X = S→O R(α)
1c X = S→O S(β)
1d X = SO_2

Carbon	*1a*	*1b*	*1c*	*1d*
2	31.2 (145 t) [b]	51.7 (142 t)	43.7 (135 t)	53.3 t
	30.0 (143 t)	50.0	–	51.8 t
3	39.7 s	58.5 s	37.6 s	47.8 s
	–	57.1	–	46.6
4	99.9 s	82.0 s	96.9 s	98.0 s
	98.7	80.6	96.0	96.5
6	57.7 (174 d)	73.9 (174 d)	67.3 (170 d)	67.4
	55.8 (172 d)	72.2 (178 d)	66.2 (172 d)	67.1 (172 d)
7	60.4 (152 d)	60.7 (152 d)	58.9 (152 d)	60.2
	59.1 (154 d)	59.1 (152 d)	57.4 (152 d)	58.4 (156 d)
8[a]	166.0 s	166.3 s	166.2 s	165.7 s
	164.8	165.1	164.8	164.0
10[a]	163.0 s	165.0 s	163.6 s	162.8 s
	161.7	163.6	162.2	161.8
11	20.9 (123 q)	18.8 (131 q)	24.2 (131 q)	22.2
	20.0 (127 q)	17.9 (131 q)	23.6 (133 q)	21.6 (130 q)
12	85.4 (143 t)	–	86.4 (141 t)	85.5
	85.1 (148 t)	–	86.4 (148 t)	85.2 (145 t)
13[a]	169.6 s	170.0 s	168.5 s	169.2 s
	168.2	168.4	167.9	168.2
14	67.9 (147 t)	67.8 (145 t)	67.3 (148 t)	68.5
	66.2 (147 t)	66.2 (146 t)	66.2 (150 t)	66.1 (147 t)
15	159.2 s	158.3 s	157.8 s	158.0 s
	157.4	157.2	157.0	157.3
16	115.1 d	115.0 d	115.1 d	115.1 d
	114.4 (160 d)	114.4 (162 d)	114.5 (162 d)	114.4 (160 d)
17	129.2 d	129.8 d	129.8 d	129.9 d
	129.2	129.2 (168 d)	129.5 (162 d)	129.5 (160 d)
18	–			
	121.0 (162 d)	121.0 (162 d)	121.4 (160 d)	121.2 (154 d)
19	66.6 (150 t)	66.8 (148 t)	67.3 (148 t)	67.4
	66.2 (147 t)	66.2 (146 t)	66.2 (150 t)	67.1 (150 t)
20	142.5 s	–	–	142.0 s
	142.2 s	142.5 s	142.2 s	142.2 s
21	129.7 d	129.3 d	129.4 d	129.4 d
	129.2 (164 d)	129.2 (168 d)	129.5 (162 d)	129.5 (160 d)
22	–			
	123.5 (172 d)	123.5 (170 d)	123.5 (170 d)	123.6 (170 d)
23	–			
	147.1 s	147.1 s	147.3 s	147.3 s

[a] For assignment of carbonyl carbons, see Refs. 19 and 20. [b] ppm values obtained in pyridine-d_5 (upper values) and in DMSO-d_6 (lower values).

Table 5. Selected ^{13}C NMR data of *1a–d*.

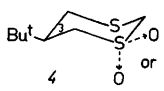
X		C-2	C-3	C-4	C-11	C-12	C-6	C-7
1a	*S*	31.2[a]	39.7	99.9	20.9	85.4	57.7	60.4
1b	*R*(α)eq S→O	51.7 (−20.7)[b]	58.5 (−18.8)	82.0 (17.9)	18.8 (2.1)	– –	73.9 (−16.2)	60.7 (−0.3)
1c	*S*(β)ax S→O	43.7 (−12.5)	37.6 (2.1)	96.9 (3.0)	24.2 (−3.6)	86.4 (−1.0)	67.3 (−9.6)	58.9 (1.5)
1d	SO_2	53.3 (−22.1)	47.8 (−8.1)	98.0 (1.9)	22.2 (−1.3)	85.5 (−0.1)	67.4 (−9.7)	60.2 (0.2)

[a] ppm values in δ units downfield from TMS (in pyridine-d_5). [b] Data in parentheses are shift differences relative to the sulfide; a positive value means an upfield shift.

field shift relative to the *R*(α)-sulfoxide. The deshielding of C-2 in sulfones (compared to the corresponding equatorial sulfoxide) varies appreciably: the effect is very small in aliphatic sulfones,[21] −0.5 ppm in the unsubstituted thiane[7,22] and −4.6 ppm in oxathiane.

The behavior of C-6 of the β-lactam ring parallels that of C-2, *i.e.* there is a greater deshielding in the *R*(α)-sulfoxide *1b*. This downfield shift of C-2 and C-6 is similar to that found in 3-chlorocephams.[3a,4]

The C-3 carbon is shielded by 2.1 ppm in the axial sulfoxide *1c* and by 20.9 ppm relative to the equatorial sulfoxide. This shielding, often referred to as the steric *γ-gauche* effect,[23] is also characteristic of other thiane-sulfoxides and sulfilimines,



e.g. it is 7.8 ppm in thianes (Table 8) and 16.3 ppm in the anchored dithiane derivative *4*.[5b] This proves the assumed conformation A, as in the other case this *γ-gauche* effect should appear in the *R*(α)-sulfoxide. However, problems arise in the sulfones,[10,11,24] for in spite of the further presence of the *γ-gauche* interaction this shielding is strongly reduced.

Sulfoxide and sulfone γ-effects. For a detailed insight into this problem, the four possible γ-effects must be taken into consideration (see Fig. 2). If it is assumed that the through-bond electronic effect of a lone-pair is smaller in the *gauche* position, *i.e.* $\gamma_{gauche}^{lone-pair} < \gamma_{anti}^{lone-pair}$, the shift difference $\Delta\delta SO_{eq} -$

SO_{ax}: $\gamma_{gauche}^{O} + \gamma_{anti}^{lone-pair}$
SO_{eq}: $\gamma_{anti}^{O} + \gamma_{gauche}^{lone-pair}$
SO_2: $\gamma_{gauche}^{O} + \gamma_{anti}^{O}$

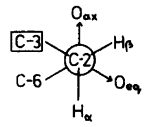


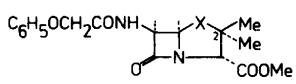
Fig. 2. γ-effects between C-3 and the sulfoxide group in configuration A.

SO_2 on C-3 will to a first approximation reflect the γ_{gauche}^{O} effect of the oxygen, while, in turn, the difference $\Delta\delta SO_{ax} - SO_2$ is characteristic of the $\gamma_{anti}^{lone-pair}$ effect. In other words, it is better to say that a ring carbon *anti*-periplanar to the lone pair of the sulfur is shifted upfield. This effect is about 6–10 ppm in thiane derivatives,[10,21,22] 8–9 ppm in sulfilimines[25] and 10 ppm in nitrogen heterocycles.[5] Thus, a clearer view of the influence of the sulfoxide lone-pair on C-3 can be obtained, if differences Δδ are formed from the shift data of the sulfone and sulfoxide derivatives, rather than using the parent sulfide as reference. In this way the influence of inductive effects of electrostatic types is decreased, as they exhibit a smaller change in the

Table 6. Shift differences for C-3 of *1b–d*.

X		C-3 ppm	$\Delta\delta_{SO-SO_2}$
1b	$R(\alpha)_{eq}$ S→O	58.5	−10.7
1c	$S(\beta)_{ax}$ S→O	37.6	10.2
1d	SO_2	47.8	

Table 7. Shift differences for Me-2 of 1-oxides of penicillin V methyl ester.



X	Me-2β ppm[a]	Me-2β $\Delta\delta_{SO-SO_2}$	Me-2α ppm	Me-2α $\Delta\delta_{SO-SO_2}$
$R(\alpha)_{eq}$ S→O	24.3	−4.3	16.0	1.7
$S(\beta)_{ax}$ S→O	19.3	0.7	18.4	−0.7
SO_2	20.0		17.7	

[a] ppm in δ units, taken from Ref. 2b.

SO_2→SO reversal than they do in the S→SO case.[26] The shift differences $\Delta\delta SO-SO_2$ for *1b–d* are shown in Table 6, and reveal that the equatorial lone-pair shields the C-3 resonance. To assess the generality of this phenomenon, let us consider the 2β- and 2α-methyl groups of penicillin derivatives (Table 7) as well as the thiane oxides (Table 8). A related *γ-anti* effect caused by second-row heteroatoms on carbon atoms is discussed in Ref. 31.

The effect can clearly be seen even in the case of penicillin sulfoxides, despite the fact that in the thiazolidine ring the dihedral angle between the *trans* methyl group and the lone-pair is smaller than that in the *anti*-periplanar arrangement in cephams.

The pyrazoline C-12 signal in the ^{13}C NMR spectrum of *1b* is not understandable. In the case of this compound a δ-effect of 3–4 ppm would be expected; in fact this signal cannot be found using different solvents and decouplings. At first sight, plausible possibilities were (1) the presence of an *N*-oxide in the pyrazoline ring, (2) a 2-pyrazoline was formed or, as a consequence of (1) or (2), (3) accidental signal coincidence. Against the first two assumptions is the fact that *1b* has a mass-spectrum characteristic of 1-pyrazolines (loss of N_2)[1] and a satisfactory ^1H NMR spectrum; in addition, formation of an *N*-oxide would have caused another type of shift.[28] On the other hand, signal coincidences could be resolved using another solvent. Probably relaxation effects are responsible for this phenomenon.

Table 8. Shift differences for C-3 of thiane 1-oxides.

X	C-3 ppm[a]	C-3 $\Delta\delta_{SO-SO_2}$
S	(27.9)[27]	(−2.8)
S→O ax	15.5[22]	9.6
S→O eq	23.3[22]	1.8
SO_2	25.1[7]	

[a] The given lit. data were obtained in $CDCl_3$ or CD_2Cl_2 solutions.

Note added in proof. A more recent X-ray study on compound *1a*, confirming the proposed structure in this article. will appear in *J. Mol. Struct.*

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Chlorination of Carboxylic Acid Derivatives. IX. Liquid Phase Chlorination of Aliphatic C_2-C_8 Alkyl Acetates. EI Mass Spectra of Monochlorinated Esters

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A series of aliphatic alkyl acetates from ethyl to octyl acetate was chlorinated in the liquid phase in order to obtain monochlorinated products. The chlorination of esters was carried out with chlorine in the liquid phase in the absence and in the presence of benzene and with sulfuryl chloride in the presence of Bz_2O_2. The products were determined by gas-liquid chromatography and gas-liquid chromatography-mass spectrometry. Chlorination is appreciably deactivated at the 1-position, particularly with SO_2Cl_2, the deactivation at the 2-position being strongest with Cl_2 in the presence of benzene. The amounts of 1-chloro and ω-chloro isomers constituted the greatest disparity between the chlorination methods. The most characteristic mass spectral fragment ions of the 35 chlorinated alkyl acetates are given.

Numerous papers have been published on the free-radical substitution in aliphatic carboxylic acids and their derivatives induced by chlorinating reagents,[1-5] but very little has been reported on the reactivity of the alcohol chain in aliphatic esters. Bruylants and his co-workers have studied the chlorination of short-chain alkyl acetates,[6-10] Brown and Ash the chlorination of propyl acetate with chlorine and sulfuryl chloride,[11] Waddle and co-workers the chlorination of propyl and butyl trichloroacetates[12,13] and Singh and Tedder the halogenation of esters of 1-butanol.[14]

Recently, the chlorination of straight-chain methyl esters from propanoic to octadecanoic acid has been reported to produce monochloro isomers and, as side products, chloromethyl esters.[1-3] The amounts of the latter were only a few per cent due to the short-chain alcohol, and quantities decreased with an increase in acid chain length, as expected.

The present work was carried out in an attempt to study the chlorination of aliphatic C_2-C_8 alkyl acetates and to compare the results with those of our previous chlorination of methyl esters of aliphatic C_3-C_9 carboxylic acids.[1,2]

EXPERIMENTAL

Samples. Ethyl, propyl, butyl and pentyl acetates were commercial products from Fluka, AG. Hexyl, heptyl and octyl acetates were obtained from commercial (Fluka) alcohols and acetyl chloride as described earlier,[15] and C_2-C_8 alkyl chloroacetates from alcohols and chloroacetyl chloride (Fluka) by the same method.[15]

Chlorinations. Alkyl acetates were chlorinated with chlorine in the liquid phase at room temperature without solvent and in 10% benzene solution as described earlier.[1] The peroxide-catalyzed reaction with SO_2Cl_2 was carried out as described by Danechrad.[16] After removal of excess chlorination reagent and the liberated hydrogen chloride, the crude reaction mixtures were analyzed by gas-liquid chromatography (GLC) and gas-liquid chromatography-mass spectrometry (GLC-MS). Owing to a deficit of chlorinating agent variable amounts of unreacted substrates were observed, the amounts of higher chlorinated products being a few per cent.

Gas-liquid chromatography. GLC analyses were run on a Varian Model 2400 gas-liquid chromatograph, adapted for glass capillary work under the following conditions: Injector temperature, 220 °C; flame ionization detector, 240 °C; nitrogen carrier gas flow-rate, 1 ml/min; splitting ratio, 1:25; chart speed 10 mm/min; 5% Carbowax

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Table 1. The relative quantities[a] of monochloro isomers formed in the chlorinations of aliphatic C_2-C_8 alkyl acetates.

Chain length	Method[b]	Isomeric monochloro esters 1-Cl	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl
C_2	A	1.7	1.0						
	B	1.8	1.0						
	C	0.7	1.0						
C_3	A	0.5	1.2	1.0					
	B	0.4	1.8	1.0					
	C	0.4	1.8	1.0					
C_4	A	0.3	0.9	1.8	1.0				
	B	0.1	1.3	4.6	1.0				
	C	–	1.5	2.6	1.0				
C_5	A	0.3	0.7	1.6	1.8	1.0			
	B	0.1	1.0	5.6	7.1	1.0			
	C	–	1.3	2.8	2.6	1.0			
C_6	A	0.2	0.6	1.3	1.7	1.8	1.0		
	B	0.1	0.8	3.7	6.5	5.8	1.0		
	C	–	1.1	2.4	2.9	2.6	1.0		
C_7	A	0.2	0.6	1.3	1.5	1.7	1.8	1.0	
	B	0.1	0.8	3.9	6.9	8.4	6.7	1.0	
	C	–	1.2	2.4	2.9	3.1	2.8	1.0	
C_8	A	0.2	0.7	1.3	1.5	1.7	1.8	1.8	1.0
	B	0.1	0.9	3.8	7.5	10.0	10.8	7.6	1.0
	C	–	1.2	2.4	2.9	3.1	3.2	2.9	1.0

[a] Relative to the ω-chloro isomers (= 1.0). The trace amounts of alkyl chloroacetates are not taken into consideration.
[b] Chlorination methods: (A) Cl_2, neat, 20 °C, UV-light; (B) Cl_2, benzene, 20 °C, UV-light; (C) SO_2Cl_2, neat, 60 °C, Bz_2O_2, dark.

Table 2. The relative quantities[a] of monochloro isomers formed in the Cl_2-chlorinations of aliphatic C_2-C_8 alkyl acetates and methyl esters of aliphatic C_3-C_9 carboxylic acids.

Chain length	Ester	Isomeric monochloro esters							
	(A) Alkyl acetate:	1-Cl	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl
	(M) Methyl ester:	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl	9-Cl
C_2	A	1.7	1.0						
C_3	M	0.5	1.0						
C_3	A	0.5	1.2	1.0					
C_4	M	0.2	1.2	1.0					
C_4	A	0.3	0.9	1.8	1.0				
C_5	M	0.1	1.0	1.8	1.0				
C_5	A	0.3	0.7	1.6	1.8	1.0			
C_6	M	0.2	0.9	1.6	1.8	1.0			
C_6	A	0.2	0.6	1.3	1.7	1.8	1.0		
C_7	M	0.1	0.8	1.4	1.8	1.8	1.0		
C_7	A	0.2	0.6	1.3	1.5	1.7	1.8	1.0	
C_8	M	0.1	0.6	1.1	1.4	1.5	1.6	1.0	
C_8	A	0.2	0.7	1.3	1.5	1.7	1.8	1.8	1.0
C_9	M	0.1	0.7	1.0	1.3	1.4	1.5	1.5	1.0

[a] Relative quantities for the ω-chloro isomers taken as 1.0; the values for methyl esters are taken from our previous papers,[1,2] the proportions of chloromethyl esters not being taken into consideration.

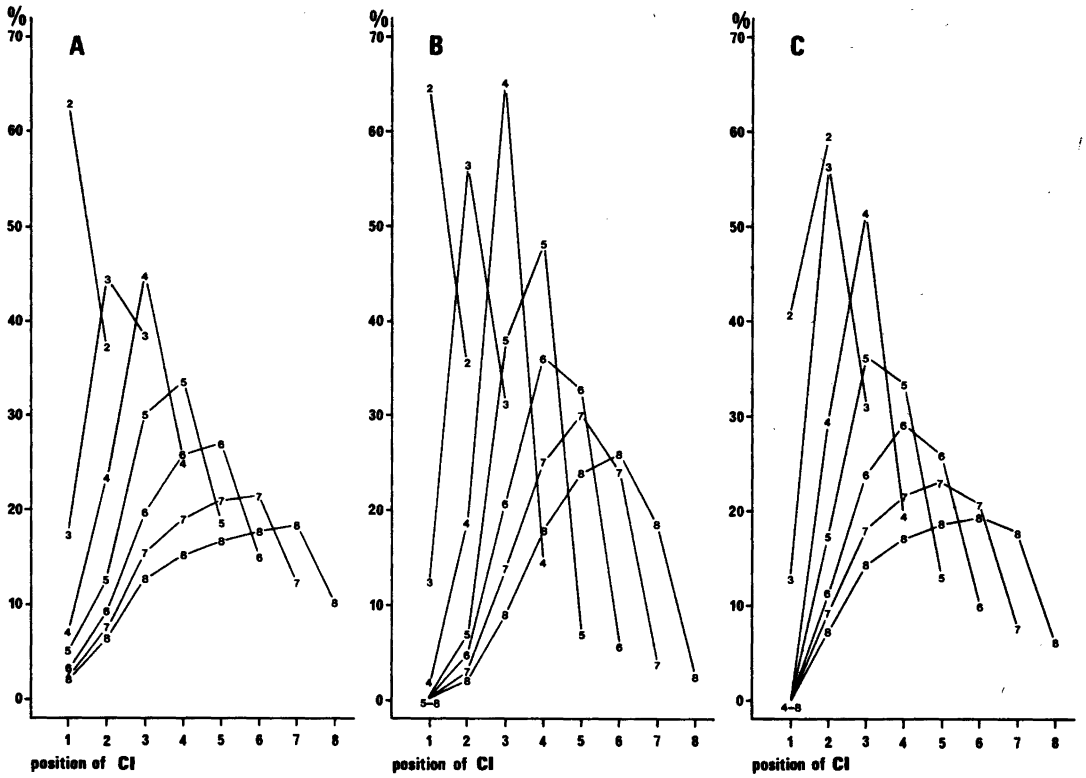

Fig. 1. Isomer distribution of monochlorinated C_2-C_8 alkyl acetates based on GLC analyses. Chlorination methods: (A) Cl_2, neat, 20 °C, UV-light; (B) Cl_2, benzene, 20 °C, UV-light; (C) SO_2Cl_2, neat, 60 °C, Bz_2O_2, dark. The number denotes the alcohol chain length.

Table 3. The relative quantities[a] of monochloro isomers in the SO_2Cl_2-chlorinations of aliphatic C_2-C_8 alkyl acetates and methyl esters of aliphatic C_3-C_9 carboxylic acids.

Chain length	Ester	Isomeric monochloro esters							
	(A) Alkyl acetate:	1-Cl	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl
	(M) Methyl ester:	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl	9-Cl
C_2	A	0.7	1.0						
C_3	M	0.9	1.0						
C_3	A	0.4	1.8	1.0					
C_4	M	0.4	2.1	1.0					
C_4	A	–	1.5	2.6	1.0				
C_5	M	0.3	1.9	2.9	1.0				
C_5	A	–	1.3	2.8	2.6	1.0			
C_6	M	0.3	2.0	3.5	3.1	1.0			
C_6	A	–	1.1	2.4	2.9	2.6	1.0		
C_7	M	0.3	1.6	2.8	3.3	2.9	1.0		
C_7	A	–	1.2	2.4	2.9	3.1	2.8	1.0	
C_8	M	0.3	1.4	2.5	3.1	3.5	2.6	1.0	
C_8	A	–	1.2	2.4	2.9	3.1	3.2	2.9	1.0
C_9	M	0.3	1.1	2.5	2.9	3.1	3.1	2.7	1.0

[a] Relative quantities for the ω-chloro isomers taken as 1.0; the values for methyl esters are taken from our previous papers,[1,2] the proportions of chloromethyl esters not being taken into consideration.

Table 4. 70 eV EI-MS data (rel. int. ⩾5%) for monochlorinated alkyl acetates. Notation of compounds: the (*e.g.* 36 denotes 3-chlorohexyl acetate).

Ion	*m/z* >38	Compound 12	22	13	23	33	14	24	34	44	15	25	35	45	55	16
$C_8H_{14}^{\cdot+}$	110															
$C_5H_9Cl^{\cdot+}$	104												6			
$C_7H_{13}^{+}$	97															
$C_7H_{12}^{\cdot+}$	96															
$C_7H_{11}^{+}$	95															
$C_4H_7Cl^{\cdot+}$	90							7	11							
$C_5H_{10}O^{\cdot+}$	86										7			5		
$C_5H_9O^{+}$	85										6		5			
$C_6H_{11}^{+}$	83															
$C_6H_{10}^{\cdot+}$	82															
$C_6H_9^{+}$	81															
$C_3H_5Cl^{\cdot+}$	76											5				
$MeCO_2CH_2^{+}$	73		12		14	6		32	6	6		19		8	9	
$C_4H_8O^{\cdot+}$	72								5							
$C_5H_{10}^{\cdot+}$	70															
$C_5H_9^{+}$	69										5	5	14	15	8	
$C_5H_8^{\cdot+}$	68												10	27	17	
$C_5H_7^{+}$	67													7	7	
$MeCHCl^{+}$	63													5		
$C_2H_3Cl^{\cdot+}$	62		10													
$[MeCO_2+2H]^{+}$	61					19			8	9			10	11	16	
$C_4H_{10}^{\cdot+}$	58										5			5		5
$C_4H_9^{+}$	57			9												
$C_4H_8^{\cdot+}$	56												12			7
$C_4H_7^{+}$	55						6	11	22	15			5	6	13	5
$C_4H_6^{\cdot+}$	54									16						
$C_4H_5^{+}$	53															
$ClCH_2^{+}$	49															
$C_3H_7^{+}$, $MeCO^{+}$	43	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
$C_3H_6^{\cdot+}$	42	5	5						9		5	5	7	7	9	
$C_3H_5^{+}$	41			5	8	12				6	9	9	21	17	12	8
$C_3H_3^{+}$	39									5			6	5		

20 M glass capillary column (22 m × 0.3 mm I. D.), prepared in our laboratory;[17] column temperature program from 50 °C at 6 °C/min until the elution of peaks ceased. The chromatographic data were analyzed with a Hewlett-Packard Model 3390A Reporting Integrator using standard programs.

Mass spectra. GLC-MS data were recorded on a Varian MAT-212 mass spectrometer connected with a Varian Model 3700 gas-liquid chromatograph. It was equipped with a 25 m × 0.22 mm (I.D.) vitreous silica SF-30 WCOT column, supplied by Scientific Glass (North Melbourne, Australia), with a helium flow-rate of 1 ml/min. The column temperature was programmed from 50 °C at 6 °C/min. Electron ionizing energy was 70 eV and ion source temperature 220 °C. Data were acquired and processed on a Spectro System MAT-188.

RESULTS AND DISCUSSION

Fig. 1 illustrates the isomer distributions of monochlorinated products formed in three different chlorination processes. The relative quantities of products are presented in Table 1, tabulated relative to the ω-chloro isomers. Values are averages of two independent experiments and agree to within ±4%.

The isomeric monochlorinated alkyl acetates have been reported[14] to elute on GLC in direct order from 1-chloro to ω-chloro compound, as do the corresponding methyl derivatives.[18] Fig. 2 illustrates the GLC patterns of monochlorinated octyl acetates formed in three different chlorination processes. As can be seen, the isomers can be completely separated on Carbowax 20 M. On a non-

first number indicates the position of the Cl-substituent, the second number the alcohol chain length

26	36	46	56	66	17	27	37	47	57	67	77	18	28	38	48	58	68	78	88
																	6		
								5			10								12
							7	7	9										
							5	11	12	12									
																6	7	5	
	11	6					13							5					
		5						8											
8		8	14	5							6				7		5	6	5
	8	22	21	10										5	10	12	21	15	8
						6	18	25	35	34	14		6	14	17	21	31	14	5
5								6							5				
23		5	7	7		16		5	6	6	8		14		5		6	5	7
	5				5						9		6		7	9	6	5	
				8			6	8	6	10	12	7	6	14	20	16	22	18	20
							5	7	9	16	12		8	17	25	23	29	31	16
	16	21	33	17			10	11	16	11	13	8		13	19	22	24	24	14
	12	10	9	14			13	10	10	10	23			11	8	9	10	13	24
					5					5									
						7	7					7		6				6	
		5		8		7	11	10	16	10	10	8		11	8	7	8	6	12
5	23	23	23	18	5	11	31	32	36	29	30	8	10	22	20	29	27	28	28
				7			18	15	22	26	8			16	13	17	22	12	6
															5				
	5		5				8												
100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
5	11	10	10	8	8		10	8	7	7	12	5	9	8	9	11	10	11	11
10	16	17	13	17	9	13	23	16	21	20	16	11	15	25	27	23	28	28	24
	7	5	5	6			7	6	8	6	5		5	8	7	7	8	7	7

polar SE-30 column, however, the isomers are eluted closer together, leading to a poor separation of 6-chloro- and 7-chloro-octyl acetates. The polarity of the isomeric monochlorinated alkyl acetates increases with the increase in distance between the ether oxygen and the chlorine substituent, which leads to the better separations of the isomers on polar than on non-polar columns.

The results of the neat chlorinations of C_3-C_5 alkyl acetates given in this work are comparable agreement with those reported in the paper by Soumillion and Bruylants,[7] the main products being always the (ω-1)-chloro isomers. Fig. 1B and Table 1 show that benzene strongly affects the selectivity of the reagent as with the chlorinations of chloromethyl esters.[19] The main products were the (ω-1)-chloro isomers for C_2-C_5 esters and the (ω-2)-chloro isomers for the longer chain esters. The amounts of the ω-chloro products were 1.1 to 4.2 times as great in the absence as in the presence of benzene. Smaller amounts of 1- and 2-chloro isomers formed in the reactions with the solvent were also observed.

It has been previously reported that the sulfuryl chloride chlorination of propyl acetate yields quite similar isomer distribution than the photochemical chlorination under similar conditions.[11] As can be seen from Fig. 1 and Table 1, a quite different isomer distribution under reaction conditions used in this work is observed. The chlorinations with sulfuryl

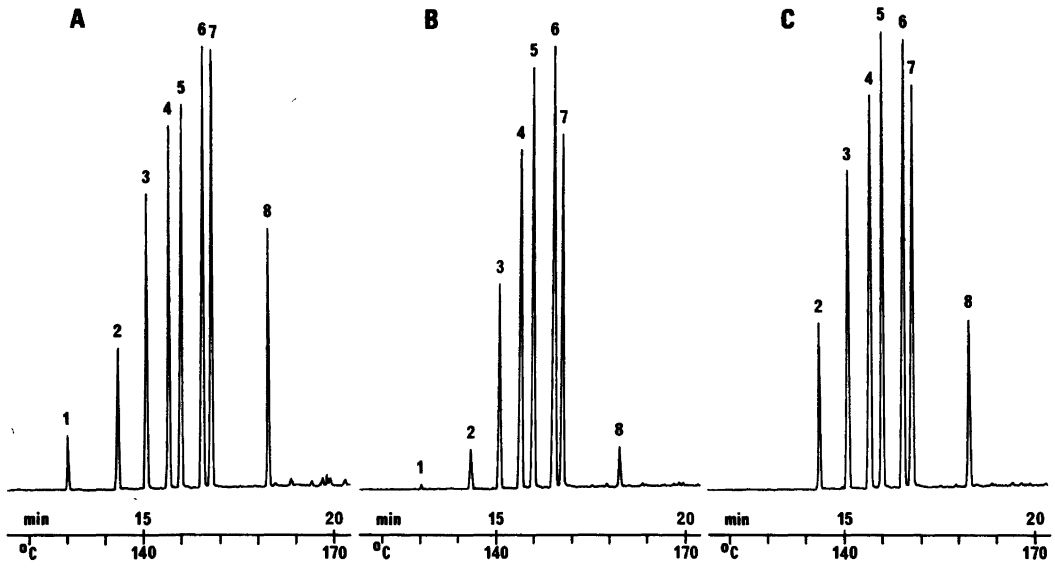

Fig. 2. GLC-patterns of monochlorinated octyl acetates formed by three different chlorination methods (A, B, C); see Table 1. Products were analyzed on Carbowax 20 M glass capillary column. The peak number indicates the position of the Cl-substituent.

chloride are appreciably deactivated at the 1-position and slightly at the 2-position. The trace amounts (or absences) of 1-chloro isomers formed at higher reaction temperature (60 °C) could be caused by the instability of products.[11] Nevertheless, greater amounts of 1-chloropropyl[11] and 1-chlorobutyl acetates[14] have been reported to be formed at higher reaction temperatures, wherefore the effect of the instability could also be negligible. Owing to the strong deactivation, the amount of ω-chloroethyl acetate was greater than that of 1-chloro isomer in the reaction with SO_2Cl_2, the other chlorination methods yielding the reverse proportion. The main products were the (ω-1)-chloro isomers for propyl and butyl acetates and the (ω-2)-chloro isomers for the other esters. The amounts of the ω-chloro isomers of C_3-C_8 alkyl acetates were 1.2 to 1.7 times as great with chlorine as with SO_2Cl_2, the ratio linearly increasing with an increase in the chain length.

Negligible amounts (<2%) of alkyl chloroacetates were found among the monochlorinated products of short-chain C_2-C_4 esters, the compounds being determined by comparison with model samples. The chlorine substitution seems to be much easier on the alcohol chain than on the acid chain, which can be seen from the relatively great amount of chloromethyl ester formed in the chlorination of methyl propanoate.[1] The chlorination of methyl acetate, for example, yielded *ca.* ten times as much chloromethyl acetate as methyl chloroacetate.[20] The process was very slow, moreover, and the total amount of chlorinated products was only 5% under the same reaction conditions as used in this work.

To compare the effects of different types of substituent, the relative quantities of monochlorinated isomers formed in the chlorinations of C_2-C_8 alkyl acetates and methyl esters of C_3-C_9 carboxylic acids are given in Tables 2 and 3. The values for methyl esters are taken from our previous papers,[1,2] the proportions of chloromethyl isomers not being taken into consideration. The results show that nearly the same isomer distributions of the corresponding esters are observed (acid chain length = alcohol chain length). Table 2, however, shows that in the reactions with Cl_2 the deactivation at the α-position seems to be stronger in methyl esters than in alkyl acetates, whereas with SO_2Cl_2 it is just the opposite (Table 3).

Mass spectra. The mass spectra of a large number of aliphatic alkyl acetates are reported in the literature,[21-27] but mass spectra for their chlorinated derivatives are few in number.[28]

EI-MS data for monochlorinated C_2-C_8 alkyl acetates are given in Table 4, all peaks greater or equal to 5% of the base peak (100%) being tabulated. Ions containing ^{37}Cl are not shown.

The molecular ion peaks of unchlorinated alkyl acetates are weak and can be seen only at lower molecular weight,[21] and $M^{\cdot+}$ is not shown by any of the chloro esters. α-Cleavage at the carbonyl group, $MeCO^+$, and to a small extent the $C_3H_7^+$ fragment ion, produce together the base peak at *m/z* 43 in all spectra. The loss of $C_3H_7^{\cdot}$ from $M^{\cdot+}$ clearly occurs most easily in the case of (ω-3)-chloro isomers (*14*, *25*, *36*, *47* and *58*), the remaining chlorine-containing fragment ion not being shown in the spectra, however.

The mass spectra of the short-chain monochlorinated alkyl acetates contain only a few additional peaks and the intensities are low. An additional α-cleavage reaction initiated by the saturated oxygen atom given $MeCO_2CH_2^+$ ions at *m/z* 73, the most intense peak in the spectra of 2-chloro isomers. The corresponding chlorine-containing fragment ion at *m/z* 107 is characteristic of 1-chloro isomers, but the intensity of the peak is very low (~1%).

The loss of acetic acid from the molecular ion *via* rearrangement of one hydrogen atom results in the formation of the $C_nH_{2n-1}Cl^{\cdot+}$ ion series at *m/z* 62, 76, 90, 104, ... This chlorine-containing fragment ion is the most abundant ion in the spectra of 2- and 3-chloro isomers of short-chain esters, but does not appear in those of long-chain esters. The subsequent loss of $Cl^{\cdot}$ or HCl gives $C_nH_{2n-1}^+$ and $C_nH_{2n-2}^{\cdot+}$ ion series. The peak at *m/z* 60, due to $MeCO_2H^{\cdot+}$, however, is not shown by any of the chlorinated acetates.

The rearrangement of two hydrogen atoms[24] is characteristic for alkyl acetates, giving the fragment ion, $[MeCO_2+2H]^+$, at *m/z* 61. This peak is the most intense peak in the spectra of the ω-chloro isomers. The chlorine substituent at 1- or 2-position, however, seems to make these hydrogen rearrangements difficult, giving rise to the smaller (<5%) peaks. Subsequent or simultaneous loss of $Cl^{\cdot}$ or HCl gives $C_nH_{2n-2}^{\cdot+}$ and $C_nH_{2n-3}^+$ ion series. These fragment ions are weak in all 1- and 2-chloro isomers.

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Multivariate Data Analysis of Substituent Descriptors

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A matrix containing seven often used substituent descriptors (Hammett sigmas *etc.*) for twenty-eight substituents is studied by multivariate statistical analysis.

The results show: (a) Strong grouping of the substituents into four separate classes; alkyls, donors, acceptors and halogens. (b) Separate models for the classes are superiour for describing the intra class structures compared to a whole set model. (c) A high collinearity between some of the descriptors.

As discussed, the found grouping and the collinearity can be limiting factors in the use of multiple regression in quantitative structure-activity and structure-reactivity studies.

In some of our previous work we investigated the application of multivariate statistics in physical organic chemistry. Substituent effects[1–6] in linear free energy relationships (LFERs), has been an area where statistical tools have shown to be valuable.[7–9] Thus, a principal component (*PC*) model with one component ($A=1$ in eqn. (1) below) has the same form as a simple linear regression equation ($M=1$ in eqn. (7) below) and as the Hammett equation.[10] Hence a *PC* model has been used to derive a unified substituent scale for isolated benzene systems, independently of a single reference reaction.[2]

The main difference between the linear regression and the *PC* analysis lies in the assumptions about the substituent parameters. In the former analysis, the values of the independent variable(s) x_i (the substituent constants) are assumed to be exactly known and 100 % relevant to the description of the data set under examination.[11] In the latter approach no assumption about the relevance of the variables x_i is required, since this relevance is obtained from the statistical analysis.

The two philosophies diverge even more when the number of explanatory variables is increased, as, for instance, in the multiple regression analysis (MRA) of dual or multiple parameter equations [see eqn. (7)]. To get statistically sound results, the application of MRA requires that the x variables are independent and fairly non-collinear of each other. However collinearity can be tested for. In contrast, *PC* analysis is insensitive to collinearities and in fact uses them to estimate the components θ. In other words in MRA one first needs to define "fundamental" effects. Thereafter one tries to interpret the results in terms of the definitions previously agreed. With *PC* analysis no such definitions are required. The results are usually interpreted just in terms of the components needed to model the data set. They can also be related to the effects currently believed to be measured by the variables used in the input.

A further statistical problem with MRA is the heavy dependence of the results upon the number of observations in relation to the number of independent variables. As discussed by Topliss and Edwards[12] this problem also arises when variables are selected or screened from a larger ensemble. Thus when the number of screened independent variables exceeds the number of observations, the risk for spurious correlations is serious. For example, with 5 independent variables and 10 observations, the probability for chance correlation, P_c, ($r^2 > 0.8$) is 0.05. If the number of screened variables is increased to 10, then $P_c = 0.30$. This problem is amplified if the independent and independent variables are grouped in the same way. If we have, say, twenty observations grouped in five classes the chance correlation approaches the case with five observations. We note (see Ref. 12) that with five observa-

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tions and five variables $P_c = 0.30$, but with twenty observations and five variables the chance correlation is negligible.

We have previously pointed out the problem of grouping for the substituent constants measuring the inductive and mesomeric effects. In the two-dimensional space defined by σ_I and σ_R the points for some twenty-four of the most common substituents are located in such a way that *ca.* 50 % of the points lie within a narrow area, and the others are spread out in various directions.[5,6] Owing to this situation, it follows that a certain number of appropriate substituents has to be used in MRA to obtain significant results.[13]

In order to investigate whether the same situation also applies to the case with an increased number of variables (substituent scales), we were tempted to make a *PC* analysis of a matrix containing some of the mostly used descriptors of substituents. In this report we describe the result obtained by applying the *PC* model to a data set formed by values of seven variables $\sigma_m^{0\,2d}$, $\sigma_p^{0\,2d}$, $\sigma_R^{0\,14}$, σ^+ or σ^{-15}, E_S^{16}, π^{16}, and molar refractivity (MR)[16] for twenty-eight common substituents. Thus the variables used are measures of various electronic, steric and polarizability effects.

METHODS

The *PC* method is presented in detail in Refs. 1, 2, 7, 17 and 18. Therefore, we will limit the presentation to a brief summary. For an introduction to multiple regression see Ref. 19.

Principal components analysis

The descriptor matrix Y contains the elements y_{ik}, where index i is used for the descriptors (variables) and index k for the substituents (objects). From this data matrix the number of cross-terms, A, and then the parameters α_i, β_{ia} and θ_{ak} in eqn. (1) are estimated by minimizing the squared residuals ε_{ik}.

$$y_{ik} = \alpha_i + \sum_{a=1}^{A} \beta_{ia}\theta_{ak} + \varepsilon_{ik} \qquad (1)$$

In this model α_i and β_{ia} are constants, which only are dependent on the descriptors and θ_{ak} are the substituent dependent parameters. The deviations from the model are expressed by the residuals ε_{ik}.

Before applying any statistical analysis, the descriptor values were auto-scaled. This means that the variables are given the same variance (fixed to unity). With this scaling all variables are given the same importance in the *PC* analysis.

First a model with $A = 0$ is fitted to the data, which means that each descriptor is given as its mean value α_i. Then the α_i value for each variable is subtracted from the matrix elements y_{ik} thus giving the residuals of dimension zero. If these residuals now contain systematic information, the $\beta_{i1}\theta_{1k}$ term is estimated. Whether the residuals contain information or not is determined by cross-validation [for the details see Ref. (18)]. Then new residuals are calculated by subtracting the term $\beta_{i1}\theta_{1k}$. If the new residuals contain systematic information additional $\beta_i\theta_k$ terms are then estimated one after the other, until the residuals just contain noise.

After a model has been determined with autoscaling, it can be refined by a reweighting of the variables, in this case by multiplying each variable with its modelling power ψ_i, defined in eqn. (2).

$$\psi_i = (1 - s_i/S_{iY}) \qquad (2)$$

Here s_i and S_{iY} are the residual standard deviations for variable i with A significant components and with $A = 0$, respectively. This means that variables for which the $\beta\theta$ terms contain no or little information, will have modelling powers close to zero. Thus with this type of reweighting, such variables are given small weights.

Once a class model is determined, a data vector y_{ip} of a substituent p can be fitted to the class parameters by MRA as in eqn. (3). The class model is characterized by the α_i and β_{ia} parameters estimated from a data set with M variables and N substituents.

$$y_{ip} - \alpha_i = \sum_{a=1}^{A} t_{ap}\theta_{ak} + e_{ip} \qquad (3)$$

How well the data vector for the substituents fits the model is expressed by the residual standard deviation s_p in eqn. (4). By comparing the size of

$$s_p = \left[\sum_{i=1}^{M} e_{ip}^2/(M - A) \right]^{1/2} \qquad (4)$$

s_p^2 with S_A^2 [eqn. (5)], with the F-test in eqn. (6), we can decide if a substituent data vector belongs to a certain class or not. S_A is the total residual standard deviation for a class with A significant components.

$$S_A=\left[\sum_i\sum_k \varepsilon_{ik}^2/(N-A-1)(M-A)\right]^{1/2} \quad (5)$$

$$F=s_p^2/S_A^2 \quad (6)$$

Multiple regression analysis

In the multiple regression model (7) a data vector y_k (the dependent variable) is fitted to a fixed number of M independent variables x_i. The model

$$y_k=c_0+\sum_{i=1}^{M} c_i x_{ik}+e_k \quad (7)$$

is characterized by the regression coefficients c_0 and c_i ($i=1-M$). For example, in structure-activity or structure-reactivity studies, y_k is a vector consisting of activities or reactivities for a set of similar compounds modified by changing substituents. The independent variables are then a set of substituent descriptors like those analyzed in this paper. Note that once a *PC* model is determined, the new θ_{ak} substituent descriptors can be used in eqn. (7) as independent variables instead of x_{ik}. The advantage is that usually $A \ll M$ and that the θ_{ak} parameters are orthogonal to each other thus avoiding collinearity problems.

RESULTS AND DISCUSSION

The application of a *PC* model to the whole data set shows that only two components are significant according to the cross-validation. *Ca.* 82 % of the total variance is described by the two components model. For the the optimized model, the parameters specific for the variables (α_i and β_{ia}) are given in Table 1 and the parameters specific for the substituents (θ_{ak}) are given in Table 2. In Fig. 1, the β_{i1} parameters are plotted against β_{i2} for each variable. It is noteworthy that the first component contains mainly the contributions of the electronic variables (seen from their high absolute values of β_{i1}) and the second component contains mainly information from the remaining three variables (seen from their high absolute values of β_{i2}). Fig. 1 also indicates the degree of collinearity between the variables. Variables highly correlated to each other lie either very near each other ($r \approx 1$) or in a symmetrical position with respect to the center of the diagram ($r \approx -1$). In the present case, we observe that the four electronic descriptors all lie within a small range, thus pointing out the redundancy of the σ scales for this data set, whereas E_S contains some non-redundant information and both π and MR fall in a third region of the diagram.

A plot of the values of the two components, θ_{1k} and θ_{2k} for each substituent, *i.e.* the values that each substituent assumes along the new dimensions of the reduced 7-dimensional space, is shown in Fig. 2. It clearly indicates that the substituents do not constitute a single homogeneous class, but are strongly grouped according to their chemical nature. Especially the first component, containing the "electronic" variables, seems to discriminate between the classes. Four separate subsets of substituents can be recognized: alkyls, halogens, acceptors and donors. Such strong grouping into four classes was also recognized in a multivariate analysis of ^{13}C NMR shifts of more than seventy monosubstituted benzenes.[20]

We note that Hansch *et al.*[21] have investigated a similar data set consisting of 8 variables and 90 common substituents by an hierarchical clustering analysis. The aim was to find substituents with similar properties. However, the number of clusters was determined in advance. Different analyses with 5, 10, 20 and 60 globular clusters were performed. This explains the difference between their results and ours. For example, in their analysis such diverse substituents as alkyls, donors and acceptors can be found within the same cluster. The present overall analysis shows that most of the variance in the data is described by a two-components model. Therefore, the grouping (clustering) of the substituents can be evaluated directly from Fig. 2, and no initial assump-

Table 1. Model parameters (α_i, β_{i1} and β_{i2}) for the whole data set.

	σ_m^0	σ_p^0	σ_R^0	$\sigma^{+/-}$	E_S	π	*MR*
w_i^a	2.80	2.23	2.03	1.06	0.537	0.410	0.056
α_i	0.719	0.415	−0.207	0.056	−0.789	0.117	0.676
β_{i1}	−0.460	−0.547	−0.314	−0.596	0.149	0.113	0.032
β_{i2}	−0.167	−0.040	0.182	0.084	−0.577	0.499	0.590

[a] The weights for the optimized model. The weights after autoscaling are; 4.30, 3.02, 4.06, 1.32, 1.10, 0.92 and 0.12.

Table 2. Components for the whole set model and residual standard deviations s_k for each substituent when (*i*) fitted to the whole class model, (*ii*) to its own class model (classes; alkyls, halogens, acceptors and donors) and (*iii*) to its next closest class. The residual standard deviations are denoted $s_k(i)$, $s_k(ii)$ and $s_k(iii)$.

k Subst.	θ_1	θ_2	$s_k(i)$	$s_k(ii)$	$s_k(iii)$	$F^b_{calc.}$
1 H	0.610	−0.698	0.423		0.32(A)	17
2 Me	0.890	−0.122	0.248	0.063	0.37(D)	4.0
3 Et	0.879	0.140	0.199	0.017	0.36(D)	3.8
4 *i*-Pr	0.879	0.515	0.164	0.061	0.37(D)	4.0
5 *t*-Bu	0.832	1.089	0.190	0.109	0.48(D)	6.8
6 CH_2Ph	0.707	1.179	0.334	0.020	0.52(D)	8.0
7 Ph	0.503	1.621	0.235	0.073	0.61(D)	11.
8 F	0.225	−0.840	0.218	0.027	0.46(D)	6.3
9 Cl	−0.119	−0.364	0.218	0.045	0.39(D)	4.5
10 Br	−0.175	−0.186	0.227	0.027	0.34(D)	3.4
11 I	−0.147	0.123	0.226	0.041	0.25(D)	1.8
12 CF_3	−1.249	0.154	0.276	0.30	0.48(H)	689
13 CO_2Me	−1.235	−0.006	0.222	0.13	0.60(H)	138
14 COPh	−1.129	1.415	0.173	0.10	0.40(H)	62
15 CHO	−1.479	−0.137	0.228	0.18	0.65(H)	162
16 CO_2R	−1.066	0.194	0.144	0.03	0.48(H)	89
17 CO_2H	−1.210	−0.108	0.287	0.18	0.62(H)	148
18 SO_2NH_2	−1.767	−0.491	0.324	0.27	0.78(D)	18
19 SO_2Me	−2.083	−0.430	0.315	0.22	0.88(D)	23
20 CN	−1.881	−0.451	0.103	0.11	0.72(H)	199
21 NO_2	−2.388	−0.055	0.193	0.18	0.82(H)	258
22 OMe	1.360	−0.557	0.083	0.16	0.39(A)	18
23 OH	1.614	−0.806	0.135	0.13	0.41(A)	120
24 OPh	1.074	1.181	0.322	0.035	0.35(A)	15
25 SMe	0.855	−0.032	0.134	0.14	0.33(A)	13
26 NH_2	2.111	−0.810	0.264	0.071	0.67(A)	53
27 NMe_2	2.374	−0.230	0.216	0.071	0.84(A)	83
28 NHAc	0.954	−0.147	0.367	0.21	0.51(A)	31

[a] Components for the whole set model. [b] *F*-Test according to eqn. (6) testing if a substituent data vector belongs to its next closest class. $F_{0.05} = 2.9$, 3.0 and 2.6 when a data vector is fitted to the alkyl, halogen or donor class models, respectively. [c] Next closest class given within parentheses A = alkyls, H = halogens and D = donors.

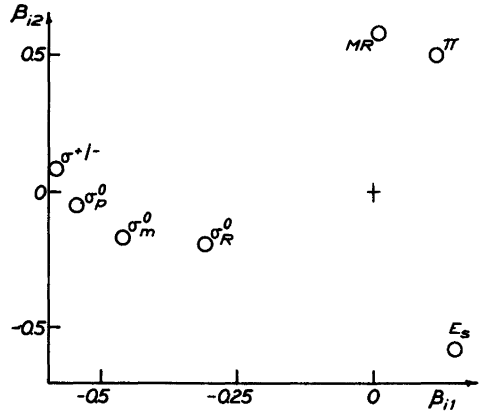

Fig. 1. A plot of β_{i1} against β_{i2} for the whole set analysis.

tion about the shape of the clusters has to be made.

Another investigation similar to the present one is that of Nieuwdorp *et al.*[22] In this paper factor analysis was applied to a data set consisting of 17 substituents and 76 reactions series, representing a wide span of reaction types. For each of the substituents three parameters were estimated from a model similar to eqn. (1) with A = 3. By plotting the three estimated constants for the substituents against each other, we find the same strong grouping of the substituents as in the present work.

If the four substituent classes are described by separate *PC* models, by pooling the variances[23] with $A = 0$ reported in Table 3, we see that 73 % of the total variance is described. The same scaling is retained as in the overall analysis to enable a comparison. Thus the conclusion is that 73 %

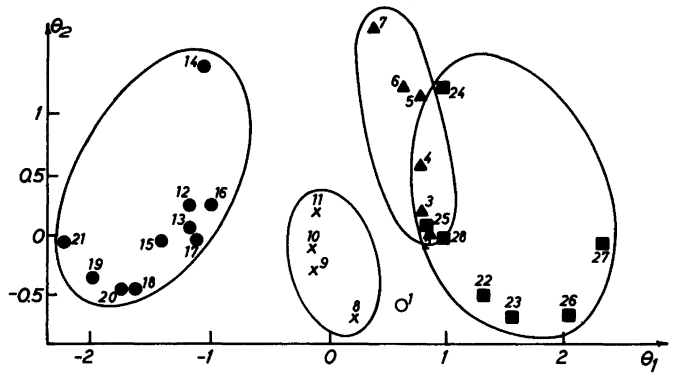

Fig. 2. Plot of the first against the second component for the whole data set model. Numbers identifying substituents as in Table 2; ●, acceptors; ■, donors; ▲, alkyls; ×, halogens.

of the total variance in the data consists of interclass variance while 27 % of the variance is due to intraclass variance. Since the overall analysis with 2 components explains 82 % of the total variance, the overall analysis mainly explains the interclass variation in data and only a minor part of the intraclass behaviour, see Fig. 3.

The high significance of the grouping is also confirmed by an analysis of variance.[23] Thus, the description of the data-set ($-H$ is not included) is significantly better ($P<0.01$) by the present four models with A = 0, than by an overall model with $A=0$.

The intraclass structures are better described by separate class models with $A=1$ in the case of the halogens and by $A=2$ for the other classes. These models describe 92 % of the variance in the whole set data or 70 % of the pooled variance for the separate class models with $A=0$ (see Fig. 3). This shows that for the given set of substituent descriptors separate class models are superior to a single overall model for describing the intraclass structure.

In addition, the separate class models can be further refined by using the same weighting strategy as for the whole class analysis. Thus as much as 93 % on an average of the intraclass structure is described if the data for each class first is autoscaled and then reweighted by multiplying each variable with its modelling power. The variance for the models with this weighting are given within paren-

Table 3. Residual standard deviation after model expansion for the whole set and for each subset with the same scaling. The value within parentheses for the separate class models refer to the optimized models with individual scaling and therefore the values in the different rows are not strictly comparable. The percentages of the variance with $A=0$, explained by models with $A=1$ and $A=2$ are denoted V_1 % and V_2 %.

Set	n^a	$S_0(A=0)^b$	$S_1(A=1)^b$	$S_2(A=2)^b$	V_1 %^{c}	V_2 %^{c}
Whole	28	0.605	0.361	0.257	64	82
Alkyls^d	6	0.316	0.197	0.092	61	92
		(0.471)	(0.252)	(0.091)	(71)	(96)
Halogens^d	4	0.174	0.051		92	
		(0.334)	(0.059)		(97)	
Acceptors^d	10	0.322	0.255	0.214	38	56
		(0.279)	(0.194)	(0.073)	(52)	(93)
Donors^d	7	0.367	0.253	0.184	52	75
		(0.473)	(0.177)		86	

^{a}Number of substituents. ^{b}Residual standard deviation for the *PC* models with $A=0(S_0)$, $A=1(S_1)$ and $A=2(S_2)$. ^{c}Percentages of the variance with $A=0$, explained by models with $A=1$ [$V_1\%=100(1-S_1/S_0)$] and by the models with $A=2$[$V_2\%=100(1-S_2/S_0)$]. ^{d}The substituents are for alkyls; 2–7, halogens; 8–11, acceptors; 13–21 and donors 22–28. For numbering see Table 2.

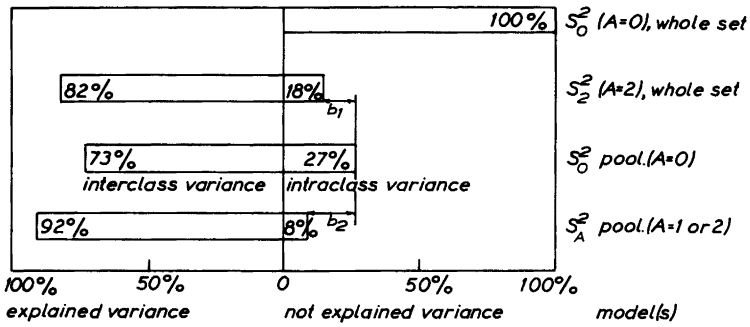

Fig. 3. Illustration of the sizes of the residual variances for different models expressed as % of the residual variance of the overall model with $A=0$. When the four separate classes are described by their mean values, 73% of the total variance is described. Thus the 73% can be assigned as interclass variance. Of the 27% intraclass variance, the whole set model only explains 9% (b_1) and the four separate models explain 19% (b_2). Thus the overall model with $A=2$ explains ~33% and the four class models ~70% of the intraclass variance.

theses in Table 3.

In the separate class models the contribution from the different variables varies considerably. For donors the "electronic" variables dominate (σ_m^0,σ_p^0 and $\sigma^{+/-}$) while for the halogens the "non-electronic" variables dominate (E_S, π and MR). For the alkyls σ_p^0, $\sigma^{+/-}$, E_S, π and MR contribute, while for the acceptors mainly σ_p^0, π and MR are important. This can be seen from the β-values and modelling powers for the different intraclass structures are not parallel to each other and consequently they are not parallel with the interclass behaviour.

The four subsets are well separated, as indicated by the residual standard deviations given in Table 2, obtained by the SIMCA[7] classification method. The standard deviations are given for each substituent (*i*) when fitted to the overall model, (*ii*) to its own class model and (*iii*) to its next closest class. The residual standard deviations in case (*i*) and (*iii*) are significantly larger ($P=0.05$) in all cases except one, compared to the standard deviation in case (*ii*). The exception is iodine that also fits the donor class, seen from the *F*-test in Table 2.

The class separation is clearly seen in Fig. 2. However, the figure is somewhat misleading with respect to substituents 25 and 28 (SMe and NHAc). These substituents are not close to the alkyls model. Their positions in the plot are due to an artifact of the projection of the data down on a plane. Hydrogen was not initially assigned to any of the classes. Indeed it does not belong to any class according to the *F*-tests (see Table 2). It is also noticable that Ph is well described by the same model as the alkyls, even if Ph formally not is an alkyl substituent.

To summarize the results: A strong grouping in the substituent descriptors is found. An overall *PC* model mainly explains interclass variation and little intraclass variation. Separate models for separate groups of substituents describe the intraclass behaviour much better than a single overall model. The separate models do not parallel with their interclass behaviour. In the overall model a high collinearity is found between some of the descriptors.

These findings will be important in structure-reactivity and structure-activity studies. With respect to the first area, we note that each subset has its own particular intraclass structure. A general theory that is able to cope with these class structures is not yet available. In structure-activity studies one rarely finds suitable model systems that are approximately linearly related to the properties of the system under investigation. Hence several substituent scales are used in a multiple regression model and the problems discussed above become serious. We also note that the present set of descriptors is widely used in structure-activity studies (see Ref. 16). If the present descriptors are used as independent variables in MRA, the result will be an unstable model which will have poor predictive ability due to the strong collinearity between some of the descriptors.[19] We also note that the statistical tests on the regression coefficients in MRA assume that the objects are not grouped. If they are grouped, the confidence intervals of the regression coefficients will be deceptively small and the correlation coefficients deceptively high.

The collinearity problem could be circumvented

by using the present θ_1 and θ_2 substituent parameters as independent variables in MRA instead. However, also in this case, the limitations for MRA are present due to the grouping of the substituents. This means that the prediction of a compound with unknown behaviour will not be much better than by using the mean value of the dependent variable for the already measured compounds in this class. However, in order to reasonably well define separate class models, at least 4–5 substituents must be present in each class. If one or more of the separate classes not are represented in the dependent variable and we want to make a prediction for a compound in the missing class, this can only be obtained if the relative position of the classes is the same in the dependent variable and independent variable. Whether this assumption is valid or not remains to be investigated.

Supplementary material available. Data used, α_i, β_{ia} and θ_{ak} for the four subclasses and classification results, can be obtained on request from the authors.

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Cyclooligomeric Condensation Products. IV.* Formation of Stereoisomeric [1_4](2,5)Furanophanes

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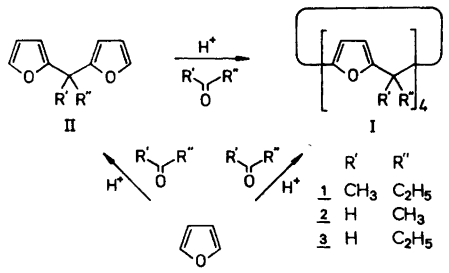
^{13}C NMR and HPLC analyses were used to show that the acid-catalyzed condensation of acetaldehyde with 1,1-di(2-furyl)ethane gives a mixture, m.p. 127–140 °C, of the four geometrical isomers of 1,7,13,19-tetramethyl[1_4](2,5)furanophane. Quantitatively, there is a small but significant deviation from a random statistical distribution between the four isomers, mainly evident from the relative enrichment in the mixture of the all-*cis* isomer, m.p. 191.0–192.0 °C. Similarly, the condensation of propanal with 1,1-di(2-furyl)propane gives a mixture, m.p. 83–88 °C, of the stereoisomers of 1,7,13,19-tetraethyl[1_4](2,5)furanophane from which the all-*cis* isomer, m.p. 152.0–153.0 °C was isolated by recrystallization. Finally the 1,7,13,19-tetraethyl-1,7,13,19-tetramethyl[1_4](2,5)furanophane, m.p. 170.0–172.0 °C, obtained by the condensation of butanone with furan, was found to be a mixture of stereoisomers too.

The formation of [1_4](2,5)furanophanes I (R′ and R″ = alkyl) by the acid-catalyzed condensation of ketones with furan or difurylalkanes II (R′ and R″ = alkyl) (see Scheme 1) has been studied extensively by W. H. Brown and co-workers.[1–7]

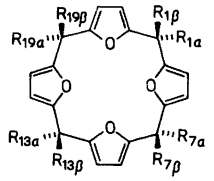
Interest in these macrocycles was further stimulated by the report that increased yields could be obtained in the presence of soluble lithium salts, suggesting the presence of a metal ion templating effect.[8] However, recently an alternative explanation was forwarded by Rest, who was able to correlate the yield of the macrocycles, not with the metal ion content but with the amount of acid added to the reaction mixture.[9]



Scheme 1.

When the furanophanes are unsymmetrically substituted (I, R′ ≠ R″) the macrocycle may exist in four different configurations Ia–Id (see Table 1). Brown observed that the reaction of butanone with

Table 1. Stereostructures Ia–Id. Configurations and number of ^{13}C NMR Peaks. R = R′ or R″ (see Scheme 1).



Position of R′ [a]		No. of peaks [b]
Ia	α,α,α,α	1/1
Ib	α,α,α,β	4/3
Ic	α,α,β,β	2/1
Id	α,β,α,β	1/1

[a] Relative positions of R′ at carbons 1,7,13 and 19 (cyclophane numbering) or 2,7,12 and 17 (IUPAC numbering). [b] Calculated number of ^{13}C NMR peaks for each type of aromatic/aliphatic carbon.

* Part III, see Ref. 14.

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furan unexpectedly gave a single product *1* (I: R′ = CH_3; R″ = C_2H_5) m.p. 174 °C, while the reaction between butanone and 2,2-di(2-furyl)propane (or acetone with 2,2-di-2-furylbutane) gave the expected mixture of *cis* and *trans* isomers, m.p. 204 and 178.5 °C, respectively, in a ratio of 1:8.[1] Recently, the reaction between acetaldehyde and 1,1-di(2-furyl)ethane was reported to give a product *2* (I: R′ = H; R″ = CH_3), m.p. 140 – 142 °C, in a yield of 13%.[10,11]

A similar reaction, the acid-catalyzed condensation of various aldehydes with resorcinol, gives tetrasubstituted [1₄]metacyclophanes with a high degree of stereoselectivity.[12-14] These macrocycles, which may be obtained in yields exceeding 80%, are related to the furanophanes not only by their mode of synthesis but also by their size and symmetry properties. Initially only two out of the four possible geometrical isomers are formed, one of which is quantitatively converted into the other (the all-*cis* isomer) as the reaction proceeds. In addition, this final single stereoisomer exists in a single stable conformation out of several possible.

The stereoselectivity of the reaction was attributed to the effect of intra-molecular non-bonded interactions leading to conformational control of the intermediates. In view of the similarities between the [1₄]metacyclophanes and the [1₄](2,5)furanophanes and the lack of stereochemical information on the latter compounds, a reinvestigation of the furanophanes seemed worthwhile in order to extend and further develop the stereocontrol concept advanced for the former system.

EXPERIMENTAL

^1H and ^{13}C NMR data of the compounds in $CDCl_3$ solutions were obtained using a Bruker WP 200 FT instrument at 200 MHz and 50.3 MHz, respectively. For the low-temperature spectra an equal mixture of $CDCl_3$ and CH_2Cl_2 was used as a solvent. The dipole moment of *2a* was calculated from determinations at three different concentrations (0.031 – 0.124 M in benzene) using a dipolemeter DM 01 (Wissenschaftlich-Technishen Werkstätten GmbH). HPLC analyses were performed on a Spectra Physics SP 8000 chromatograph equipped with a 4.6 × 200 mm Spherisorb 5 μm silica column, and a UV-detector (280 nm) using cyclohexane – methylene chloride (2:1 v/v) as an eluent at a pumping rate of 2 ml/min. R_f-values are given in seconds. The microanalyses were performed by Alab, Uppsala.

Furanophane (1). It was prepared according to the literature procedure,[1] m.p. 170 – 172 °C (lit.[1] m.p. 174 °C). ^{13}C NMR: δ 157.81, 157.69, 157.57 (C-arom), 104.15, 104.09, 103.97, 103.87 (CH-arom), 40.57, 40.55 (C), 30.00, 29.93, 29.87, 29.82, 29.77 (C-CH_3), 21.91, 21.87, 21.82, 21.70, 21.64 (CH_2), 8.28, 8.22, 8.15 (CH_2-CH_3).

Furanophane (2). 1,1-Di(2-furyl)ethane[3,10] (5.0 g, 31 mmol) was added to a stirred, ice-chilled solution of aq. acetaldehyde (5 ml 90% (v/v), 90 mmol) dissolved in 105 ml of a 2*M* solution of $LiClO_4 \cdot 3H_2O$ (32.0 g) in a mixture of 60% $HClO_4$, H_2O and EtOH (1:4:16 v/v). After 25 min the ice-bath was removed and the mixture was stirred for another 41 h and then poured into a mixture of 10% $NaHCO_3$ (100 ml) and water (300 ml). The product was taken up in 400 ml of diethyl ether, 50 ml of light petroleum was added and the organic solution was washed two times with 200 ml of water and finally with brine before it was dried over anhydr. $MgSO_4$. Evaporation yielded 6.84 g of crude product which was first purified by flash chromatography[15] on 180 g of silica gel using an equal mixture of toluene and light petroleum as eluent. The final fraction eluted, gave upon evaporation 2.27 g of a yellow powder which was further purified by sublimation at 120 °C/10 Pa yielding 31% (1.83 g, 4.9 mmol) of a mixture of stereoisomers *2*, m.p. 127 – 140 °C. Anal. $C_{24}H_{24}O_4$: C, H. IR and MS as published.[10] ^1H NMR:[11] δ 5.97, 5.95(5), 5.95 (8H, three s, arom), 4.04, 4.01, 4.00, 3.99 (4H, four q, rel. int. 1.1:1.4:1.0:2.0, *J* 7 Hz, CH), 1.51, 1.50, 1.49 (12H, three d, *J* 7 Hz, CH_3). ^{13}C NMR: δ 155.53, 155.51, 155.44, 155.40, 155.39, 155.31 (C-arom), 104.89, 104.84, 104.77, 104.75, 104.67, 104.66, 104.55, 104.51 (CH-arom), 32.90, 32.80, 32.69 (CH), 17.56, 17.48, 17.29, 17.24, 16.99, 16.94 (CH_3) (rel. area: 1.5:1:2.4:3.2:1.7:2.5).[16] HPLC (R_f-value in s, rel. area): 214(29), 242(49), 376(22).

Furanophane (2a).[17a] Recrystallization of the isomer mixture *2* three times from hexane and seven times from light petroleum yielded 150 mg (2.5%) of the pure stereoisomer *2a*, m.p. 191.0 – 192.0 °C. Anal. $C_{24}H_{24}O_4$: C, H. IR and MS as *2*. ^1H NMR: δ 5.95(6) (8H, s, arom), 4.04 (4H, q, *J* 7 Hz, CH), 1.52 (12H, d, *J* 7 Hz, CH_3). ^{13}C NMR: δ 155.54 (C-arom), 104.48 (CH-arom), 32.65 (CH), 16.95 (CH_3). HPLC (R_f in s, rel. area): 376(100).

The combined mother-liquors were evaporated and recrystallized from light petroleum yielding a mixture *2′*, m.p. 133.0 – 134.5 °C. Anal. $C_{24}H_{24}O_4$: C, H. IR and MS as *2*. Partial ^{13}C NMR:[18] δ 17.56, 17.47, 17.29, 17.22, 16.97 (CH_3) (Ref. area: 1.0:1:3.8:2.7:1.4). HPLC (R_f in s, rel. area): 217(46), 251(54).

Furanophane (3). It was prepared in a manner similar to *2* from 1,1-di(2-furyl)propane (5.25 g, 30 mmol) and propanal (4.5 g, 77 mmol). However, the eluent used for flash chromatography was a mixture

of toluene and light petroleum 3:10 v/v). The yield of *3*, m.p. 83–88 °C was 25% (1.62 g, 3.8 mmol). Anal. $C_{28}H_{32}O_4$. IR. (KBr) 2955, 2925, 2870, 1565, 1460, 1185, 1010, 970, 955, 790, 770 cm^{-1}. MS [IP 70 eV] $m/z = 432(M^+)$. ^1H NMR δ 5.94(4), 5.94, 5.93 (8H, three s, arom), 3.70, 3.69 (12H, two t, J 7.8 Hz, CH), 1.93(5), 1.92, 1.91(5) (8H, three quintets, J 7.8 Hz, CH_2), 0.87, 0.86(7), 0.86, 0.85, 0.84(5) (12H, five t, J 7.3 Hz, CH_3). ^{13}C NMR δ 154.42, 154.34, 154.32, 154.25, 154.21 (C-arom), 105.51, 105.48, 105.46, 105.40, 105.32 (CH-arom), 40.62, 40.58, 40.56 (CH), 24.36, 24.32, 24.26, 24.21, 24.18 (CH_2), 12.15, 12.08, 12.01 (CH_3). HPLC: three peaks.[18]

*Furanophane (*3a*).*[17b] The mixture *3* was recrystallized four times from light petroleum to yield 16 mg (0.2%) of the pure stereoisomer *3a* m.p. 152.0–153.0 °C. Anal. $C_{28}H_{32}O_4$: C, H. IR and MS as *3*. ^1H NMR δ 5.94 (8H, s, arom), 3.70 (4H, t, J 7.8 Hz, CH), 1.93 (8H, quintet, J 7.6 Hz, CH_2), 0.84(6) (12H, t, J 7.3 Hz, CH_3). ^{13}C NMR: δ 154.23 (C-arom), 105.45 (CH-arom), 40.60 (CH), 24.32 (CH_2), 11.98 (CH_3). HPLC: one peak with the same R_f-value as the last peak of *3*.[18]

RESULTS

The reaction of acetaldehyde with the difurylethane was quite facile at room temperature in the presence of perchloric acid and lithium perchlorate. From the mixture of condensation products a macrocyclic fraction *2* ($C_{24}H_{24}O_4$), m.p. 127–140 °C, was isolated in a fairly good yield (31%) by flash chromatography followed by sublimation *in vacuo*. Its ^1H NMR spectrum exhibited multiple overlapping resonances. The ^{13}C NMR showed multiple resonances too for each type of carbon with eight and six separate singlets for the aromatic CH-carbons and the aliphatic CH_3-carbons, respectively.

TLC analysis (silica plates) of *2* did not give any indication of the presence of more than one compound. However, after extensive optimization of the elution parameters, HPLC analysis of *2* gave three well-resolved peaks with a relative area of 29:49:22. In one chromatogram, using a somewhat less polar eluent mixture, the first peak was slightly resolved, indicating the presence of two components in a relative ratio of 1:3.

Repeated recrystallization of *2* gave a small (2.5%) fraction *2a* ($C_{24}H_{24}O_4$), m.p. 191.0–192.0 °C. Its ^1H NMR showed a singlet in the aromatic region and a single AX_3 system in the aliphatic. The ^{13}C NMR consisted of a single peak for each type of carbon. Its ^1H and ^{13}C low temperature NMR spectra, recorded at −75 and −65 °C, respectively, exhibited only minor changes relative to the spectra recorded at room temperature. (The methyl doublet of the ^1H NMR spectrum was slightly more broadened than the TMS-singlet.) The dipole moment of *2a* was also measured and found to be zero. HPLC analysis of *2a* gave one peak with the same R_f-value as the last fraction of the mixture *2*.

The combined mother-liquors from the recrystallization of *2a* were evaporated and recrystallized to yield a fraction *2'* ($C_{24}H_{24}O_4$), m.p. 133.0–134.5 °C. Its HPLC analysis showed two peaks with the same R_f-values as those of the first two of the original mixture *2* (rel. ratio 46:54). The ^1H and ^{13}C NMR spectra were similar to those of *2* with respect to the shift values, but with all peaks belonging to *2a* missing. However, the relative intensities of the peaks were different from those of *2*.

The corresponding tetraethyl substituted furanophanes were obtained by the reaction of propanal with 1,1-di(2-furyl)propane. The product *3* ($C_{28}H_{32}O_4$), m.p. 83–88 °C, was obtained in 25% yield. Its ^1H and ^{13}C NMR spectra exhibited multiple resonances too but were less well resolved than those of *2*. HPLC analysis gave three separate peaks, the first two slightly overlapping each other. A small fraction (0.2%) *3a* ($C_{28}H_{32}O_4$), m.p. 152.0–153.0 °C, was obtained by recrystallization of *3*. Its ^1H and ^{13}C NMR spectra showed similar symmetry properties to those of *2a*. HPLC analysis gave a single peak with the same R_f-value as that of the last peak of *3*.

Finally the butanone–furan condensation product *1*, m.p. 170.0–172.0 °C, prepared according to the literature procedure,[1] was investigated by ^{13}C NMR analysis. The spectrum exhibited multiple resonances for each type of carbon.

DISCUSSION

Both HPLC and NMR analyses show that the products *1*–*3* indeed are mixtures of stereoisomers. The minute structural differences between the isomers, resulting in very similar physical properties, prevented their complete separation. Interestingly several of the mixtures exhibited fairly narrow melting point ranges, a feature also experienced by earlier workers in the field.

The ^{13}C NMR spectra were particularly useful in determining the compositions of the mixtures. The spectrum of *2* contains the full number of aromatic

CH (eight) and aliphatic CH_3 (six) resonances calculated for a mixture of all four stereoisomers possessing structures Ia–Id; (R′=H; R″=CH_3), while there is some accidental overlapping of the signals of the other two types of carbons. *A priori* these shift differences might be expected to have a twofold origin; anisotropic aromatic ring-current effects (conformational differences) and the shielding effects of the methyl groups (configurational and conformational differences). Qualitatively the relative shift differences of the six CH_3 singlets can be rationalized by evoking non-bonded through-space shielding effects from the methyl groups alone (*vide infra*).

The symmetry properties of the ^{13}C NMR spectrum of *2a* (as well as of *3a*) reveal that this isomer possesses either configuration Ia or Id. A further analysis of the ^{13}C shifts of the methyl carbons shows that the peak corresponding to *2a* has the lowest chemical shift out of the six in the mixture *2* containing all four stereoisomers. This suggests that in *2a* each methyl group experiences the maximum cumulative through-space shielding effects from the remaining three methyl groups *i.e.* the methyl groups are all on the same side of the macrocycle as in configuration Ia.

Independent support for this assignment is obtained from the result of the HPLC-analysis. Most likely the macrocycles bind to the silica surface through the intra-annular ether oxygens. Both hydrogen bonding and dipole–dipole interactions are possible. The relative binding energies depend on the number of ether oxygen of the macrocycle that simultaneously can be oriented into binding positions. The number of methyl groups that sterically interfere with binding to the surface depends on the configuration of the macrocycle. The maximum binding strength is attained in the all-*cis* isomer in which all four ether oxygens can participate in the binding. All four methyl groups are then on the opposite side of the macrocycle pointing away from the silica surface. The remaining three isomers will have one or two methyl groups poking into the silica surface. They will thus exhibit correspondingly lower binding strengths due to the decreased number of ether oxygens being able to simultaneously participate in the binding. From these considerations follows that upon chromatography the all-*cis* isomer, which is the most strongly absorbed one, will be eluted last of the four. Thus this supports the assignment of structure Ia (R=H; R=CH_3) to isomer *2a*. It can further be argued by similar reasoning that the second last peak is due to the isomer possessing the *cis-cis-trans* configuration Ib. The first peak which seems to contain two components would then be comparised of the two most hindered isomers possessing configurations Ic and Id.

Independent of the HPLC determination the relative composition of *2* can also be calculated from the peak areas of the CH_3 singlets of the ^{13}C NMR spectrum. By this method the ratio between the isomers possessing configuration Ia–Id was 20:52:20:8, in close agreement with the HPLC data (22:49:21:8). These numbers should be compared with the statistically expected distribution 12.5:50:25:12.5 and point to a small but significant enrichment of the all-*cis* isomer *2a*. By the NMR method the composition of the mixture *2′* was calculated to be 0:51:39:10. The effect of the repeated recrystallization of the combined mother-liquors from the recrystallization of *2a* thus led to a relative enrichment with respect to the isomer possessing configuration Ic. This enrichment was, however, insufficient to obtain it in a pure state.

A key factor, which was suggested to govern the stereochemistry in the formation of the [1_4]metacyclophanes, was the conformational control originating from intra-molecular non-bonded interactions at first in the triphenylmethane or diphenylalkane units and then subsequently in the higher condensation intermediates. Inspection of CPK-models indicates that these interactions decrease when the resorcyl groups are replaced by the furyl groups. We suggest that the low degree of stereoselectivity in the formation of the [1_4](2,5)furanophanes is a consequence of this lowering of the conformational barriers.[19] Also, for the same reason, the activation energy for pseudorotation of a macrocycle possessing the all-*cis* configuration Ia is expected to be lower than for the corresponding [1_4]metacyclophane isomer ($\Delta G^{\ddagger}_{306K} = 60.3$ kJ mol^{-1}). Thus the result of the low-temperature NMR of *2a*, which failed to demonstrate the presence of such a conformational barrier, does not rule out the proposed all-*cis* configuration. The lack of any measurable dipole moment of *2a* indicates either that the method is too insensitive to the small structural effects or that it is the result of the combined configurational and conformational effects.

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16. Calculated intensities for a statistically formed mixture (*i.e.* Ia – Id = 1:4:2:1): 1:1:2:2:1:1.
17. Systematic names: (a) *r*-2,*c*-7,*c*-12,*c*-17-tetramethyl-21,22,23,24-tetraoxapentacyclo[16.2.1.-$1^{3,6}.1^{8,11}.1^{13,16}$]tetracosane. (b) *r*-2,*c*-7,*c*-12,*c*-17-tetraethyl-21,22,23,24-tetraoxapentacyclo-[16.2.1.1^{3,6}.1^{8,11}.1^{13,16}]tetracosane.
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The Crystal Structure of Bis(6-hydroxy-4,4-dimethyl-2-oxo-6-cyclohexenyl) Selenide

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The crystal structure of the title compound, $(C_8H_{11}O_2)_2Se$, has been established by single-crystal X-ray diffraction techniques. The compound crystallizes in the tetragonal space group $P4_2bc$ with unit cell dimensions $a=13.420(10)$ Å, $c=9.278(5)$ Å and $Z=4$. The structure was solved by the heavy atom method and refined to a final R value of 0.045 for 518 symmetry independent reflections. The molecule has C_2 symmetry, with selenium atom in the special position.

The Se–C distance is 1.916(10) Å and the C–Se–C angle 100.9(4)°. The bond lengths in the enol system show considerable degree of delocalization. Short intramolecular contacts indicate bifurcated Se-containing hydrogen bonds between the two six-membered rings of the molecule.

Selenium dioxide oxidation of some cyclic α- and β-diketones have afforded new organic selenium compounds: 1,3-oxaselenole derivatives,[1-3] a selenoether of a reductone [1] and a representative of the 7-selenabicyclo[2.2.1]heptane series.[3,4] Each model compound has been subjected to X-ray analysis.[3-6] The title compound *1* [7,8] and bis(2-hydroxy-4,4,6,6-tetramethyl-3-oxa-1-cyclohexenyl) selenide *2* reported earlier [6] can be regarded as derivatives of the respective reductones (Fig. 1). Selenide *1* is a β-diketone derivative and selenide *2* an α-diketone derivative. To determine the crystal structure of *1* and to compare the structural properties of these closely related selenides was the purpose of this present study.

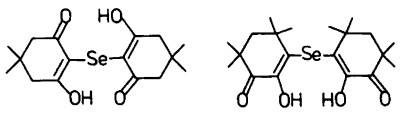

Fig. 1. Selenides *1* and *2*.

EXPERIMENTAL

The title compound was prepared as described earlier and recrystallized from ethanol to give white crystals.[7,8]

Preliminary photographic investigations indicated tetragonal symmetry and systematically absent reflections showed the space group to be $P4_2bc$ or $P4_2/mbc$. The crystal selected for the data collection had approximate dimensions $0.12\times0.12\times0.4$ mm^3. The determination of the unit cell parameters and the data collection were carried out on a Syntex $P2_1$ four-circle diffractometer using graphite monochromatized Mo$K\alpha$-radiation. ω-scanning technique ($5°<2\theta<50°$) and variable scan speed (2–20°/min) were employed. Reflections having $h\leq k$ were measured and, of those, 518 had $F>3\sigma(F)$ and were used in the structure analysis. The data were corrected for Lorentz and polarization effects.

CRYSTAL DATA

$(C_8H_{11}O_2)_2Se$, FW = 357.31,
space group $P4_2bc$ (No. 106).
$a=13.420(10)$, $c=9.278(5)$ Å, $Z=4$
$D_{obs}=1.43$ g cm^{-3}, $D_{calc}=1.420$ g cm^{-1},
μ(Mo$K\alpha$) = 24.3 cm^{-1}.

STRUCTURE DETERMINATION AND REFINEMENT

The structure analysis showed the space group to be acentric $P4_2bc$. The structure was solved by the

0302-4369/83 $2.50

Table 1. Fractional atomic coordinates ($\times 10^4$) and thermal parameters[a] ($\times 10^3$) for the non-hydrogen atoms.

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Se	0	5000	2500[b]	49(1)	53(1)	36(1)	8(1)	0	0
O1	1525(6)	6372(6)	4045(13)	48(6)	32(5)	115(8)	−6(3)	−9(6)	2(6)
O2	512(6)	3015(6)	4180(11)	44(5)	39(4)	77(6)	−5(4)	−6(5)	6(5)
C1	1055(7)	4686(7)	3815(12)	22(5)	35(5)	32(6)	17(4)	−5(5)	2(5)
C2	1711(9)	5457(9)	4265(16)	57(8)	32(6)	52(8)	−9(6)	10(8)	−4(7)
C3	2630(8)	5207(8)	5207(17)	41(6)	47(8)	48(8)	0(5)	8(7)	−16(8)
C4	2982(7)	4114(7)	5011(19)	35(5)	36(6)	56(7)	15(4)	12(9)	15(10)
C5	2071(8)	3445(7)	5223(17)	47(6)	33(5)	35(9)	10(9)	15(6)	11(6)
C6	1179(9)	3741(9)	4352(12)	39(7)	41(7)	36(5)	5(5)	9(6)	−6(6)
C7	3439(10)	3994(11)	3489(19)	43(8)	42(8)	82(11)	10(6)	18(8)	25(8)
C8	3764(11)	3849(12)	6165(18)	40(9)	68(11)	78(11)	4(7)	8(8)	−9(9)

[a] The anisotropic thermal parameters are given by $\exp[-2\pi^2(h^2a^{*2}U_{11}+\cdots+2hka^*b^*U_{12}+\cdots)]$. [b] The z-coordinate of selenium has been fixed.

heavy atom method. The selenium atom occupies the special position 0,1/2,z and for fixing of the origin the z-value was fixed at 0.25. After anisotropic least-squares refinements of all non-hydrogen atoms the R factor was 0.059. In the difference Fourier map a maximum of 0.38 e Å^{-3} was found at a distance of 0.94 Å from O2 and the other hydrogen atom maxima were found in the expected places, the C−H distances varying between 0.86 and 1.15 Å. Because of the poor observed reflections/parameter ratio the coordinates and the termal parameters (0.05 Å^2) of the hydrogen atoms were fixed. For the same reason, the estimated standard deviations of the bond lengths and angles are somewhat large. The estimated standard deviations of the O−C and C−C bond lengths are 0.014−0.018 Å except of those including methyl carbons (0.021 and 0.024 Å). The deviations of the Se−C−C and C−C−C bond angle values are 0.8 and 0.8 − 1.2°, respectively. The final R value was 0.045.

Scattering factors for Se, O and C were from Cromer and Mann[9] and those for H from Stewart, Davidson and Simpson.[10] The calculations were carried out on a Univac 1108 computer using the X-RAY 76 program system.[11]

DISCUSSION

Positional parameters are listed in Table 1. Fig. 2 shows the molecule with the important structural parameters and intramolecular hydrogen bonds. A list of structure factors is obtainable from the authors on request.

The asymmetric unit of the structure comprises one half of the molecule. The structure consists of discrete molecules having C_2 symmetry with the

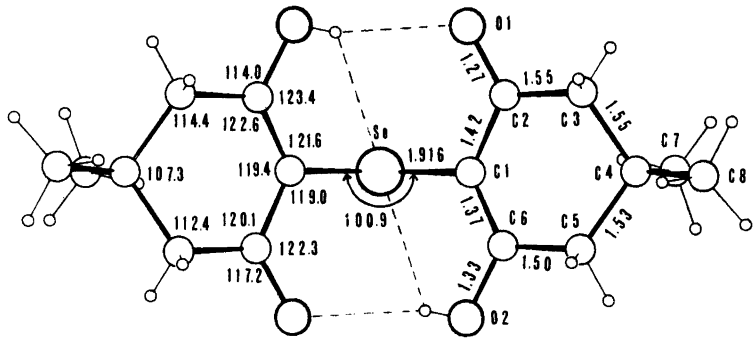

Fig. 2. A view of the molecule of *1* along the z-axis.

selenium atom in the special position. Although the estimated standard deviations of the bond parameters are fairly large, all bond lengths and angles in the conjugated system as well as the position of the hydroxyl hydrogen atom maxima indicate the C_{2v} symmetry to be impossible.

Geometrical constraints evidently determine the intramolecular contacts: the Se–O1 distance of 3.101(10) Å and the Se–O2 distance of 3.162(9) Å. The O1*–O2 distance (O1* at $-x,1-y,z$) 2.857(12) Å and the distances of the H1 atom from O2 (0.94 Å), from selenium (2.53 Å) and from O1* (2.23 Å) suggest a bifurcated hydrogen bond between these atoms. Generally, the ability of selenium to participate in H-bonding is small.[12,13] The resonance structure where selenium carries a negative charge coupled with the general trend of H-atoms to be donated to regions of large electron density might explain this arrangement.[12] The partial negative charge was assumed to explain also that the high field ^{77}Se NMR chemical shift of *1* appears nearly in the region of selenole anions.[14]

To allow comparison of the structural parameters and the solid state interactions of *1* and *2*, the molecular packing diagram of *2* with the numbering scheme and some relevant bond lengths is shown in Fig. 3.

That *1* is intramolecularly and *2* intermolecularly hydrogen bonded may be due to the tendency of the molecules to maximize H-bonding. The hydrogen bonding may affect the electronic structure of the participating moieties elsewhere in the molecule, too.[15] Such changes are however small, about 0.01 Å in the bond lengths. Additional changes may occur when hydrogen bonds are formed between molecules having delocalizated π-bonding systems. The selenide *1* displays considerable degree of delocalization whereas *2* does not show any delocalization owing to the lack of a suitable conjugated system. A comparison of *1* and dimedone[16,17] reveals that the Se atom and the differences in the H-bonding have only a minor effect on the conjugated system also in the solid state and both compounds display a similar extent of delocalization. Only small differences in the planarity of the conjugated systems of the two compounds can be discovered.

The Se–C bond lengths in *1* and *2* are equal (1.916(10), 1.916(4) and 1.917(4) Å), but the C–Se–C angle in *2* (103.9(2)°) is opened compared with *1* (100.9(4)°). The magnitudes of these parameters are as usually found in aromatic selenides (*ca.* 1.93 Å[18] and 96–106°[19]). The dihedral angle C2–C1–Se–C1* of 91.3(10)° in *1* (C1* at $-x,1-y,z$) and the dihedral angles C2–C1–Se–C11 of 41.6(4)° and C1–Se–C11–C12 of 46.9(3)° in *2*[6] (*cf.* Table 2) show roughly differences in the distortion of the six-membered rings around Se–C bonds. The planarity of the conjugated systems in *2* and the conformation of the six-membered rings of both compounds can be seen from Table 4. The conformation of the rings in *1* can be described in the first place as half-boat, the conformation of the rings in *2* as half-chair. Small differences in the geometry of the rings in *2*[6] (*cf.* Table 2) are reflected in the dihedral angle values of O1–C2–C3–O2 (1.5(6)°) and O3–C12–C13–O4 (9.5(5)°).

In the liquid state both selenides are fully

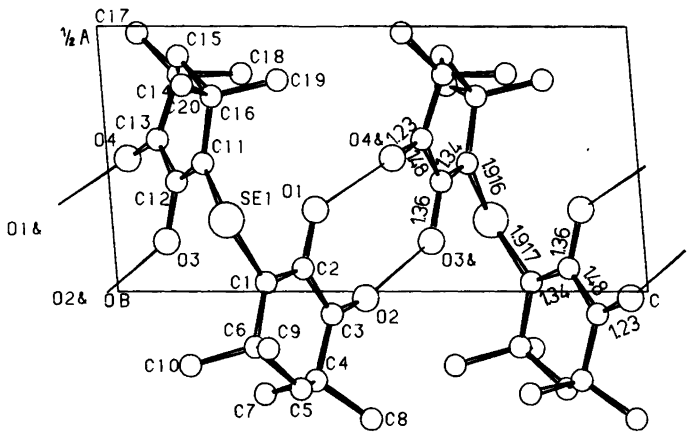

Fig. 3. Molecular packing diagram of *2*.

Table 2. Deviations (Å) of atoms from the least-squares planes.

Compound *1*

Plane through C2, C3, C5 and C6.

C2	C3	C5	C6	C1	C4	O1	O2	Se
−0.01	0.01	−0.01	−0.01	−0.09	−0.65	0.17	0.17	−0.29

Compound *2*

Plane through C1, C2, C3 and C6.

C1	C2	C3	C6	C4	C5	O1	O2
−0.03	0.03	−0.01	0.01	−0.21	0.42	0.09	0.03

Compound *2*[a]

Plane through C11, C12, C13 and C16.

C11	C12	C13	C16	C14	C15	O3	O4
0.02	−0.02	0.01	−0.01	0.35	−0.30	−0.07	−0.18

[a] The values for *2* have been calculated from coordinates previously published.[6]

enolized[3,8] and *1* appears as C_{2v} symmetric due to the rapid proton exchange on the NMR time scale.

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Letter

Nitration of Polycyclic Aromatic Hydrocarbons with Dinitrogen Tetroxide. A Simple and Selective Synthesis of Mononitro Derivatives

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Polycyclic aromatic hydrocarbons (PAH:s) are generally nitrated with HNO_3 in acetic acid or acetic anhydride.[1] Yields are normally good, but work-up procedures are often tedious and polynitration is sometimes encountered.[1,2] A few reports on the reaction between PAH:s and nitrogen oxides have appeared,[3,4] most recently 9-nitrophenanthrene being detected in the complex reaction mixture from the UV-irradation of a solution of N_2O_4 and phenanthrene in CCl_4.[3c] The exposure of some PAH:s to gaseous NO_2/N_2O_4 led to the formation of nitro PAH:s, some of which have been discussed in relation to the carcinogenic effects of automobile exhaust and tobacco smoke.[4,5] In this letter we present our initial studies on the reaction between some PAH:s and N_2O_4 in Cl_2Cl_2 solution. The reaction is very clean and rapid and provides, under mild

Table 1. Nitration of polycyclic aromatic hydrocarbons with dinitrogen tetroxide.

PAH	Catalytic amount of CH_3SO_3H added	Reaction time/h	Yield[a]/%	Isomer distribution[c]/%	
Perylene	No	0.2	95[b]	3-nitro	99.2
				1-	0.8
Pyrene	No	0.5	97[b]	1-	100
Anthracene	No	1	>90[c,d]	9-	100
				6-	97
Chrysene	No	24	>90[c]	other mononitro	3
Naphtalene	No	48	59[c]	1-	96
				2-	4
				2-	90
Fluorene	No	24	>90[c]	3-	1
	Yes	2	92[b]	4-	9
Fluoranthene	No	24	75[c]	3-	63
	Yes	0.4	90[c]	8-	27
				other mononitro	10
Binaphtyl	No	24	>90[c]	4-	100
	Yes	1	89[b]		
Triphenylene	No	120	50[c]	1-	22
	Yes	2	92[b]	2-	78

[a] Yield based on PAH; identity and purity confirmed by MS[e] and GLC.[f] [b] Isolated yield. [c] Determined by GLC. [d] 5–7 % of 9,10-anthraquinone was also formed. [e] Finnegan 4021 spectrometer operating at 70 eV. [f] HP 5380A gas chromatograph equipped with an HP 18850A integrator. 0.5 m×1.8 mm glass-lined column; 5 % OV 1701 on Chromosorb W.

0302-4369/83 $2.50

Table 2. Isomer distribution in the nitration of some PAH:s with HNO_3 in acetic anhydride and with N_2O_4 in CH_2Cl_2.

PAH	Fluorene			Triphenylene		Chrysene		Fluoranthene		
Isomers	2-	3-	4-	1-	2-	6-	other mono-nitro	3-	8-	other mono-nitro
Conditions for nitration										
HNO_3/Ac_2O [a]	69	2	29	54 [c]	46	90	10	44	27	29
HNO_3/Ac_2O [b]	71	1	28	55	45	89	11	45	26	29
N_2O_4/CH_2Cl_2	90	1	9	22	78	97	3	63	27	10

[a] Fluorene: 0 °C;[7a] triphenylene: 60 °C;[7b] chrysene: 0 °C;[7c] fluoranthene: 25 °C.[7d] [b] 0 °C, this work. [c] A value of 50:50±5 % has also been reported.[7e]

conditions, almost quantitative yields of mononitrated PAH:s after a simple work-up procedure that minimizes handling of these hazardous compounds.

We have earlier reported[6] that the reaction between N_2O_4 and naphthalene in CH_3CN or CH_2Cl_2 yields mononitronaphthalenes with a 1/2 ratio of 25 and that the reaction is acid catalyzed. During the continuation of these studies with more reactive substrates we found that perylene was very rapidly nitrated by N_2O_4 in nearly quantitative yield in the absence of any acid catalyst. We therefore decided to extend our studies to some other PAH:s (Table 1), and found that pyrene and anthracene were also rapidly nitrated without added acid while the other compounds in the study required longer reaction times and/or acid catalysis. The reaction shows high positional selectivity (Table 2), and in the case of triphenylene nitration takes place predominantly at the less hindered but less reactive 2-position (as does sulfonation, acylation and bromination), while nitration with HNO_3 in acetic anhydride gives a small excess of 1-nitrotriphenylene.[7b,e] Mechanistic studies on the reaction are in progress.

Experimental. Materials. The PAH:s used were of highest commercial quality available and used without further purification. Dichloromethane (Merck zur Rückstandsanalyse) was dried and stored over 3 Å molecular sieves. Solutions of N_2O_4 were made up as described previously.[6a]

Nitrations with N_2O_4. The PAH (2.5 mmol) in 125 ml CH_2Cl_2 and 2.7 mmol of N_2O_4 in 25 ml CH_2Cl_2 were mixed and allowed to stand at room temperature for the appropiate time. In some cases 0.5 mmol CH_3SO_3H was added. Most of the solvent was evaporated and 1 g of silica gel 60 (Merck, 230–400 mesh) was added. After completed evaporation the yellowish powder was placed on top of a column packed with silica gel and eluted with CCl_4 (containing up to 10 % CH_2Cl_2). Order of elution: Triphenylene, 1-, 2-; fluorene, 3-, 4-, 2-; perylene, 1-, 3-; fluoranthene, 1-, 7-, 3-, 8-; naphthalene, 1-, 2-;

Nitrations with HNO_3/Ac_2O. To the PAH (5 mmol) in 5 ml Ac_2O at 0 °C was added 0.33 ml of concentrated HNO_3 in 1.67 ml Ac_2O over 30 min. After another 30 min of stirring the reaction mixture was poured onto ice/CH_2Cl_2 and the organic layer washed with water and analyzed by GLC.

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Short Communications

Chemical Studies on Bryophytes. 23. ^{13}C NMR Analysis of a Biflavone from *Dicranum scoparium*

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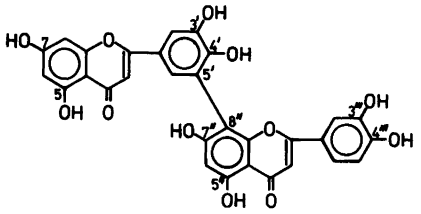
The isolation and identification of 5′,8″-biluteolin (*1*) from the moss *D. scoparium* has been reported earlier.[1] Most of the naturally occurring biflavonoids have carbon–carbon interflavonoid linkages. Their structures are difficult to determine, especially whether C–6 or C–8 is involved in the carbon–carbon linkage. In most cases, synthesis is necessary to confirm the structures.



^{13}C NMR spectroscopy has proved to be a superior method for the structure determination of biflavonoids.[2–5] The ^{13}C chemical shift values of the natural product 5′,8″-biluteolin (*1*), three synthetic biluteolin octamethyl ethers and two other related compounds are given in Tables 1 and 2.

The chemical shifts in Table 1 were assigned on the basis of proton noise-decoupled and off-resonance decoupled spectra and by comparison with the data published earlier for luteolin and carbon–carbon linked biflavonoids.[2–6] The presence of two signals at 181.9 and 182.3 ppm due to two flavone carbonyl carbon atoms, C–4 and C–4″, in the ^{13}C NMR spectrum of *1*, and the similarity of the remaining signals to the signals of luteolin indicated that *1* must be a dimer of luteolin.

In ^{13}C NMR spectra of 5,7-dihydroxyflavonoids, the signals for C–6 and C–8 are always found between 90 and 100 ppm, with the signal for C–6 found downfield from C–8.[2–8] Thus, the signal at 99.3 ppm is assigned to C–6 and C–6″, and that at 94.3 ppm to C–8. The absence of a signal in the range 90 to 96 ppm indicates that C–8″ is substituted. The C-arylation of C–6 or C–8 in 5,7-dihydroxyflavonoids shifts the signal of the corresponding aglycone carbon downfield by 4 to 10 ppm and does not markedly affect the signal of the other carbon.[2–5] Thus, the intense signal at 104.2 ppm, which is not split in the off-resonance decoupled spectrum, is assigned to C–8″. By comparison with luteolin, the signal at 104.2 ppm also represents the C–4a and C–4a″ carbons. The two signals at 103.5 and 103.0 ppm are assigned to C–3 and C–3″.

There are eight signals in the range 112.7 to 122.7 ppm. Five of these, at 112.7, 114.1, 116.1, 119.1 and 122.7 ppm, are split into doublets in the off-resonance decoupled spectrum, and are assigned to C–2′, C–2‴, C–5‴, C–6′ and C–6‴, respectively. Among the remaining three intense signals, at 120.6, 121.4 and 122.4 ppm, the lowfield signals are due to C–1′ and C–1‴. The signal at 120.6 ppm is assigned to the C–5′ carbon, which is shifted downfield by 4.5 ppm due to the substitution effect of the interflavonoid linkage.

The lowfield signals between 145.9 and 164.4 ppm due to oxygenated carbons have been assigned (see Table 1) by comparison with the signals in the spectrum of luteolin.

Permethylated *1* showed a more complicated spectrum than 5′,5‴- and 8,8″-biluteolin octamethyl ether, which both have very simple spectra owing to high symmetry. In the spectrum of 5′,5‴-biluteolin octamethyl ether the signal for C–5′ and C–5‴ has moved downfield to 132.2 ppm. Compared with that of luteolin 5,7,3′,4′-tetramethyl ether, this shift of 20.7 ppm is much larger than the corresponding shift of 5.9 ppm in 8,8″-biluteolin octamethyl ether and those reported previously in C–C linked biflavonoids [2–5]

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Table 1. ^{13}C NMR chemical shifts of biflavones and related compounds (ppm, TMS=0).

Compound	C-2 C-2″	C-3 C-3″	C-4 C-4″	C-5 C-5″	C-6 C-6″	C-7 C-7″	C-8 C-8″	C-8a C-8a″	C-4a C-4a″	C-1′ C-1‴	C-2′ C-2‴	C-3′ C-3‴	C-4′ C-4‴	C-5′ C-5‴	C-6′ C-6‴
Luteolin[6]	165.1	103.9	182.6	161.6	99.9	164.3	94.9	158.2	104.8	123.1	114.4	146.0	149.8	117.1	120.1
5′,8″-Biluteolin (*1*)	164.4	103.5[a]	182.3[b]	161.8[c]	99.3	164.4	94.3	157.7	104.2	121.4[d]	112.7	145.9	148.6	120.6[d]	119.1
	164.4	103.0[a]	181.9[b]	161.0	99.3	162.1[c]	104.2	155.0	104.2	122.4[d]	114.1	146.2	149.8	116.1	122.7
Luteolin 5,7,3′,4′-tetramethyl ether	160.1	107.0	175.6	159.6	93.3	163.5	96.1	159.1	108.2	123.0	109.1	148.9	151.5	111.5	119.2
5′,5‴-Biluteolin octamethyl ether	160.6	108.5	177.3	160.0	92.7	163.8	96.2	159.6	109.0	126.5	109.3	149.3	152.8	132.2	120.9
8,8″-Biluteolin octamethyl ether	161.0	106.8	177.7	161.6	91.6	164.8	102.0	160.4	107.7	123.4	107.7	148.8	151.4	111.0	118.9
5′,8″-Biluteolin	160.7[a]	108.1	177.3	160.1	92.8	163.9	96.2	159.6	109.1	–[e]	109.1	149.4	152.9	–[e]	122.4
octamethyl ether	160.9[a]	106.9	177.3	160.9[a]	91.6	164.6	–[e]	160.7[a]	107.7	–[e]	107.7	148.8	151.4	111.0	119.2

[a,b,c,d] Assignments bearing the same superscript in any spectrum may be reversed. [e] Chemical shifts not assigned due to the small amount of sample available.

Table 2. ^{13}C NMR chemical shifts of the methoxyl carbons.

Compound	Chemical shifts (ppm)
Luteolin 5,7,3′,4′-tetramethyl ether	55.7, 55.9, 55.9 and 55.9
5′,5‴-Biluteolin octamethyl ether	55.8, 56.0, 56.3 and 60.9
8,8″-Biluteolin octamethyl ether	55.8, 56.0, 56.1 and 56.5
5′,8″-Biluteolin octamethyl ether	55.4, 55.8, 56.0, 56.0, 56.0, 56.5, 56.5 and 60.9

and might be explained by steric effects.

In the spectrum of permethylated *1* the chemical shifts for the C–5′ and C–8″ carbons could not be observed due to the small amount of sample available, but the absence of signals corresponding to unsubstituted C–5′ and C–8″ confirmed the carbon–carbon linkage between these carbons.

The chemical shifts of the methoxyl carbons of the permethylated biflavones are shown in Table 2. Most of the chemical shifts of methoxyl carbons in flavones usually occur between 55.5 to 56.0 ppm.[8] In some cases the signals are shifted downfield due to steric crowding when the methoxyl group is *ortho*-disubstituted with bulky substituents.[9–13] Thus, the signal at 60.9 ppm in 5′,5‴- and 5′,8″-biluteolin octamethyl ether are assigned to the methoxyl groups in the 4′- and 4‴-position. No individual assignment is possible for the remaining methoxyl carbons.

Experimental. ^{13}C NMR spectra were recorded as described earlier.[14] Chemical shifts were referred to external TMS on the basis of the chemical shifts of DMSO-d_6 and $CDCl_3$ (39.5 and 77.0 ppm, respectively). Both proton noise-decoupled and off-resonance decoupled spectra have been recorded. The methyl ethers were measured in $CDCl_3$ (23 °C), and *1* in DMSO-d_6 (50 °C).

Isolation of 5′,8″-biluteolin (*1*) from the moss *D. scoparium*, syntheses of luteolin 5,7,3′,4′-tetramethyl ether, 5′,5‴-biluteolin octamethyl ether, 8,8″-biluteolin octamethyl ether and 5′,8″-biluteolin octamethyl ether were described earlier.[1]

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The Nitrous Acid Deamination of Methyl 2-Amino-2-deoxy-α-D-glucopyranoside, 2-Amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose and 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylamine in Glacial Acetic Acid

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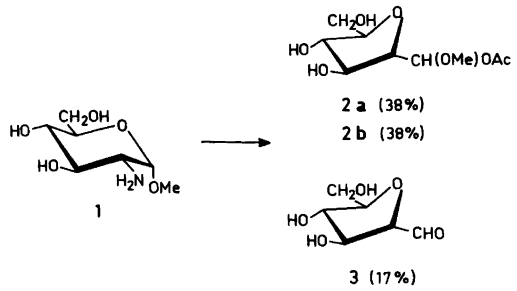
The deamination of carbohydrate amines has been reviewed by Williams.[1] In hexopyranoses, rearrangement by participation of an atom antiperiplanar to the nitrogen atom of the amine often predominates, especially when the amino group is equatorial. Most studies on deamination of carbohydrates have been performed in water or water-containing solvents.

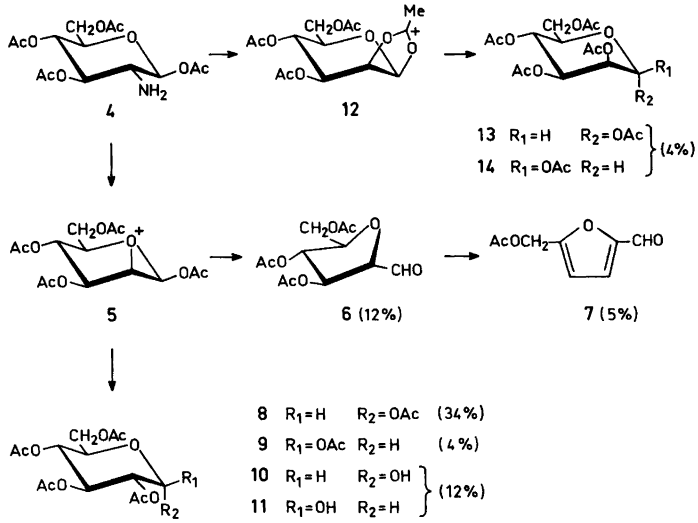
Nitrous acid deamination of polyfunctional amines usually results in complex mixtures owing to the high reactivity of the carbonium ion that is generated by heterolysis of the diazonium ion. Rearrangement reactions generally increase with the polarity of the solvent used.[2] We now report on the deamination of 2-amino-2-deoxy-D-glucose and D-glucosylamine derivatives in a non-aqueous solvent (anhydrous acetic acid).



Scheme 1. Treatment of methyl 2-amino-2-deoxy-α-D-glucopyranoside (*1*) with sodium nitrite in anhydrous acetic acid.

Treatment of methyl 2-amino-2-deoxy-α-D-glucopyranoside (*1*) with sodium nitrite in anhydrous acetic acid yielded the two anomers of 1-*O*-acetyl-2,5-anhydro-1-*O*-methyl-D-mannose (*2a* and *2b*) and 2,5-anhydro-D-mannose (*3*) in the proportions 2:2:1 (Scheme 1). No ring-contraction products involving participation of C-4 as observed for the corresponding reaction in water [3] were obtained. The formation of mixed acetals of *3* has been observed on brominolysis of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-iodo-α-D-glucopyranoside in acetic acid.[4]

Deamination of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (*4*) resulted in a more



Scheme 2. Deamination of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (*4*).

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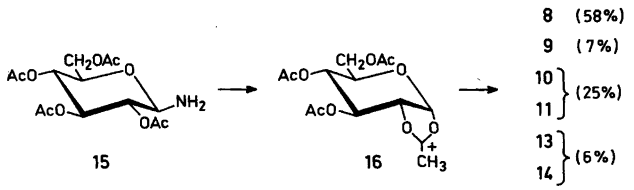

Scheme 3. Deamination of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (*15*).

complicated reaction mixture, which was investigated by ^{1}H NMR, GLC and sugar analysis. Part of the product was not deaminated, most probably because of acetyl migration to nitrogen. The main products were acetates of D-glucopyranose (*8*, *9*, *10* and *11*) and some mannopyranose derivatives (*13* and *14*). Ring-contracted products, namely 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose (*6*) and its product formed on elimination of acetic acid (*7*),[5] 5-(acetoxymethyl)-2-furaldehyde, were also formed. Yields are depicted within parentheses in Scheme 2. The formation of β-D-glucopyranose pentaacetate (*9*) may be accounted for by postulating an attack of acyl ion (or acetic acid) upon the oxonium ion *5*. The α-anomer (*8*) obtained in high yield is probably an anomerization product. Tetraacetates, with free hydroxyl group on C-1, (*10* and *11*) were also formed, probably by hydrolysis of *8* and *9* by water formed in the diazotation reaction. The formation of small amounts of D-mannopyranosyl pentaacetates indicates either a direct attack of acetyl ion (or acetic acid) upon the diazonium ion derived from *4* or on C-1 of the acetoxonium ion *12*.

The low yield of ring-contraction products on deamination of *4* is due both to the solvent and to the presence of an acetoxyl group on C-1. Similar results have been observed previously.[3]

On deamination of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (*15*) in anhydrous acetic acid, mainly acetates of D-glucopyranose (*8*, *9*, *10* and *11*) were formed probably *via* attack on C-1 of the diazonium ion derived from *15* or on the acetoxonium ion *16* (Scheme 3). The formation of small amounts of D-mannose derivatives (*13* and *14*) may be accounted for by an attack of acyl ion (or acetic acid) on C-2 of *16*.

Experimental. General methods. Melting points are uncorrected. Concentrations were carried out under reduced pressure at bath temperatures not exceeding 40 °C. NMR spectra were recorded in D_2O, $CDCl_3$ or acetone (d_6) at 30 °C using a JEOL FX 90 Q instrument. TLC and column chromatography were performed on silica gel F_{254} (Merck) and silica gel 60 (Merck), respectively. Optical rotations were determined with a Perkin Elmer 141 polarimeter. For GLC a Packard 427 instrument and glass capillary columns (25 m × 0.3 mm) coated with OV-225 were used.

Deamination of methyl 2-amino-2-deoxy-α-D-glucopyranoside (1). Compound *1* (800 mg) was dissolved in glacial acetic acid (80 ml) and sodium nitrite (3.0 g) was added gradually. The reaction mixture was stirred at room temperature for 1 h and filtered. The filtrate was evaporated to give a syrup (780 mg) which showed two major spots on TLC; the anomeric forms of 2,5-anhydro-1-acetyl-1-methoxy-D-mannose (*2a* and *2b*). Fractionation on a silica gel column (3 × 50 cm) irrigated with ethyl acetate – methanol (9:1 v/v) afforded pure fractions of *2a* (123 mg) and *2b* (82 mg) along with fractions containing a mixture of *2a* and *2b* (370 mg).

Compound *2a*, $[\alpha]_{578}^{20}$ +55.5° (*c* 0.93, CH_3OH), was a syrup which was homogeneous on TLC and GLC. ^{1}H NMR (acetone-d_6): δ 5.82 (d, 1 H, $J_{1,2}$ 5.9 Hz, H-1), 4.01 – 4.17 (m, 2 H, H-3, H-4), 3.80 – 3.93 (m, 2 H, H-2, H-5), 3.63 – 3.71 (m, 2 H, H-6, H-6'), 3.42 (s, 3 H, OMe) and 2.09 (s, 3 H, Ac). Acetylation of *2a* yielded a syrup (*2a'*), $[\alpha]_{578}^{20}$ +46.3° (*c* 0.52, $CHCl_3$), lit.[4] $[\alpha]_D$ +49.4°. ^{1}H NMR ($CDCl_3$): δ 5.90 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 5.36 (t, 1 H, $J_{2,3}$ 3.7 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.16 (t, 1 H, $J_{4,5}$ 2.8 Hz, H-4), 4.19 – 4.21 (m, 3 H, H-5, H-6, H-6'), 4.13 (dd, 1 H, H-2), 3.49 (s, 3 H, OMe), 2.13 (s, 3 H, Ac) and 2.09 (s, 9 H, 3 Ac).

Compound *2b*, $[\alpha]_{578}^{20}$ +33.2° (*c* 0.81, methanol) was obtained as a syrup and was homogeneous on TLC and GLC. ^{1}H NMR (acetone-d_6): δ 5.87 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.05 – 4.22 (m, 2 H, H-3, H-4), 3.80 – 3.98 (m, 2 H, H-2, H-5), 3.68 (m, 2 H, H-6, H-6'), 3.42 (s, 3 H, OMe), and 2.09 (s, 3 H, Ac). Acetylation of *2b* yielded *2b'* which was also obtained as a syrup and had $[\alpha]_{578}^{20}$ +41.1° (*c* 0.73, $CHCl_3$), lit.[4] $[\alpha]_D$ +46.3°. ^{1}H NMR ($CDCl_3$): δ 5.86 (d, 1 H, $J_{1,2}$ 5.9 Hz, H-1), 5.43 (t, 1 H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.12 (dd, 1 H, $J_{4,5}$ 4.8 Hz, H-4), 4.11 – 4.26 (m, 3 H, H-5, H-6, H-6'), 4.10 (dd, 1 H, H-2), 3.48 (s, 3 H, OMe), 2.12 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), and 2.09 (s, 6 H, 2 Ac).

Compound *1* (20 mg) was dissolved in glacial acetic acid (2.5 ml) and sodium nitrite (120 mg) was added. The reaction mixture was stirred for 1 h at room temperature. After evaporation to dryness

myo-inositol (3 mg) was added as internal standard and the reaction mixture was treated with pyridine and hydroxylamine hydrochloride followed by acetic anhydride. In this reaction *2a* and *2b* were acetylated and *3* transformed into the acetylated nitrile. The reaction mixture was evaporated and analyzed by GLC. The yield of *2a*, *2b* and 2,5-anhydro-D-mannose (*3*) was 38, 38 and 17%, respectively. The detector responses for acetylated *2a*, *2b* and *3* as nitrile were determined relative to *myo*-inositol and were found to be 0.55, 0.50 and 0.39, respectively.

Deamination of 2-amino-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (4). Compound *4* (40 mg) was dissolved in anhydrous acetic acid (6 ml) and sodium nitrite (250 mg) was added together with 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside as internal standard. The reaction mixture was stirred at room temperature for 1 h, then concentrated to dryness, extracted with chloroform (3 × 5 ml) and filtered. The filtrate was divided into three parts.

The first part was dissolved in $CDCl_3$ and analyzed by ^1H NMR spectroscopy. Doublets at δ 5.72 ($J_{1,2}$ 7 Hz) and δ 6.32 ($J_{1,2}$ 4 Hz) in the relative proportions 1:9 were attributed to the anomeric protons of *9* and *8*, respectively.

The first part was also analyzed by GLC showing α- and β-D-glucose pentaacetate (*8* and *9*, 34 and 4%, respectively) and 5-(acetoxymethyl)-2-furaldehyde (*7*, 5%).

The second part was concentrated to dryness and treated with an excess of sodium borohydride (20 mg) in aqueous methanol.[4] After 18 h at room temperature, the reaction mixture was treated with an excess of acetic acid. The reaction mixture was concentrated to dryness, codistilled with methanol (3 × 10 ml) and acetylated. GLC revealed the presence of 1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-mannitol (12%).

In the third part, the components were deacetylated.[6] Sugar analysis [7] revealed the presence of D-mannose (4%) and D-glucose (50%).

In the preparative experiment compound *4* (200 mg) was dissolved in glacial acetic acid (30 ml) and sodium nitrite (1.25 g) was added. The reaction mixture was stirred at room temperature (1 h), concentrated to dryness and extracted with chloroform (3 × 50 ml). The extract was filtered and concentrated to a small volume. Separation on a silica gel column (3 × 25 cm) with chloroform – methanol (20:1, v/v) yielded *7*, 10 mg. The H^1 NMR spectrum was in accordance with literature values. ^{13}C NMR ($CDCl_3$). δ: 177.75 (CHO), 170.27 ($CH_3C=O$), 152.96 (C-2), 121.32 (C-3), 112.49 (C-4), 155.48 (C-5), 57.80 (C-6), 20.61 ($CH_3C=O$). M.S. had *inter alia* peaks at *m/e* 43 (100), 79 (23), 97 (18), 109 (22), 126 (62) and 168 (2).

Deamination of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine (15). Compound *15* (40 mg) was deaminated in the presence of 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose (10 mg) and analyzed as described above. The reaction mixture contained 58% of *8* and 7% of *9*. Sugar analysis of the deacetylated reaction mixture yielded 6% D-mannose and 90% D-glucose, indicating the presence of 6% of *13* and *14* and 25% of *10* and *11* in the original reaction mixture.

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Simple Preparation of Azetidino-[1.2-*d*]benzodiazepines

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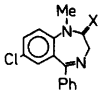
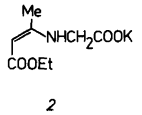
Both 1,4-benzodiazepines and condensed β-lactams are compounds of great interest. This prompted us to synthesize new derivatives having a tricyclic azetidino[1.2-*d*]benzodiazepine structure. The new heterocycle fused to the "*d*" face of the parent molecule [7] may cause an interesting influence on pharmacological activities as well as provide a new starting site for further chemical transformations.

The reaction between ketene or diketene and diazepam (*1a*) is reported to yield an oxazino-benzodiazepine adduct.[1] Several methods are known for the introduction of an amino-substituted β-lactam ring; a very useful one was discovered by Bose *et al.*[2] and Sharma *et al.*[3], namely the use of glycine Dane-salts:[4] *i.e.* on appropriate activation potassium α-methyl-β-ethoxycarbonylethenylaminoacetate (*2*) and a Schiff base give rise to the corresponding azetidinone. Phosphorus oxychloride was found useful as a reagent for activating *2*. Thus, if a mixture of diazepam and *2* was treated with $POCl_3$ in the presence of excess triethylamine, 54% of *3a* was isolated. In the case of medazepam (*1b*), the oily *3b* was obtained in about 70% yield and was converted directly to *3d*. When *4* was used as starting material, the yield of *5* was significantly lower; only some percent of the desired *5* could be isolated by thick-layer chromatography. In this case the 1-NH group presumably interfered with the active intermediates.



1a X = O
1b X = H_2



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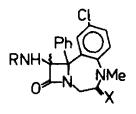
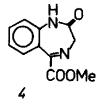


3a R =  X = O
3b R = X = H_2
3c R = TsOH·H- X = O
3d R = TsOH·H- X = H_2
3e R = $ClCH_2CO$- X = O



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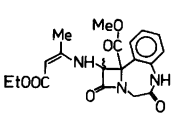

5

Trifluoroacetic anhydride could also be used [5] for the activation of *2*. In this method, *3a* resulted in a yield of only 18%.

Regeneration of the amino group was performed with *p*-toluenesulfonic acid.[6] Thus, *3c* was obtained in nearly quantitative yield as its *p*-toluenesulfonate salt, and *3d* in somewhat lower yield. *3c* was converted to its *N*-chloroacetyl derivative (*3e*) by standard acylation. Compounds of type *3* exhibit spectroscopic evidence of the β-lactam ring, *i.e.* in their ^1H NMR spectra the β-lactam proton appears at 5.1 – 5.7 ppm, as a doublet coupled ($J \sim 10$ Hz) to the NH proton. In the IR spectra the carbonyl absorption appears at 1750 – 1785 cm^{-1}.

We have also tried to add *N*-chloroacetylglycine to *1a* instead of the Dane-salt *2*, using $POCl_3$ and NEt_3, but only traces of *3e* could be observed. The preparation of similar compounds using differently substituted benzodiazepine compounds is in progress.

Experimental. General. Melting points were determined on a Kofler apparatus and are uncorrected. ^1H NMR spectra were obtained on a JEOL FX60 or Bruker WP 200 SY spectrometer with TMS as internal standard. IR spectra were recorded on a Perkin-Elmer 283B spectrometer, using KBr discs. TLC were run on precoated plates (Merck Silica Gel F_{254}) with toluene-ethyl acetate 1:1.

9-Chloro-4,5,6,10b-tetrahydro-1-(α-methyl-β-ethoxycarbonylethenyl)amino-6-methyl-10b-phenylazetidino[1,2-d][1,4]benzodiazepine-2,5-dione (3a). To a mixture of *2* (2.25 g), diazepam (1.4 g) and triethylamine (2.05 g) in dry CH_2Cl_2 (30 ml), $POCl_3$ (1.54 g) in CH_2Cl_2 (12 ml) was added dropwise, while the temperature was kept at 0 °C. After the addition the suspension was stirred overnight at room temperature and was then washed with water and 5% $NaHCO_3$ solution. After removal of the solvent, the resulting oil crystallized on standing. Recrystallization from $CHCl_3$ – light petroleum led to 1.2 g (54%) of the title compound, m.p. 185 – 186 °C. IR (KBr): 1766 (s), 1721 (s), 1662 (s), 1262 (s), 1167 (s) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 1.12 (3 H, t), 2.04 (3 H, s), 2.78 (3 H, s), 3.78 (H, d, J_1 14 Hz), 3.89 (2 H, q), 4.45 (H, d, J_1 14 Hz), 4.41 (H, s), 5.41 (H, d, J_2 10 Hz), 7.1 – 7.8 (8 H, m), 8.34 H, (d, J_2 10 Hz).

9-Chloro-4,5,6,10b-tetrahydro-1-(α-methyl-β-ethoxycarbonylethenyl)amino-6-methyl-10b-phenylazetidino[1,2-d][*1,4*]*benzodiazepine-2-one* (3b). Prepared as for *3a* yielding 2.2 g of thick oily crude product. Its ^1H NMR spectrum revealed two doublets, at 5.17 and 8.62 ppm (J 10.7 Hz), corre-

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sponding of the β-lactam and NH protons. The compound contained some free ethyl acetoacetate and was converted directly to *3d* without purification.

9-chloro-4,5,6,10b-tetrahydro-1-amino-6-methyl-10b-phenylazetidino[1,2-d][1,4]benzodiazepine-2,5-dione tosylate (3c). *3a* (1.02 g) and *p*-toluenesulfonic acid (0.47 g) were dissolved in acetone (20 ml) and 6 drops of water were added. The mixture was stirred overnight and next day the precipitate formed was collected and washed with CCl_4: 1.11 g (96.5 %). M.p. 238–239 °C (from MeOH – ether). Anal. $C_{25}H_{24}N_3O_5SCl$: N, Cl. IR (KBr): 1784 (br s), 1642 (s), 1484 (s), 1225 (br s), 1220 (s), 1009 (s) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_b): δ 2.33 (3 H, s), 2.41 (3 H, s), 4.02, 4.16 (2 H, ABq, *J* 15 Hz), 5.55 (H, s), 7.1–8.1 (12 H, m), 8.55 (H, br s).

9-Chloro-4,5,6,10b-tetrahydro-1-amino-6-methyl-10b-phenylazetidino[1,2-d][1,4]benzodiazepine-2-one tosylate (3d). Crude *3b* (4.4 g) was dissolved in acetone (75 ml) and H_2O (0.5 ml). *p*-Toluenesulfonic acid (1.9 g) was added and the gelatinous suspension formed was briefly warmed to ~60 °C, with vigorous stirring. It was allowed to react overnight at room temperature. The precipitate was collected, washed with a little CCl_4 and recrystallized from a minimum amount of hot acetone – MeOH, giving 2.3 g of off-white product, m.p. 184–186 °C. Anal. $C_{25}H_{26}N_3O_4SCl$: N, Cl. IR (KBr): 1747 (br s), 1600 (m), 1500 (s), 1178 (s), 1008 (s) cm^{-1}. ^1H NMR (200 MHz, CD_3OD): δ 2.35 (3 H, s), 2.74 (3 H, s), 2.8–4.0 (4 H, AA'BB'), 5.15 (H, s), 7.1–7.8 (12 H, m).

9-Chloro-4,5,6,10b-tetrahydro-1-chloroacetamido-6-methyl-10b-phenylazetidino[1,2-d][1,4]benzodiazepine-2,5-dione (3e). To a mixture of *3c* (0.51 g) and triethylamine (0.33 ml) in dry CH_2Cl_2 (30 ml), chloroacetyl chloride (0.25 g) in CH_2Cl_2 (3 ml) was added dropwise, and the mixture was stirred for 1 h and washed with brine, 5 % $NaHCO_3$ solution and 5 % H_2SO_4. Evaporation and recrystallization from acetone – ether yielded 0.32 g (78 %) of white material, m.p. 221–222 °C. IR (KBr): 1776 (s), 1678 (s), 1489 (m), 1412 (m), 1205 (m) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_b): δ 2.46 (3 H, s), 3.62, 3.84 (2 H, ABq, *J* 13 Hz), 3.94, 4.14 (2 H, ABq, *J* 13.3 Hz), 5.75 (H, d, *J* 8 Hz), 6.6–8.3 (8 H, m), 9.04 (H, d, *J* 8 Hz).

4,5,6,10b-Tetrahydro-1-(α-methyl-β-ethoxycarbonylethenyl)amino-10b-methoxycarbonylazetidino-[1,2-d][1,4]benzodiazepine-2,5-dione (5). This was prepared as for *3a* from 0.3 g of *4*. After work-up, the oily residue was purified by thick-layer chromatography (silica gel, twice benzene – ethyl acetate 1:1), yielding 17 mg of pure *5* (3.2 %), m.p. 177–179 °C. ^1H NMR (60 MHz, DMSO-d_6): 1.15 (3 H, t), 2.08 (3 H, s), 3.63 (3 H, s), 4.02 (2 H, q), 4.16 (2 H, br s), 4.65 (H, s), 5.81 (H, d, *J* 10.1 Hz), 6.7–7.9 (4 H, m), 9.0 (H, d, *J* 10.1 Hz), 10.05 (H, s).

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Preparation of 4-(2-Deuterio-2-propyl)anisole Through Cathodic Cleavage of a Sulfone in the Presence of Deuterium Oxide

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In connection with other work, the title compound was needed. Several attempts to prepare this acid-sensitive, α-deuterated isopropyl compound from 2-(4-methoxyphenyl)-2-propanol failed, except for reduction with a mixture of aluminium chloride and lithium tetradeuteridoaluminate, which gave a mediocre yield of the desired material.

It occurred to us that cathodic cleavage of a sulfone in a deuterium oxide-containing solvent might be a feasible procedure, since acidic conditions are avoided. The route illustrated in Scheme 1 was devised and found to furnish the title compound in an isotopic purity of 98.5 %.

Dialkylation of the known [1] *p*-methoxybenzyl *p*-tolyl sulfone with butyllithium and iodomethane was straightforward. Upon cathodic reduction of the dimethyl compound, a radical anion is initially formed. The latter undergoes fragmentation to a benzylic radical and a sulfinate anion. A second electron transfer to the radical yields a carbanion, which is rapidly protonated by the strongest acid present in the medium. We used a solution of 4 % deuterium oxide in anhydrous DMF (*N,N*-dimethylformamide), containing tetraethylammonium perchlorate (0.25 M).

The possibility that tetraethylammonium ion from the supporting electrolyte might compete as the proton donor was a matter of concern, since it would lead to inferior isotopic purity. A small but measurable participation by tetraethylammonium bromide has been reported [2] in the cathodic cleavage of iodobenzene in DMF solution containing ordinary or heavy water. It was suggested [2] that hydroxide (or deuteroxide) ions, formed in the catholyte upon electrolysis, cause Hofmann elimination, whereby protons are formed. In order to avoid this possibility, we performed the electrolysis at ice-bath temperature (5 °C) and interrupted it after 35 % extent of reaction, before too much base had accumulated. In this way, a material of sufficient isotopic purity was secured.

Experimental. GLC analyses were performed on a Perkin Elmer Model 900 instrument, NMR spectra on a Bruker WH 270 instrument, MS either on a GC-MS Finnigan 4021 or a VG analytical ZAB instrument. Voltammetry and preparative electrolyses were carried out as described previously.[3]

The reaction between sodium *p*-toluenesulfinate and *p*-anisyl alcohol in formic acid solution gave a quantitative yield of *p*-methoxybenzyl *p*-tolyl sulfone as reported.[1] A solution of 4 g (14.5 mmol) of this materal in 25 ml of dry THF was added to 14.5 mmol of butyl lithium (15 % solution in hexane) in 20 ml of dry THF at –10 °C during 15 min. After another 15 min at –10 °C, 0.95 ml (15 mmol) of iodomethane was added, and the mixture was allowed to attain room temperature. After 30 min, it was again cooled to –10 °C, and the treatment with butyl lithium and iodomethane was repeated. After evaporation of the solvent, the lithium iodide was removed by washing with water, and the residue was recrystallized from ethanol. The yield was 3.7 g (85 %), m.p. 120–121 °C, ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.76 (6 H, s), 2.37 (3 H, s), 3.80 (3 H, s), 6.79 (2 H, d, *J* 9 Hz), 7.13 (2 H, d, *J* 8 Hz), 7.24–7.28 (4 H, m). Anal.: *C, H, S.*

In order to determine a suitable electrolysis potential, a cyclic voltammogram at a hanging mercury drop electrode was recorded. At a sweep speed of 25 mV s^{-1}, an irreversible peak was obtained at –2.18 V (*vs.* SCE) in 0.1 *M* tetraethylammonium perchlorate in DMF. The

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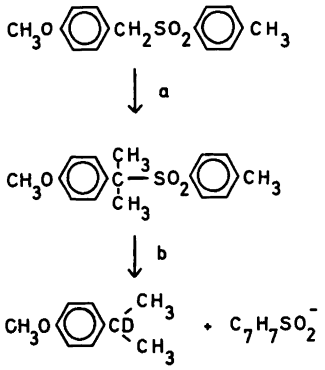

Scheme 1. Synthesis and cathodic cleavage of 2-(4-methoxyphenyl)-2-propyl 4-tolyl sulfone. Conditions: a. C_4H_9Li, CH_3I, then repeat; b. Hg cathode at –2.3 V *vs.* SCE, 0.25 *M* $(C_2H_5)_4NClO_4$ in DMF, 4 % D_2O.

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preparative electrolysis was performed at the cathode potential –2.3 V *vs.* SCE in an H-cell with 20 cm^2 mercury cathode surface. The catholyte was 100 ml 0.25 *M* tetraethylammonium perchlorate in DMF, containing 4 ml of deuterium oxide (Ciba-Geigy, 99.7 atom % D), and 3 g (10 mmol) of 2-(4-methoxyphenyl)-2-propyl 4-tolyl sulfone. The anolyte, separated by a Nafion® 125 cation exchange membrane, contained 0.25 *M* tetraethylammonium bromide so as to avoid formation of protons at the carbon anode. The cell was cooled in an ice-bath. After introduction of 700 As (the amount of charge necessary for cleavage of 35 % of the substrate), the run was interrupted (6 h). The catholyte was diluted with 300 ml of water and extracted with ether. The organic layer was dried over sodium sulfate, and the solvent was evaporated. A residue of 2.5 g, consisting of product and starting material in the approximate ratio 1:2, was obtained. Chromatography on a column of alumina with hexane as the eluent yielded 0.45 g (30 %) of 4-(2-deuterio-2-propyl)-anisole, pure according to GLC. ^1H NMR (270 MHz, $CDCl_3$): δ 1.21 (6 H, t, J 0.9 Hz), 3.78 (3 H, s), 6.85 (2 H, d, J 9 Hz), 7.14 (2 H, d, J 9 Hz). MS [IP 70 eV; m/e (% rel. int.)]: 151 (27.4), 136 (100), 106 (21.1), 92 (18.3), 78 (11.4), 51 (10). Mol. wt. obs. 151.10806, calc. for $C_{10}H_{13}DO$ 151.11074.

Through quantitative analysis the isotopic purity was determined at 98.5 %. The signal for the residual α protons was measured at high amplification.

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Modification of Carboxyl Groups Induces Self-association of Fibronectin *in vitro*

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Soluble fibronectin deposits *in vitro* and *in vivo* as fibrillar protein network. Fibrillar protein polymers form in fibronectin preparations during storage, and also when fibronectin interacts with polyamines [Vuento *et al.*, *Eur. J. Biochem*, *105* (1980) 33]. We have studied the self-association of fibronectin as a direct binding of ^{125}I-labelled fibronectin to fibronectin-Sepharose. This binding had an apparent dissociation constant of 10^{-11} M. It was enhanced by lowering pH to the protonation range of carboxyl groups, by chemically blocking carboxyl groups with carbodiimide, and by spermine at low ionic strength. The results support the view that deposition of fibronectin is essentially a self-assembly process, which could be initiated by modification of the molecular charge of the protein.

Fibronectin is a dimeric protein with a mol. wt. of 440 000 which is present in a concentration of 300 mg/l in blood plasma, but also as an insoluble (deposited) protein in the pericellular matrix of *in vitro* cultured cells and in connective tissue.[1–3] The origin of this dual existence could reside in molecular differences between the circulatory and the cell surface forms of the protein. This hypothesis is supported by some studies[4,5] showing distinct differences in the migration rates of the two forms in polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. However, the biochemical and physicochemical properties of circulatory and cell surface fibronectins are very similar.[6–9] Quite recently it has become obvious that the circulatory protein is capable of forming protein deposits indistinguishable from those observed in cell cultures. This deposition of soluble fibronectin has been demonstrated by immunochemical methods both *in vitro*[10] and *in vivo*.[11] The findings indicate that soluble and deposited fibronectins could have a common precursor protein. The subsequent deposition of fibronectin could be controlled by interactions of fibronectin with other molecules. Several interactions potentially important in this respect are already known. Fibronectin is capable of binding to collagen, fibrin, glycosaminoglycans and actin (reviewed in Ref. 3). It has been suggested[3] that the deposition of fibronectin might be affected by multiple interactions with some of these macromolecules.

We have earlier suggested that self-association of fibronectin could be an important factor in the deposition of this protein.[12] Electron microscopy studies have demonstrated the presence of protein filaments in the preparations of purified fibronectin and in fibronectin aggregates formed with polyamines.[12] These results indicate that the deposition of fibronectin in a polymeric form would not necessarily need a macromolecular matrix, but could proceed as a self-assembly process. In this paper we show that protonation or modification of the carboxyl groups of fibronectin enhances the self-association of the protein. The results support the view that post-translational modification of fibronectin is sufficient for its deposition.

MATERIALS AND METHODS

Fibronectin was purified from citrated human plasma as described previously.[13] The purity of the material was controlled by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate.[14] Concentrations of solutions of purified fibronectin were determined by photometry[15] assuming A_{280} (1 %; 1 cm)=12.8. The

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method of Lowry *et al.*[16] was used in other protein determinations. Fibronectin was labelled with ^{125}I by lactoperoxidase catalyzed iodination.[17] Details of the procedures used will be given elsewhere.[18] Proteins were coupled to CNBr-activated agarose gel as described.[18] Approximately 0.5–1 mg of protein was bound per ml of gel. Some of the experiments described below were carried out with immobilized proteins further modified chemically. In these instances a suspension of Sepharose-linked protein was treated with the modifying reagents which were then removed by washing the gel with buffer. The experiments involving modification of carboxyl groups were carried out with soluble fibronectin or with fibronectin coupled to Sepharose. Carboxyl groups were modified with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Sigma, St. Louis, MO., U.S.A.) at pH 4.75 in the presence of glycine ethyl ester.[19] Fibronectin was first dialyzed against 0.9% NaCl, or fibronectin-Sepharose was washed with this solution. Neutralized glycine ethyl ester was added, and pH was adjusted to 4.75. The mixture was slowly stirred on a magnetic stirrer. Carbodiimide was added to a final concentration of 0.1 M, and pH was kept at 4.75 by adding dilute HCl. After an appropriate incubation time (routinely 1 h was used), the reaction was stopped by dialysis against 10 mM Tris-HCl, pH 7.5/0.9% NaCl/0.02% NaN_3. The extent of the reaction was measured by analyzing the incorporation of radioactive ^{14}C-labelled glycine ethyl ester (glycine residue labelled, specific activity 1.65 GBq/mmol, New England Nuclear, Dreieich, Germany). In these experiments the total concentration of glycine ethyl ester was 20 mM to ensure a high enough specific radioactivity in the reaction mixture. In other experiments glycine ethyl ester was used at a concentration of 1 M. The concentration of fibronectin was initially 1 mg/ml.

The self-association of fibronectin was studied using a solid-phase radioassay. A detailed description of the assay will be published elsewhere.[18] Aliquots of Sepharose-linked proteins (20 μl) were incubated in plastic tubes (7 × 50 mm) with labelled fibronectin. The volume of the incubation mixture was 1.0 ml. Bovine serum albumin (Sigma) was added to buffers to a concentration of 5 mg/ml. This was necessary in order to prevent binding of labelled protein to tube walls. The tubes were mixed by end-over-end rotation. After incubation for 16–20 h the gel was sedimented by centrifugation (1000*g*, 1 min) and supernatant was carefully removed by suction. The gel was rapidly washed once with 1 ml of buffer. The tubes with their contents were counted in a scintillation counter. Initial experiments showed that the binding was reversible. We therefore used the method of Scatchard[20,21] for estimation of dissociation constants. Fibronectin used in these experiments was freshly prepared. The binding experiments were done within 1–2 days after radiolabelling.

Affinity chromatography was performed at room temperature using columns (3 ml) of fibronectin-Sepharose or albumin-Sepharose equilibrated with 10 mM Tris-HCl, pH 7.5/0.9% NaCl/0.02% NaN_3. Fifty ml of freshly prepared pooled human serum was passed through the columns, which were then extensively washed with the above buffer. Elution was achieved with 8 M urea in the above buffer. Eluted proteins were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate,[14] and transferred electrophoretically from polyacrylamide gel to nitrocellulose membrane[22] (Bio-Rad, Richmond, CA., U.S.A.). The

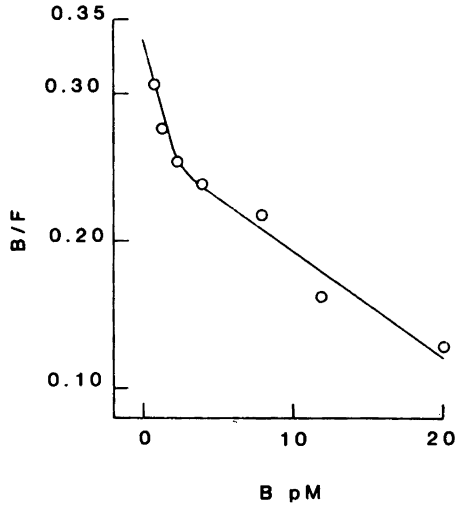

Fig. 1. Scatchard plot of binding of ^{125}I-labelled fibronectin to fibronectin-Sepharose. The ratio of the concentrations of bound and free labelled fibronectin (B/F) has been plotted against the concentration of bound labelled fibronectin (B). The linear portions of the curve fitted to the experimental points have been used to estimate dissociation constants *K*. The slope of such a line is equal to $-K^{-1}$. Abscissa intercept of the line is equal to the concentration of binding sites.

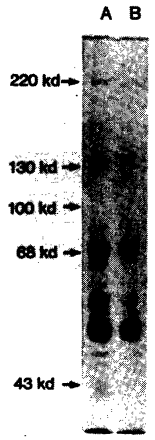

Fig. 2. Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. Protein material bound from serum to and eluted from fibronectin-Sepharose (A) or albumin-Sepharose (B) was analyzed after reduction with mercaptoethanol. Migration is from top to bottom. Migration positions of molecular weight markers are shown on the left. The gel was stained with Coomassie Blue.

protein bands were then visualized using predetermined dilution of anti-fibronectin antiserum[23] and peroxidase-conjugated antibodies against rabbit immunoglobulins[22] (Dako, Denmark).

RESULTS AND DISCUSSION

Previous studies using electron microscopy have demonstrated formation of filamentous protein polymers from purified fibronectin.[12] In accord with these findings, we demonstrate here a direct binding of labelled fibronectin to fibronectin-Sepharose (Fig. 1). In Fig. 1, the ratio of concentrations of bound and free ^{125}I-labelled fibronectin (B/F) has been plotted against concentration of bound ^{125}I-labelled fibronectin.[21] The curve shows deviation from linearity, indicating binding site heterogeneity.[21] The linear portions of the curve were used to estimate dissociation constants. The slope of the line is equal to $-K^{-1}$, K being the dissociation constant. Abscissa intercepts of the lines (not shown) are equal to the concentrations of binding sites. Such calculations indicated that the "high" affinity binding sites have an apparent dissociation constant of 3×10^{-11} M. The "low" affinity sites have an apparent dissociation constant of 1×10^{-10} M. This is actually a fairly high affinity. As the immobilization of fibronectin to solid phase may affect the binding and make the reaction mechanism complicated, the values obtained for dissociation constants should be taken as approximative.

Self-association of fibronectin was also observed in affinity chromatography experiments. Approximately 1 and 0.5 mg of protein were recovered from columns of fibronectin-Sepharose and albumin-Sepharose, respectively. Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate revealed two closely spaced polypeptide bands having mobility similar to that of fibronectin in the protein material eluted from fibronectin-Sepharose (Fig. 2). Immunoblotting using antibodies against fibronectin confirmed the presence of fibronectin in the material eluted from fibronectin-Sepharose column, while the material bound to and eluted from albumin-Sepharose did

Table 1. Effect of spermine and heparin on the binding of ^{125}I-labelled fibronectin to fibronectin-Sepharose. Experimental details are given in the Materials and Methods section. The diluent buffer used in these experiments was 5 mM Tris-HCl, pH 7.5, containing 5 mg/ml of bovine serum albumin. Concentration of fibronectin was 5 ng/ml. Results for albumin-Sepharose and plain Sepharose serve as controls. Abbreviation: n.d., not determined.

Reagent added		Binding of ^{125}I-labelled fibronectin to derivatized Sepharose expressed as % of total radioactivity		
		Fibronectin-Sepharose	Albumin-Sepharose	Sepharose
None		18.7	3.0	0.8
Spermine	0.1 mM	50.1	9.9	2.2
	1.0 mM	51.6	14.2	6.1
	10 mM	27.2	4.9	2.0
Heparin	1 μg/ml	18.6	n.d.	0.8
	20 μg/ml	2.6	n.d.	0.6

Fig. 3. Immunoblotting of the material eluted from fibronectin-Sepharose and albumin-Sepharose. Material eluted from fibronectin-Sepharose (A) or from albumin-Sepharose (B) were fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate, and then transferred electrophoretically from polyacrylamide gel to nitrocellulose membrane. Immunoreactive fibronectin was visualized on nitrocellulose membrane after reaction with anti-fibronectin antibodies (anti-FN) by using peroxidase-conjugated antibodies against rabbit immunoglobulins. Control experiment was performed using nonimmune rabbit serum (NRS) with subsequent staining with peroxidase-conjugated anti-immunoglobulin antibodies. Migration positions of molecular weight markers are shown on the left (arrows).

not seem to contain fibronectin (Fig. 3). Other investigators also have demonstrated fibronectin-fibronectin-interactions using affinity chromatography.[24]

Also in accordance with previous results [12] we observed an enhancement of the self-association of fibronectin by spermine at low ionic strength (Table 1). Interestingly, no enhancement but a clear inhibition was observed when heparin was included in the incubation buffer (Table 1). Because heparin has been shown to interact with fibronectin,[25] this result may indicate the involvement of the same fibronectin domain in self-assembly and in the interaction with heparin.

The binding of labelled fibronectin to fibronectin-Sepharose was strongly dependent on pH, as shown in Fig. 4. The optimum pH appeared to be between 4 −6. The results obtained with different buffers varied to some extent. Thus more extensive binding was observed in phosphate buffer than in citrate buffer. The reason for this behaviour is not known. Binding of labelled fibronectin to nonsubstituted Sepharose gel was not significant at any pH value tested (Fig. 4). Similar low binding (approximately 1% of total radioactivity) was observed with albumin-Sepharose (not shown). As the pK values

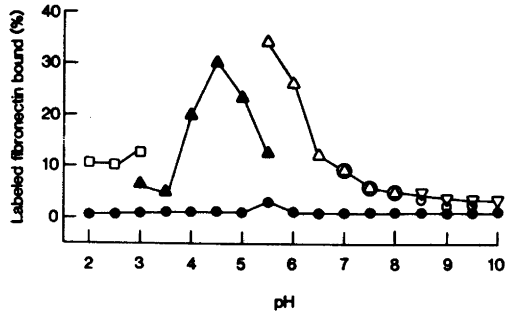

Fig. 4. Effect of pH on the binding of ^{125}I-labelled fibronectin to fibronectin-Sepharose. Aliquots of fibronectin-Sepharose (20 μl, 20 μg of fibronectin) were equilibrated with ^{125}I-labelled fibronectin (5 ng) for 16 h at room temperature in various buffers (1.15 ml). The concentration of the buffers was 50 mM. All buffers contained 5 mg/ml of bovine serum albumin. The buffer systems used were glycine-HCl, pH 2.0 − 3.0 (□), sodium citrate, pH 3.0 − 5.5 (▲), potassium phosphate, pH 5.5 − 8.0 (△), Tris-HCl, pH 7.0 − 9.5 (○), and glycine-NaOH, pH 8.5 − 10.0 (▽). Sepharose 4B gel was used as a control (●).

for dissociation of free carboxyl groups of glutamic acid and aspartic acid are in the pH range of 4 − 5 in proteins, the results suggest that protonation of some critical carboxyl groups enhances the self-association of fibronectin. Our previous studies have shown that fibronectin binds to carboxyl-modified proteins [18] and that such modified

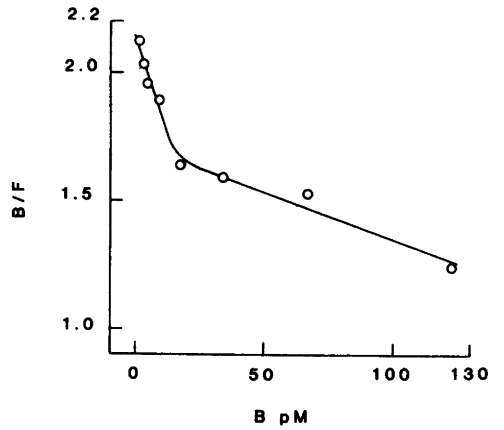

Fig. 5. Scatchard plot of binding of ^{125}I-labelled fibronectin to carboxyl-modified fibronectin-Sepharose. Experimental details are given in the Materials and Methods section and in the legend to Fig. 1.

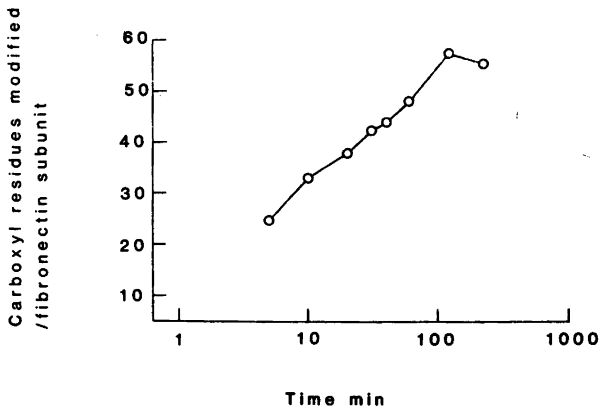

Fig. 6. Modification of carboxyl residues of fibronectin with carbodiimide. Modification of fibronectin (1 mg/ml) was carried out at pH 4.75 with 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in the presence of 20 mM glycine ethyl ester. The reaction mixture also contained 3.7 kBq/ml of ^{14}C-glycine ethyl ester. The reaction was stopped after indicated incubation times by dialysis against 10 mM Tris-HCl, pH 7.5 -0.9% NaCl -0.02% NaN_3. After an extensive dialysis, the protein concentration and radioactivity of the samples were determined. The extent of modification (residues modified per fibronectin subunit) was calculated using a mol. wt. of 220 000 for the fibronectin subunit.

proteins bind selectively fibronectin from serum.[18] In accordance with these findings, the binding of labelled fibronectin to carbodiimide-treated fibronectin-Sepharose was greatly enhanced as compared with binding to native fibronectin-Sepharose (Fig. 5). Carbodiimide-treatment of fibronectin gave rise to modification of up to 50 carboxyl residues per fibronectin subunit, as shown in Fig. 6. Scatchard analysis of binding data (Fig. 5) again suggested the presence of more than one type of binding sites on modified fibronectin-Sepharose, with apparent dissociation constants of 3×10^{-11} M ("high" affinity sites) and 3×10^{-10} M ("low" affinity sites). These values are similar to those obtained with native fibronectin-Sepharose (Fig. 1). Because the same parent fibronectin-Sepharose gel was used in both experiments, and because the volumes of the gels used in the experiments were known, we could calculate the apparent increase in the amount of high-affinity binding sites occurring during modification to be about 100-fold.

The present results are in agreement with the suggestion[12] that the deposition of fibronectin could be essentially a self-association process which would not necessarily need a pre-existing macromolecular matrix. Our results raise the possibility that such a self-association could be initiated by modification of the molecular charge of fibronectin.

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On the Accuracy and Significance in Determination of the Temperature Dependence of Activation Energy in Neutral Ester Hydrolysis and Solvolytic Substitution Reactions

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Various experimental methods (conductometry, spectrophotometry, titrimetry and polarimetry) employed for the accurate rate determinations required in the investigation of the temperature dependence of activation energy are discussed. A conductometric method which requires only simple apparatus in addition to an accurate conductometer and a reaction cell is described. The theoretical non-linearity between the degree of reaction and conductance, caused by the effect of ionic strength in the case of strong electrolytes and by the changes in the degree of dissociation of weaker electrolytes, are shown to lead only to minor errors in the rate parameters, provided that the initial ester concentration is sufficiently low.

The neutral hydrolysis of methyl trifluoroacetate in 7 different dimethyl sulfoxide – water mixtures at a wide temperature range have been studied. For $\Delta C_p^{\ddagger}$ a precision better than 10 J mol^{-1} K^{-1} is achievable and reproducible. Further, it is concluded that $\Delta C_p^{\ddagger}$, as the derivative of $\Delta H^{\ddagger}$, gives information, *e.g.*, about solvent effects not available from $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ alone.

It is well known that many solvolytic reactions do not exactly follow the Arrhenius equation. In this respect especially the S_N solvolyses of organic halides and sulfonates [1] and the neutral ester hydrolysis [2–6] have been thoroughly studied. Resently Albano and Wold criticized the methods employed in these studies, questioning their accuracy [7] and even their significance in general.[8] As our experience of ester hydrolysis, gained during two decennaries, gives evidence of sufficient accuracy in such measurements and because we believe that valuable new information, *e.g.*, on solvent structure can be obtained from them, we want to describe our experimental method, compare it with other methods, and consider the significance of such studies. In the following, we use the terminology of the transition-state theory and discuss the deviations from the Arrhenius equation in terms of the heat capacity of activation $\Delta C_p^{\ddagger} = \mathrm{d}\Delta H^{\ddagger}/\mathrm{d}T$ and its derivatives. In this connection we will not consider the question of whether they are caused by a true temperature dependence of activation energy or by a complex reaction mechanism nor how these phenomena are connected with solvent structure.

DETERMINATION OF RATE CONSTANTS

Conductometric method. The calculation of $\Delta C_p^{\ddagger}$ requires an accurate determination of rate constants over a large temperature range. Conductometry is by far the most commonly employed experimental method in this connection. The method requires that the conductance of the solution changes regularly during the reaction. This is the case in the hydrolysis of, *e.g.*, organic halides and sulfonates. Robertson *et al.*[1a,9] have developed an accurate conductometric method widely employed for these solvolyses. In the case of the neutral hydrolysis of carboxylic esters, the method can be applied only in the case of esters like 1-haloalkyl carboxylates or alkyl trihaloacetates when a sufficiently strong acid is produced in the reaction.[2,5,10,11]

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In theory, the conductance G is proportional to $c\Lambda$ and is not a precisely linear function of the concentration c, because for dilute solutions of strong electrolytes as given by eqn. (1), the limiting

$$\Lambda/\Lambda_o = 1-(\alpha^* + \beta^*/\Lambda_o)\sqrt{c} \quad (1)$$

equivalent conductance Λ_o depends on the solvent, electrolyte and temperature, and the coefficients α^* and β^* on the solvent and temperature.[12] If $(\alpha^* + \beta^*/\Lambda_o)\sqrt{c}$ is not $\ll 1$, an error is introduced into the rate constants calculated from conductances. On the basis of the available data for HCl in water [12] it can be estimated that the rate constants are less than 0.1 % too high, provided that the reaction is followed conductometrically as described in the Experimental section and that the initial ester concentration is $\leq 10^{-4}$ M. Further, the effect is quite similar at different temperatures and introduces to $\Delta C_p^{\ddagger}$ an error which is less than 0.5 J mol^{-1} K^{-1}.

In the case of weaker acids also the changes in dissociation must be taken into account. The hydrolysis of chloromethyl dichloroacetate, extensively studied by us, produces equal amounts of hydrochloric and dichloroacetic acids. From the dissociation constants of dichloroacetic acid in water at different temperatures [13] it can be calculated that its degree of dissociation varies between 0.999 and 0.997 during the reaction if the initial ester concentration is 10^{-4} M. With the aid of synthetic model runs, it can be estimated that the errors introduced to the rate constants and $\Delta C_p^{\ddagger}$ values are less than 0.1 % and 0.5 J mol^{-1} K^{-1}, respectively. Trihaloacetic acids formed in the hydrolysis of alkyl trihaloacetates are moderately strong acids and thus almost wholly dissociated in dilute solutions. *E.g.*, from the ionization constant 0.23 for trichloroacetic acid in water at 298 K the value 0.9996 is obtained for its degree of dissociation in a 10^{-4} M solution. In accordance with the consideration by Winter and Scott,[14] it can be concluded that also in this case the small variation in the degree of dissociation during the reaction does not affect the calculated rate parameters, including $\Delta C_p^{\ddagger}$.

Although the use of sufficiently low concentrations of the reactant (10^{-4} M or below) usually precludes significant errors, it is possible that deviations from linearity between concentration and conductivity cannot be avoided, *e.g.*, in the case of weaker acids. The use of a calibration procedure is then possible,[10,11] but it makes the measurements more elaborate and may decrease the accuracy essentially. Our conductometric method, based on the use of low concentrations and an accurate conductometer, is described in the Experimental section.

According to Albano and Wold [7] an important objection against the conductometric method is that "in order to get good results, the experimental solution must be buffered with salt to give a salt concentration which is almost constant during the reaction". However, the above considerations show that good results will be achieved by using sufficiently low substrate concentrations without salt buffering. Robertson *et al.*[1a,15] have used salt solutions as backing electrolytes but the concentrations they used were only from 0.0001 to 0.003 M. In our case the only "salt buffering" has been caused by the electrolyte produced in the reaction and left in the cell after most of the solution was poured out without flushing, because in our experience a high salt concentration only decreases the accuracy. In their attempts to show the disadvantages of salt buffering, Albano and Wold employed a salt concentration of 0.2 M.[7] Such buffering with salt really changes the structure of the solvent and thus makes the interpretation of the activation parameters obscure.

Spectrophotometric method. Spectrophotometric methods have only seldom been employed in $\Delta C_p^{\ddagger}$ determinations,[16,17] evidently because accurate temperature control over a large temperature range is not easily achieved. If the substrate has a good chromophoric group, as vinyl and phenyl esters have, this method is useful although less accurate than the conductometric method; also similar initial concentrations can be used.

Titrimetric method. Analysis by titrating samples, taken at intervals from the reaction mixtures, has been employed by us for sufficiently slow reactions and was found to produce $\Delta C_p^{\ddagger}$ values which compare well with those obtained by other methods. Especially argentometric titration of halide ions from halomethyl esters was found to be useful.[18]

Polarimetric method. Albano and Wold [7] employed a polarimetric method, which in principle has many advantages although its use is limited to optically active compounds and seems to require a high initial substrate concentration (0.2 M) which inevitably leads to major changes in the medium during the reaction.

Differential method. Albery and Robinson [19]

developed a differential method of measuring small differences in reaction rate which allows the variation of $\Delta H^{\neq}$ with T to be measured. Thus, values of $\Delta H^{\neq}$ could be found at different temperatures directly and $\Delta C_p^{\neq}$ could be obtained more accurately. This method was applied both in spectrophotometry and conductometry. The method seems not to have been applied later to $\Delta C_p^{\neq}$ determinations.

Calculation of rate constants. A prerequisite for accurate rate constants is that the kinetic form is known and that the rate equation is strictly obeyed. Solvolytic reactions mostly follow first-order kinetics, although, *e.g.*, the common ion effect in S_N1 solvolyses may lead to exceptions from it. Most often the integrated first-order eqn. (2) is used in the

$$k_t = \frac{1}{t} \ln \frac{c_0}{c_t} = \frac{1}{t} \ln \frac{y_0 - y_\infty}{y_t - y_\infty} \qquad (2)$$

calculation of the rate constants. Here y can be any quantity directly proportional to the concentration c of the reactant or product. Because y_0 and y_∞ are included in every k_t value and are often the most inaccurate y values (*e.g.*, in the case of conductometry, adsorption or desorption may cause some drift in longer time periods thus effecting an error mostly in y_∞), their elimination is preferable. Adams and Sheppard[20] recommend non-linear least-squares fitting of data to an equation of the form $y = A - B\ e^{-kt}$. We have preferred Guggenheim's method,[21] when also the possible errors in rate constants caused by the non-linearity between conductance and concentration are partly compensated. However, systematic errors may be masked in the calculated k value and lead to a too low error estimate. Therefore we always draw a large-scale plot of $\ln(y_{t+\Delta} - y_t)$ *vs.* t, if the calculated standard deviation of k exceeds 0.05%, and the run in question is rejected if any curvature can be seen. Albano and Wold[7] have employed a "local determination of rate constants" using spline functions, but also it suffers from the fact that every k_t includes y_∞ (see eqn. (4) in Ref. 7).

If there is any drift in the calculated rate constants, *e.g.*, due to catalysis or retardation by reaction products, extrapolations are sometimes performed. They may cause diminished accuracy and even give erroneous rate constants. The extrapolations should be performed as similarly as possible for all of the constants to be compared with each other. When the temperature dependence is studied, the extrapolation must be done from the same stages of the reaction, not from the same time intervals at different temperatures as Albano and Wold[7] did, thus probably getting uncomparable and inaccurate k_0 values.

EXPERIMENTAL

Apparatus. Conductances were measured with a Beckman RC-18 Conductometer. The readings of conductances (G) in μS are indicated directly on decade dials and the accuracy is $\pm 0.05\%$ of the reading. The advantage of the conductometer used by us is its great accuracy, but it has the disadvantage that the compensation of capacitance changes and the balancing of the bridge must be made by hand. Thus very fast reactions ($t_{1/2} < 40$ s) cannot be measured. The times were measured with a Casio CQ-2 digital clock. The time accuracy was 0.1 s.

Since the aim of our work is to determine the temperature dependence of the rate with a great accuracy, the temperature stability and its measurement during the reaction are very important. At reaction temperatures from 273 K to 293 K the thermostat used was Lauda GP S15/17 and the bath was filled with glycol – water. The cooling was done by Lauda DLK 15. At higher temperatures the thermostat was Heto 02 PT 623 and the cooling, if necessary, was done with thermostated water; the bath was filled with water. The temperature was stable to about 0.01 K. Temperatures were measured with mercury thermometers graduated in hundredths of a degree and calibrated with a Hewlett-Packard quartz crystal thermometer.

Conductivity cell. The cell was a "glass bottle" with platinum electrodes (Fig. 1). The cell constant was about 1. Before use, electrocleaning was accomplished with HCl solution. Then the electrodes were platinized with a solution of hexachloroplatinic acid and lead acetate in distilled water so that the electrodes looked velvety black. Thereafter the reaction cell was treated with water or with the water – organic cosolvent mixture in which the ester was hydrolysed, to produce a similar concentration of ions as in the kinetic runs. When the cell was properly accomodated to reaction conditions it could be used repeatedly for the same ester and solvent composition without any salt buffering. The cell was never allowed to dry, and between the kinetic experiments the reaction solution was left in the cell.

Performance of kinetic measurements. Before beginning the reaction, 15 ml of the solvent was placed in the reaction vessel A (see Fig. 1) from where it was pumped into the cell B till it was full.

Table 1. Example of experimental data for the neutral hydrolysis of methyl trifluoroacetate in a dimethyl sulfoxide–water mixture with the mole fraction 0.973 of water at 298.19 K.

t/s	$G_t/\mu S$	$G_{t+\Delta}/\mu S$ [a]	t/s	$G_t/\mu S$	$G_{t+\Delta}/\mu S$
0.0	30.00	82.77	88.4	58.00	86.40
4.9	32.00	83.02	97.4	60.00	86.66
9.9	34.00	83.28	107.0	62.00	86.92
14.9	36.00	83.54	117.4	64.00	87.18
20.2	38.00	83.80	128.4	66.00	87.44
25.8	40.00	84.06	140.5	68.00	87.70
31.5	42.00	84.32	153.7	70.00	87.96
37.5	44.00	84.58	168.3	72.00	88.22
43.9	46.00	84.85	184.4	74.00	88.48
50.4	48.00	85.11	202.7	76.00	88.74
57.2	50.00	85.37	223.7	78.00	89.00
64.5	52.00	85.63	248.1	80.00	89.26
72.1	54.00	85.89	277.8	82.00	89.51
80.0	56.00	86.15			

[a] $\Delta = 290$ s; $k = (7.018 \pm 0.002) \times 10^{-3}$ s^{-1}.

The solvent was allowed to thermostate for 30 min at the reaction temperature and then sucked back into A. The neat ester or, in the case of sparingly soluble or highly volatile esters, its about 10% solution in acetonitrile or methanol was injected with a microsyringe into the reaction vessel below the surface of the solvent so that the initial ester concentration was about 10^{-4} M or less. The solution was then shaken thoroughly and pumped to and fro between A and B. In the case of slightly soluble esters shaking and pumping were repeated. Finally B was filled with the reaction mixture. To

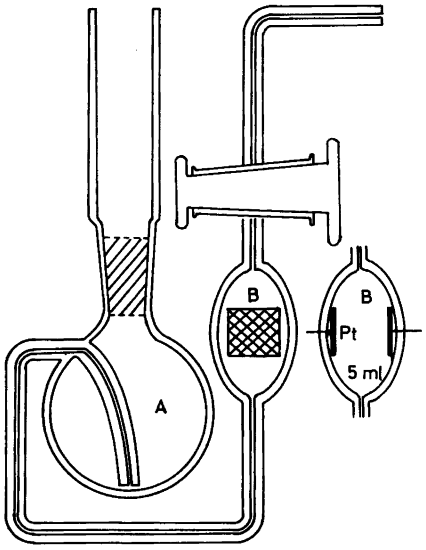

Fig. 1. The cell used in conductometric measurements. A is the reaction vessel and B the cell with platinum electrodes. Parts A and B are connected with a capillary tube so that diffusion is at minimum during the reaction.

Table 2. First order rate constants k for the neutral hydrolysis of methyl trifluoroacetate in a dimethyl sulfoxide–water mixture with the mole fraction 0.973 of water.

T/K	$10^3\ k/s^{-1}$
273.22	1.3252±0.0006
275.69	1.6106±0.0004
278.16	1.9317±0.0010
280.70	2.3261±0.0007
283.22	2.7558±0.0005
283.32	2.7785±0.0004
285.44	3.2124±0.0011
288.14	3.8269±0.0010
290.61	4.493 ±0.002
293.14	5.226 ±0.002
295.81	6.121 ±0.003
298.19	7.018 ±0.002
300.78	8.108 ±0.005
303.13	9.196 ±0.007
305.73	10.576 ±0.007
308.17	11.950 ±0.005
310.77	13.631 ±0.009
313.16	15.318 ±0.008
315.68	17.200 ±0.007

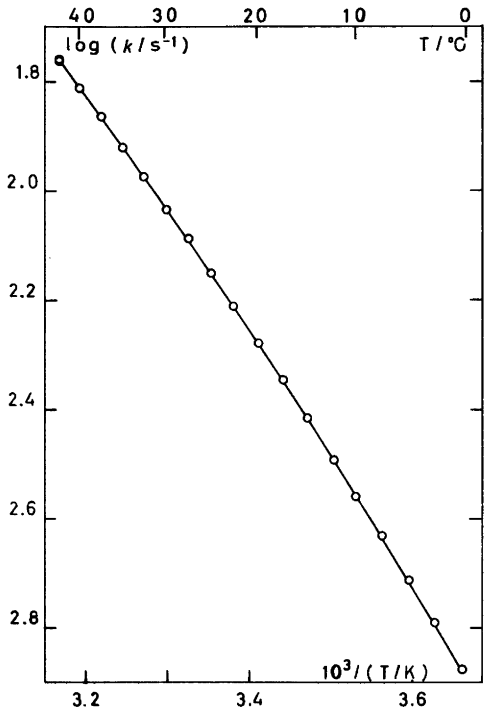

Fig. 2. Arrhenius plot for the neutral hydrolysis of methyl trifluoroacetate in a dimethyl sulfoxide –water mixture with the mole fraction 0.973 of water.

confirm the highest possible similarity between runs at different temperatures the conductance measurements were begun after about one half of the half-life of the reaction. The measurements were arranged for Guggenheim's calculation method [21] so that the first period was 2.5 to 3 times the half-life of the reaction ($=\Delta$) and the successive conductance changes were constant. In the second period the timetable of the first period was followed as seen from the example presented in Table 1. The rate constants were calculated by the method of least squares, the error estimates being the standard deviations of k. The above-mentioned theoretical and other possible systematic errors are, of course, not included in this estimate.

Materials. Methyl trifluoroacetate (zur Synthese, E. Merck AG.) was distilled before use. Dimethyl sulfoxide (zur Analyse, E. Merck AG.) was a commercial product, which was used without further purification. The solvent mixtures were prepared by diluting a known weight of distilled water with dimethyl sulfoxide to a known volume in a volumetric flask at 293 K and the resulting solvent mixture was weighed.

Experimental data. The first-order rate constants for the hydrolysis of methyl trifluoroacetate in a dimethyl sulfoxide–water mixture are given in Table 2. It is seen that the standard deviations of the calculated rate constants vary from 0.014 to 0.076%. In general in our experiments they are found to be less than 0.05% but sometimes at higher temperatures about 0.1%.

CALCULATION AND ACCURACY OF $\Delta C_p^{\ddagger}$

When the deviations from the Arrhenius equation are studied it is customary to measure accurate rate constants over as large a temperature range as possible at a high number of temperatures. From an Arrhenius plot one can control the regularity of the experimental points (as an example, see Fig. 2). Then an appropriate equation is fitted to the data employing a suitable calculation method, *e.g.*, an extended Arrhenius equation,[2] eqn. (3), after

$$\ln k = A + B/T + C \ln T + DT + ET^2 + \ldots + \varepsilon \qquad (3)$$

orthogonalization by the method of Clarke and Glew.[22] By studying the standard errors and residuals the suitability of the equation and the required number of parameters can be tested (*cf.* Table 3).

Albano and Wold [7] measured the rate constants at 3 temperatures only. As there are then no degrees of freedom, the calculated value of $\Delta C_p^{\ddagger}$ is questionable and no true estimation of its accuracy is possible. Adams [23] has described a technique for assessing the stability of $\Delta C_p^{\ddagger}$ and $d\Delta C_p^{\ddagger}/dT$.

Blandamer *et al.*[24] have examined the temperature dependence of $\Delta C_p^{\ddagger}$ using a "new" equation, which is, in fact, the basic equation of the method of Clarke and Glew.[2,22] By using in succession every experimental temperature as fixed, the equation was "capable of correctly sensing changes in $\Delta C_p^{\ddagger}$ with temperature". We fitted some synthetic data, calculated from eqn. (3) with 4 appropriately selected parameters, to their equation. A representative case is presented in Fig. 3. It can be seen, as already stated by Blandamer *et al.*,[24] that the method underestimates the true value of the temperature effect on $\Delta C_p^{\ddagger}$, but in some cases it may be useful, although only of limited value. Being a linear equation, it is much simpler to use than eqn. (3) with 4 or 5 parameters. Because the error of $\Delta C_p^{\ddagger}$ calculated by the new method [24] arises

Table 3. Temperature range (K), number of kinetic runs, calculated values of k (s^{-1}), activation parameters ΔG^* (J mol^{-1}), ΔH^* (J mol^{-1}), ΔS^* (J mol^{-1} K^{-1}), ΔC_p^* (J mol^{-1} K^{-1}), and first two derivatives of the heat capacity of activation, $d\Delta C_p^*/dT$ (J mol^{-1} K^{-2}) and $d^2\Delta C_p^*/dT^2$ (J mol^{-1} K^{-3}) at 298.15 K in neutral hydrolysis of methyl trifluoroacetate in dimethyl sulfoxide–water mixture at the mole fraction x_{H_2O} of water.

A. (The present work)

x_{H_2O}	0.984	0.973	0.951	0.920	0.849	0.790	0.681
Temperature range	273–316	273–316	273–321	273–338	273–338	273–338	273–338
Number of runs	25	19	22	30	27	28	35
10^3 k	7.588	7.010	6.049	4.831	2.750	1.571	0.450
ΔG^*	85120	85330	85680	86250	87650	89040	92130
ΔH^*	39590(20)	39560(40)	39020(40)	38840(40)	37770(30)	37370(40)	37990(50)
$-\Delta S^*$	152.7(1)	153.5(1)	156.5(1)	159.0(1)	167.3(1)	173.3(1)	181.6(2)
$-\Delta C_p^*$	245(4)	249(6)	272(6)	257(3)	233(3)	205(4)	179(4)
$d\Delta C_p^*/dT$	0.4(10)	4.5(11)	0.2(14)	−0.7(6)	−0.3(5)	0.2(7)	−0.7(7)
$d^2\Delta C_p^*/dT^2$	−0.2(4)	−0.2(4)	−1.0(4)	0.04(15)	0.1(1)	−0.2(2)	0.3(2)

B. (Cleve[25])

x_{H_2O}			0.950	0.920		0.790	0.681
Temperature range			268–328	268–338		268–348	268–348
Number of runs			9	12		18	12
10^3 k			6.04	4.83		1.57	0.468
ΔG^*			85700	86230		89050	92030
ΔH^*			39160(70)	38760(80)		37770(170)	38330(70)
$-\Delta S^*$			156.1(2)	159.2(3)		172.0(6)	180.1(3)
$-\Delta C_p^*$			270(8)	264(8)		229(15)	183(6)

mainly from the use of the "wrong equation", it cannot be a true measure of the precision of ΔC_p^* as claimed by Albano and Wold.[7]

The calculated kinetic parameters for our experimental case are given in Table 3A. For the sake of comparison the corresponding data[25] obtained about ten years ago are given in Table 3B. It is seen that the independent new results obtained by the improved conductometric method are in good agreement with the older ones (differences in ΔC_p^* 2 to 7 J mol^{-1} K^{-1}), except in the mol fraction 0.790 of water. This small disagreement (difference in the ΔC_p^* values is 24 J mol^{-1} K^{-1}, sum of their standard deviations being 19 J mol^{-1} K^{-1}) may arise from the fact that the older data include two k values at 280.09 K which deviate more than usual (3%). Further, it is seen from Table 3 that the values of ΔC_p^* differ from zero at a very high level of probability (>0.999999 according to Student's t-test). On the other hand, only in one case $d\Delta C_p^*/dT$

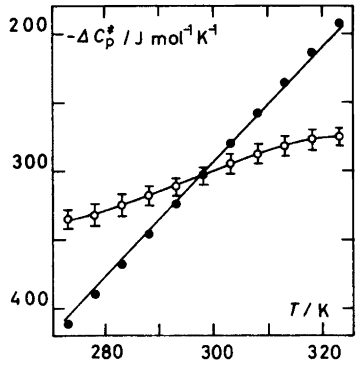

Fig. 3. Plots of ΔC_p^* *vs.* T for synthetic data ($\Delta C_p^* = -293$ J mol^{-1} K^{-1} at 298.15 K, $d\Delta C_p^*/dT = 4.184$ J mol^{-1} K^{-2}; straight line). ○ Calculated with the aid of eqn. (7) in Ref. 24; standard deviations indicated by bars. ● Calculated by the method of Clarke and Glew[22] with 4 parameters.

differs from zero at an 80% level. Therefore eqn. (3) with three parameters A, B and C, as modified by Clarke and Glew,[22] was used. This situation is typical also in other cases studied by us. Another comparison of independent $\Delta C_p^{\ddagger}$ determinations can be obtained from the neutral hydrolysis of ethyl trichloroacetate in water: Kurz and Ehrhardt [6] got the value -230 ± 4 J mol^{-1} K^{-1}, which compares well with the value -238 ± 17 previously determined by Cleve.[26]

DISCUSSION

We have developed a conductometric method which requires only simple apparatus in addition to an accurate conductometer or conductivity bridge. As manual operations are needed, the measurements are time-consuming and in fast reactions two operators are required. The rate coefficients calculated by Guggenheim's method are accurate, the standard deviation of k being about 0.05%. As usual in kinetic measurements, the reproducibility of k is lower, in extreme cases parallel runs may differ by 0.5%. The method compares, however, well with other conductometric methods and evidently is superior to other available kinetic methods for solvolytic reactions.

In our opinion, the best method for the calculation of the heat capacity of activation is that described by Clarke and Glew.[22] It has been recently criticized,[24] but in principle it is entirely correct, if the reaction rate is controlled by one step. The numerical difficulties sometimes met have their basis in the fact that the small deviations from the Arrhenius equation (*cf.* Fig. 2), in an inevitable limited temperature range, do not allow accurate determination of too many parameters whatever the functional form is. Because experimental data always have random and often also systematic errors, the only way is to extend the temperature range and to make measurements at a high number of temperatures. Neutral ester hydrolysis is an advantageous object because of its low activation energy.

Our experience has led to the impression that for $\Delta C_p^{\ddagger}$ a precision better than 10 J mol^{-1} K^{-1} (*ca.* 2 cal mol^{-1} K^{-1}) is achievable and reproducible. This is comparable with the accuracy obtained by Robertson *et al.* It is therefore sure that $\Delta C_p^{\ddagger} \neq 0$ for most solvolytic reactions and that the effects of temperature, structure of the reactant and compositon of solvent mixture on $\Delta C_p^{\ddagger}$ can be considered. Although the above discussion is in terms of $\Delta C_p^{\ddagger}$, it is possible, on the basis of more complicated mechanistic models, to explain the found deviations from the Arrhenius equation without assuming that the activation energy of any single step depends on temperature.[27]

Albano and Wold [8] have performed a multivariate data analysis of $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ for solvolysis reactions in H_2O and D_2O and investigated the possibility to substitute $\Delta C_p^{\ddagger}$ by parameters derived from them. They claim that it is sufficient to use only $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ from runs in water to get the same information as from $\Delta C_p^{\ddagger}$. Furthermore, they say that $\Delta G^{\ddagger}+\Delta H^{\ddagger}$ is correlated to $\Delta C_p^{\ddagger}$ and "that it is unnecessary to use the parameter $\Delta C_p^{\ddagger}$ to probe the solvent participation in hydrolysis reactions". Because of our interest in solvent effects, we have studied whether similar correlations could be found in these, too (Fig. 4). No simple correlation can be seen from the plots. It must also be noted that activation parameters have strongly different temperature dependences and therefore such plots will be of

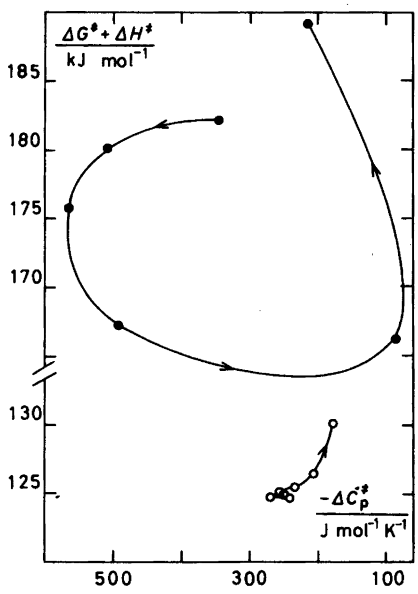

Fig. 4. Plots of $\Delta G^{\ddagger}+\Delta H^{\ddagger}$ *vs.* $\Delta C_p^{\ddagger}$ for the neutral hydrolysis of methyl trifluoroacetate in dimethyl sulfoxide–water mixtures with the mole fraction of water, x_{H_2O}, from 0.984 to 0.684 at 298.15 K (○) and for the solvolysis of *tert*-butyl chloride [9] in isopropyl alcohol–water mixtures with x_{H_2O} from 1 to 0.80 at 283.15 K (●).

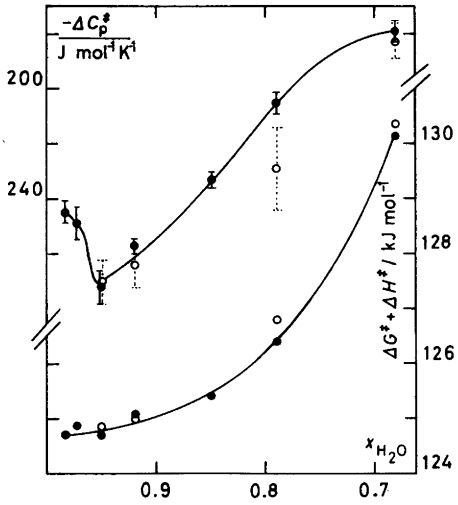

Fig. 5. Plots of $\Delta C_p^{\ddagger}$ and $\Delta G^{\ddagger}+\Delta H^{\ddagger}$ *vs.* the mole fraction of water, x_{H_2O}, for the neutral hydrolysis of methyl trifluoroacetate in dimethyl sulfoxide – water mixtures at 298.15 K: ○ Cleve,[25] ● this work.

variable complexity at different temperatures.[28] We therefore conclude that $\Delta C_p^{\ddagger}$ as the derivative of $\Delta H^{\ddagger}$ gives information that is not available from $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ alone and we are convinced that $\Delta C_p^{\ddagger}$ is a more sensitive indicator of solvent effects than the other parameters (see Fig. 5). Unfortunately, it does not seem to be true that "$\Delta C_p^{\ddagger}$ is redundant and the demanding experiments for its determination are not necessary" and that "the difficulties in determining $\Delta C_p^{\ddagger}$ are avoided".[8]

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Synthesis of α-Haloalkyl Aryl Ethers from *O,S*-Acetals

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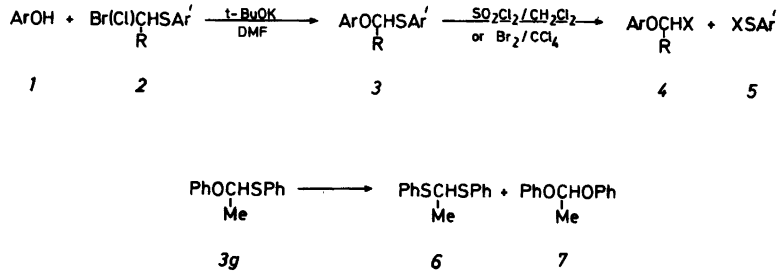
α-Haloalkyl aryl ethers are prepared under mild conditions in high yields by selective cleavage of *O,S*-acetals using sulfuryl chloride or bromine. The intermediate *O,S*-acetal is prepared from the phenol by *O*-alkylation using a readily accessible α-haloalkyl aryl thioether.

The methods reported for the synthesis of α-haloalkyl aryl ethers are either inconvenient and laborious, or they lack generality.[1] We very recently reported on a much improved synthesis of chloromethoxybenzenes by chlorotris(triphenylphosphine)rhodium(I) catalytic decarbonylation of phenoxyacetyl chlorides.[2] The decarbonylation requires heating (150–170 °C), however, which excludes heat sensitive substrates. In our continued search for improvements in the synthesis of α-haloalkyl aryl ethers under mild conditions, we have investigated cleavage reactions of carbon–sulfur bonds which represent an important type of reaction in organic synthesis.[3,*]

The substitution of the phenylthio group by a halogen has been demonstrated in the synthesis of *N*-(α-chloroalkyl)phthalimides from *N*-[(α-phenylthio)alkyl]phthalimides on treatment with sulfuryl chloride at room temperature.[4] Because divalent oxygen is a hard base and divalent sulfur is a soft base,[5] it was expected that the carbon–sulfur bond in *O,S*-acetals could be selectively cleaved under mild reaction conditions to give an α-haloalkyl ether and its complementary sulfenyl halide using reagents like sulfuryl chloride or chlorine.

On treatment of the *O,S*-acetals *3a–3g* with equimolar amounts of sulfuryl chloride or bromine at room temperature in dichloromethane or tetrachloromethane, the carbon–sulfur bond was selectively cleaved in the course of a few minutes to give the α-haloalkyl aryl ethers *4a–4i* and the sulfenyl halide *5* (Scheme 1, Table 1). The highly reactive sulfenyl halides were trapped by the reaction with an olefin *in situ* to give a high boiling liquid from which the α-haloalkyl aryl ethers could be separated by distillation; yield of adduct 73–97%. Cyclohexene was used as the trapping agent for sulfenyl chlorides, whereas styrene was used for the bromides since the bromocyclohexane adduct was found to codistil with the α-bromoalkyl aryl



Scheme 1.

* Protection of primary hydroxyl groups as methylthiomethyl ethers;[3b] benzylthiomethyl as an *S*-protecting group;[3c] dethioacetalization with bromodimethylsulfonium bromide;[3d] dethioacetalization with soft acid metal salts;[3e] synthesis of sulfonyl chlorides;[3f] synthesis of sulfenyl chloride;[3g,3h] transformation of thioethers into ethers by thallium(III) nitrate.[3i]

0302-4369/83 $2.50

Table 1. Yields in the synthesis of α-haloalkyl aryl ethers *4* and intermediate *O,S*-acetals *3*.

	Ar	R	Ar'	X	Yield (%) *3*	*4*
a	Ph	H	4-ClPh	Cl	64	77
b	4-MePh	H	4-ClPh	Cl	58	87
c	4-ClPh	H	4-ClPh	Cl	64	85
d	3-CF_3Ph	H	4-ClPh	Cl	71	89
e	4-AcPh	H	4-ClPh	Cl	68	72
f	2-naphthyl	H	4-ClPh	Cl	57	76
g	Ph	Me	Ph	Cl	47	70
h	Ph	H	4-ClPh	Br		86
i	4-MePh	H	4-ClPh	Br		75

ethers *4h* and *4i*. In ^{1}H NMR there is a downfield shift of *ca.* 0.5 ppm from the methylene protons of the *O,S*-acetals *3* (δ 5.1–5.4) to the corresponding protons of the α-halo ethers *4*.

The α-bromo ethers *4h* and *4i* easily decompose when heated and become highly coloured in storage, even in the cold.

From Table 1 it is seen that α-haloalkyl aryl ethers *4* with both electron attracting and donating substituents in the aryl moiety can be synthesized by this method. Furthermore, the nature of the aryl substituents appears to have little influence on the yields of the reaction. Thus satisfactory yields were obtained for the compounds *4e*, *4f* and *4g* which were difficult to prepare by the previously reported method of catalytic decarbonylation of phenoxyacetyl chlorides.[2]

The cleavage reaction of *3* is very rapid at room temperature and no product from α-halogenation of the *O,S*-acetal *3* was seen. This is notable since in the reaction of the *S,S*-acetal 1,3-dithiane with sulfuryl chloride the main product is 2-chloro-1,3-dithiane, and the product due to carbon–sulfur bond cleavage is only present as a minor impurity.[6]

The *O,S*-acetals *3* were prepared by the reaction between potassium phenolates *1* and readily available α-haloalkyl aryl sulfides *2*. (Table 1; *3*) The *O,S*-acetals were stable compounds except for the product *3g* derived from acetaldehyde. *3g* could be distilled, but the distillate invariably contained some of the corresponding dithioacetal *6*. ^{1}H NMR showed that the distillate of *3g* in storage at 5 °C after 14 days had lost the quartet at δ 5.50 (*CH*Me); a new quartet was present at δ 4.48 due to the dithioacetal *6*, and another quartet at δ 5.86 due to the acetal *7*. Corresponding shifts in the signals from the protons of the methyl group were also observed. On further storage for 2 weeks the signals from the acetal *7* gradually disappeared again; the thioacetal *6* was then isolated for identification.

The instability of *3g* can in part be explained by the phenomenon termed "symbiosis" which means that ligands of the same hardness or softness tend to flock together on the same acceptor atom.[5] Hence both *6* and *7*, according to the HSAB principles, are expected to be thermodynamically more stable than *3g*, which has a combination of hard and soft ligands on the same centre.

The disproportionation was not observed for the formyl derived *O,S*-acetals *3a–3f* in storage. In principle, however, the same reaction is to be expected but was not further pursued. Elimination of the phenoxy or phenylthio group with the formation of a cationic structure may be the initial step, in which case the added stabilization of this species from the methyl derivative *3g* may explain in part the differences in stability between *3a–3f* and *3g*. Alternatively, the reaction may proceed *via* a vinyl phenyl ether (thioether) intermediate after initial elimination of thiophenol (phenol) from *3g*.

EXPERIMENTAL

Procedure for the synthesis of the O,S-*acetals* 3a–3f. 1-Bromomethylthio-4-chlorobenzene[7] (20 mmol) was added to a solution of the potassium phenolate (20 mmol) in DMF (50 ml). The mixture was stirred at 80° for 1–2 h before the solvent was distilled off. The residue was triturated with water, extracted into ether and washed with water (4×). The dried ($MgSO_4$) solution was evaporated and the residue distilled or recrystallized. *3a:* B.p. 110–112 °C/0.01 mmHg.[8] Anal. $C_{13}H_{11}ClOS$: C, H. ^{1}H NMR ($CDCl_3$): δ 5.32 (CH_2), 6.7–7.4 (Ar). MS [70 e V, *m/z*

(% rel. int)]: 250 (15,M), 159(35), 158(8), 157(100), 77(16), 45(26). *3b:* M.p. 43–44 °C (light petroleum). Anal. $C_{14}H_{13}ClOS$: C,H. ^1H NMR ($CDCl_3$): δ 2.23 (CH_3), 5.30 (CH_2), 6.7–7.4 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 264 (15,M), 159(36), 158(8), 157(100), 121(5), 91(11), 45(26). *3c:* B.p. 135–140 °C/0.01 mmHg. Anal. $C_{13}H_{10}Cl_2OS$: C,H. ^1H NMR ($CDCl_3$). δ 5.29 (CH_2), 6.6–7.4 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 284 (3,M), 159(36), 157(100), 115(15), 105(13), 72(21), 45(51). *3d:* B.p. 118–122 °C/0.01 mmHg. ^1H NMR ($CDCl_3$): δ 5.13 (CH_2), 7.1–7.5 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 318 (2,M) 159(37), 157(100), 145(21), 121(7), 108(8), 75(14). *3e:* M.p. 56 °C (MeOH). Anal. $C_{15}H_{13}ClO_2S$: C,H. ^1H NMR ($CDCl_3$): δ 2.50 (4-Ac), 5.38 (CH_2), 6.8–7.8 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 292 (3,M), 159(36), 158(8), 157(100), 121(5), 77(4), 45(24). *3f:* M.p. 70 °C (EtOH). Anal. $C_{17}H_{13}ClOS$: C,H. ^1H NMR ($CDCl_3$): δ 5.46 (CH_2), 7.0–8.7 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 300 (23,M), 159(36), 157(100), 144(7), 123(13), 115(7).

1-Phenoxy-1-phenylthioethane 3g. 1-Chloro-1-phenylthioethane[9] (12.0 g, 72 mmol) was added dropwise during 5 min. to a solution of potassium phenolate (72 mmol) in DMF (200 ml). The mixture was stirred for 2 d at room temperature and 2 h at 80 °C before the solvent was distilled off. The residue was triturated with water, extracted into ether and washed with water. The dried ($MgSO_4$) solution was evaporated and the residue distilled under vacuum. Yield 7.8 g (47%), b.p. 99–103 °C/0.01 mmHg. ^1H NMR ($CDCl_3$): δ 1.64 (d, CH_3, *J* 6 Hz), 5.50 (q, CH, *J* 6 Hz), 6.8–7.5 (Ar).

Procedure for the synthesis of the α-chloroalkyl aryl ethers 4a–4e. Sulfuryl chloride (10 mmol) in dry dichloromethane (10 ml) was added dropwise during 5 min to a mixture of the *O,S*-acetal (10 mmol) in dry dichloromethane (35 ml). The mixture was stirred for 10 min before cyclohexene (11 mmol) in dry dichloromethane (10 ml) was added dropwise during 5 min at 5 °C. The mixture was stirred for 10 min at room temperature before the solvent was removed and the residue distilled under vacuum. *4a:* B.p. 92–94 °C/20 mmHg.[2] ^1H NMR ($CDCl_3$): δ 5.82 (CH_2), 6.8–7.3 (Ar). *4b:* B.p. 42–44 °C/0.01 mmHg.[1] ^1H NMR ($CDCl_3$): 2.21 (4-Me), 5.70 (CH_2) 6.8–7.2 (Ar). *4c:* B.p. 40–42 °C/0.01 mmHg.[7] ^1H NMR ($CDCl_3$): δ 5.80 (CH_2), 6.8–7.3 (Ar). *4d:* B.p. 88–90 °C/15 mmHg. ^1H NMR ($CDCl_3$). δ 5.82 (CH_2), 7.26 (Ar). MS [70 e V *m/z* (% rel. int.)]: 212/210 (10/35), M). 191(12), 175(100), 145(76), 133(13), 127(19), 114(11), 113(11), 95(15), 75(20). *4e:* B.p. 89–92 °C/0.01 mmHg.[2] ^1H NMR ($CDCl_3$): δ 2.53 (4-Ac), 5.85 (CH_2), 7.05 (d, *J* 8 Hz, H-4, H-5), 7.87 (d, *J* 8 Hz, H-2, H-6).

The 1-chloro-2-(4-chlorophenylthio)cyclohexane had b.p. 118–122 °C/0.01 mmHg.

2-Chloromethoxynaphthalene 4f. Procedure as above, but the sulfenyl chloride was trapped with styrene; b.p. 82–95 °C/0.03 mmHg.[4] ^1H NMR ($CDCl_3$): δ 5.87 (CH_2), 7.0–7.7 (Ar). The 1-chloro-1-phenyl-2-(4-chlorophenylthio)ethane had b.p. 120–135 °C/0.03 mmHg.

(1-Chloroethoxy)benzene 4g. Procedure as above; b.p. 94–96 °C/20 mmHg.[1,f] ^1H NMR ($CDCl_3$): δ 1.96 (d, CH_3, *J* 5 Hz), 6.13 (q, CH, *J* 5 Hz), 6.8–7.3 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 158/156 (3/9, M), 135(8), 121(48), 120(64), 94(100), 91(56), 77(47). The 1-chloro-2-phenylthiocyclohexane had b.p. 98–100 °C/0.01 mmHg.

Procedure for the synthesis of the α-bromoalkyl aryl ethers 4h *and* 4i. Bromine (10 mmol) in dry tetrachloromethane (10 ml) was added dropwise during 20 min at room temperature to a solution of the *O,S*-acetyl in dry tetrachloromethane (40 ml). The mixture was stirred for 1 h at room temperature before styrene (10 mmol) in dry tetrachloromethane (10 ml) was added dropwise during 15 min at 5 °C. The mixture was stirred for 1 h at room temperature before the solvent was distilled off, and the residue distilled under vacuum. *4h:* B.p. 110–112 °C/20 mmHg. ^1H NMR ($CDCl_3$): δ 5.92 (CH_2), 6.9–7.4 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 188/186 (6/6, M), 142(11), 107(100), 104(14), 94(11), 79(15), 77(70). *4i:* B.p. 48–52 °C/0.001 mmHg. ^1H NMR ($CDCl_3$): δ 2.28 (4-Me), 5.91 (CH_2), 6.8–7.3 (Ar). MS [70 e V, *m/z* (% rel. int.)]: 202/200 (7/7, M) 188(8), 186(15), 185(86), 184(8), 183(81), 121(53), 107(55), 104(100), 103(51), 91(32), 77(47). The 1-bromo-1-phenyl-2-(4-chlorophenylthio)ethane had m.p. 47–49 °C (light petroleum).

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Addition of Nitrite Ions to 1-Methoxy-1,2,3-triazolium Salts. Formation of Nitro and Hydroxy Substituted Triazoles

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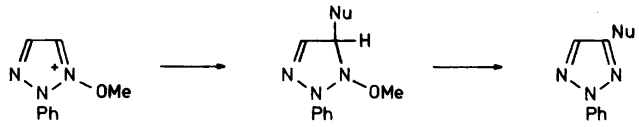
Potassium and silver nitrite add to 1-methoxy-2-phenyltriazolium salts as ambident nucleophiles to give 4-nitro- and 4-hydroxytriazoles. If unsubstituted, the latter, *via* nitrosation and oxidation, affords 4-hydroxy-5-nitro-2-phenyltriazole. The hydroxytriazoles add to unchanged starting material producing bistriazolyl ethers. A nitro-substituted derivative of the latter displays photochromic properties.

1-Methoxy-2-phenyl-1,2,3-triazolium tetrafluoroborates react with a variety of nucleophiles with addition followed by elimination of methanol to produce 4-substituted 2-phenyltriazoles (Scheme 1).[1] This paper deals with the reaction of the salts (*1*; X = H or Me) with the ambident nitrite ion to give several products.

Thus, the 1-methoxytriazolium salt (*1*; X = Me), when treated for 3 d at 20 °C with potassium or silver nitrite in acetonitrile, yields the nitrotriazole (*2*; X = Me) and the hydroxytriazole (*4*; X = Me) in substantial amounts (yields in Table 1). These are the result of an attack of nitrite nitrogen and oxygen, respectively, the hydroxytriazole arising by loss of nitrogen oxide from initially formed nitrit (*3*; X = Me). The bistriazolyl ether (*5*; X = Y = Me) is isolated as a byproduct most likely arising by addition of initially formed hydroxytriazole (*4*; X = Me) to unchanged starting material (*1*; X = Me), followed by elimination of methanol, a known reaction.[1] Finally, the triazole-1-oxide (*9*; X = Me) is formed by *O*-dealkylation of the starting material (*1*; X = Me).[1] Silver nitrite is superior to potassium nitrite producing higher yields of both nitro- and hydroxytriazole. In addition, the silver salt produces a higher *N*-addition – *O*-addition ratio (0.78) than potassium nitrite (0.51) as is the case when the two nitrites are used in aliphatic nucleophilic substitution reactions.[2]

The 1-methoxytriazolium salt (*1*; X = H) when treated with potassium nitrite yields the corresponding products, yet supplemented with the nitrohydroxytriazole (*7*) and the nitro-substituted bistriazolyl ether (*5*; X = H, Y = NO_2), both formed in substantial quantities (Table 1). The former compound may arise when one molecule of the intermediate nitrite (*3*; X = H) nitrosates a second. This affords hydroxynitrosotriazole (*6*), which in turn is oxidized to *7*. Most likely, the oxidation takes place during the acidic work-up which is essential for the isolation of *7*. Nitrous acid, evolved by the acidification, is known to be able to oxidize nitroso to nitro groups.[3–6] That nitrosation, in contrast to oxidation, takes place prior to work-up appears from the fact that the hydroxytriazole (*4*; X = H), though susceptible to electrophilic substitution as shown by its ready nitration by nitric acid (see Experimental), remains unchanged upon treatment with aqueous nitrous acid under the conditions of work-up. The hydroxytriazole (*4*; X =



Scheme 1.

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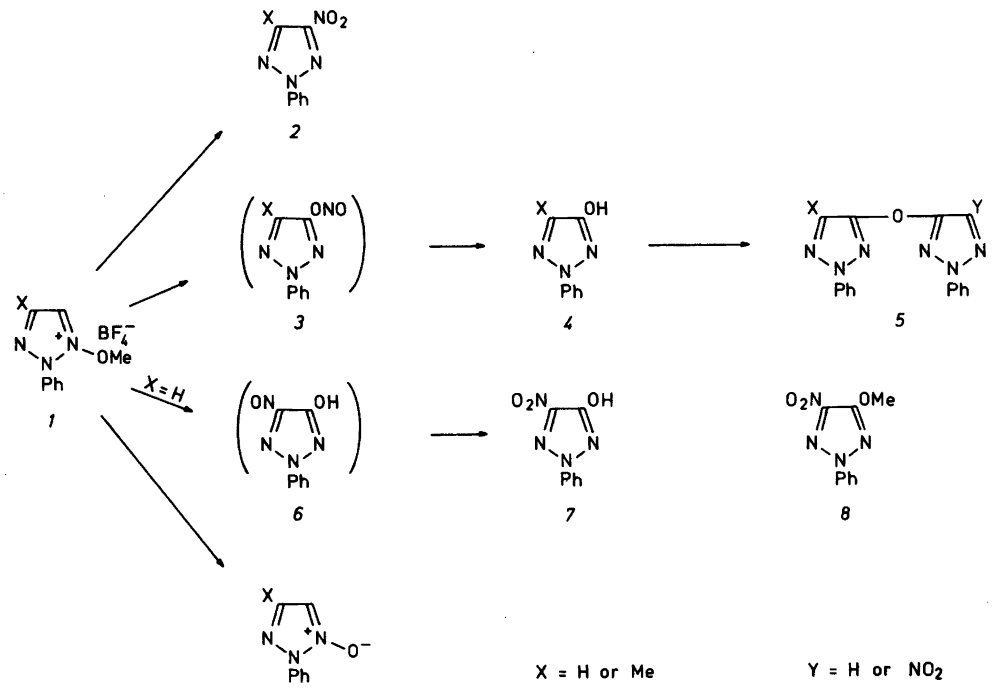
Table 1. Yields of products obtained by reaction of 1-methoxy-2-phenyl-1,2,3-triazolium tetrafluoroborates (*1*; X=H or Me) with potassium or silver nitrite.

Starting material	Product	Yield; KNO_2 as nucleophile %	Yield; $AgNO_2$ as nucleophile %
1; X=Me	*2*; X=Me	14	27
	4; X=Me	24	32
	5; X=Y=Me	7	5
	9; X=Me	48	14
1; X=H	*2*; X=H	18	23
	4; X=H	1	9
	5; X=Y=H	8	8
	7	32	46
	5; X=H, Y=NO_2	19	0
	9; X=H	11	10

H) could not be nitrosated with iso-amyl nitrite in ethanol, neither under neutral conditions nor in the presence of sodium ethoxide. Nitrosation prior to work-up is also indicated by the absence of the nitro substituted bistriazolyl ether (*5*; X=H, Y=NO_2) when the starting material (*1*; X=H) is treated with hydroxynitrotriazole *7* under the conditions of work-up while *5*; (X=H, Y=NO_2) is obtained in 69 % yield when *1* and *7* are reacted under non-aqueous basic conditions.

When the 1-methoxytriazolium salt (*1*; X=H) is treated with silver nitrite, no significant change in the *N*-addition–*O*-addition product ratio is observed (KNO_2 0.32; $AgNO_2$ 0.33) but total yields of nitro-, hydroxy-, and nitrohydroxytriazole (*2*; X=H, *4*; X=H, and *7*) increase. In contrast, no



Scheme 2.

nitro-substituted bistriazolyl ether (*5*; X=H, Y=NO_2) is formed (Table 1).

When applicated on a TLC plate the nitro-substituted bistriazolyl ether (5; X=H, Y=NO_2) turns intensively emerald green on irradiation with UV-light of wavelength below 540 nm.

EXPERIMENTAL

Solvents were removed *in vacuo*. Flash chromatography was performed as described.[1] The purity and identity of all compounds were confirmed through TLC, m.p., IR, ^{1}H NMR and MS. ^{1}H NMR spectra were recorded on a Bruker HX-90 instrument. Mass spectra were obtained on a V. G. Micromass 7090 F instrument.

1-Methoxy-4-methyl-2-phenyl-1,2,3-triazolium tetrafluoroborate (*1*: X=Me)[1] (0.37 g), potassium nitrite (0.34 g), and dry acetonitrile (3.6 ml) were stirred for 3 d. The acetonitrile was removed and the residue extracted with acetone (4 × 10 ml). The residue was acidified (hydrochloric acid), the water removed, and the residue extracted with acetone (4 × 10 ml). Both acetone solutions were combined and the solvent removed. Preparative TLC (dichloromethane–hexane [1:1]) gave 38 mg (14 %) of *4-methyl-5-nitro-2-phenyl-1,2,3-triazole* (*2*; X=H), m.p. 113–114 °C. Recrystallization (ethyl acetate–hexane) gave a product with m.p. 114 °C. Anal. $C_9H_8N_4O_2$: C, H, N. ^{1}H NMR ($CDCl_3$) δ 8.15–8.0 and 7.65–7.35 (2H, m, and 3H, m, Ph), 2.68 (3H, s, Me). MS (70eV) 204 (100 %, M^+). The subsequent fraction contained 16 mg (7 %) of bis(4-methyl-2-phenyltriazol-5-yl) ether (*5*; X=Y=Me), identical with the material described below. Further elution with ethyl acetate–hexane (1:4) gave 55 mg (24 %) of 4-hydroxy-5-methyl-2-phenyltriazole (*4*; X=Me), identical with the material below, and 0.11 g (48 %) of 4-methyl-2-phenyltriazole-1-oxide (*9*; X=Me), identical with the material described previously.[1]

A mixture of *1*; (X=Me) (0.33 g), silver nitrite[7] (0.57 g), and acetonitrile (3.3 ml), after stirring for 3 d and removal of the acetonitrile, gave a residue which was extracted with acetone (5 × 10 ml). Removal of the acetone and preparative TLC (dichloromethane–hexane [1:1]) gave 4-methyl-5-nitro-2-phenyltriazole (*2*; X=Me), and an oil. The plate was then eluted with ethyl acetate which caused the band at the base line to move. It contained 4-methyl-2-phenyltriazole-1-oxide (*9*; X=Me). The above-mentioned oil upon preparative TLC (toluene) produced pure bis(4-methyl-2-phenyltriazol-5-yl) ether (*5*; X=Y=Me). The residue from the extraction of the product mixture with acetone was acidified (4 M hydrochloric acid, 4 ml). Filtration, extraction of the precipitate with acetone (4 × 5 ml), and removal of the acetone furnished 4-hydroxy-5-methyl-2-phenyltriazole (*4*; X=Me). Yields of all products are given in Table 1.

1-Methoxy-2-phenyl-1,2,3-triazolium tetrafluoroborate (*1*; X=H)[1] (0.16 g), potassium nitrite (0.15 g), and acetonitrile (1.6 ml) were stirred for 3 d. The acetonitrile was removed and the residue extracted with acetone (4 × 10 ml). The residue was acidified (hydrochloric acid), the water removed, and the residue extracted with acetone (4 × 10 ml). Both acetone solutions were combined and the solvent removed. Acidification (hydrochloric acid), evaporation to dryness, and flash chromatography (ethyl acetate–hexane [1:1]) gave a fraction which contained *2*; (X=H), *5*; (X=Y=H), *5*; (X=H, Y=NO_2), and *8* (see below). The second fraction contained 1 mg (1 %) of 4-hydroxy-2-phenyltriazole (*4*; X=H), identical with the material described previously.[1] Subsequent elution with ethyl acetate gave 11 mg (11 %) of 2-phenyltriazole-1-oxide (*9*; X=H), identical with the material described previously.[1] Finally, elution with ethyl acetate–methanol (1:1) gave 7 to which 4 M hydrochloric acid (2 ml) was added. The water was removed, the residue extracted with dichloromethane, and the solvent removed to give 40 mg (32 %) of 4-hydroxy-5-nitro-2-phenyltriazole (*7*), m.p. 117–119 °C. Recrystallization (toluene–hexane) raised the m.p. to 126–127 °C. The compound was identical with the material described below. Preparative TLC of the mixture of *2*; (X=H), *5*; (X=Y=H), 5; (X=H, Y=NO_2), and *8* (dichloromethane–hexane |1:1.5|) gave 21 mg (18 %) of *4-nitro-2-phenyl-1,2,3-triazole* (2; X=H), m.p. 124–126 °C. Recrystallization (ethyl acetate–hexane) gave an analytical specimen, m.p. 129–130 °C. Anal. $C_8H_6N_4O_2$: C, H, N. ^{1}H NMR δ ($CDCl_3$) 8.34 (1H, s, H-5), 8.2–8.0 and 7.65–7.45 (2H, m, and 3H, m, Ph). MS (70 eV) 190 (100 %, M^+). The subsequent fraction contained *5* (X=Y=H) and *8* (see below). The third fraction contained 21 mg (19 %) of 4-nitro-2-phenyl-5-(2-phenyltriazol-4-oxy)triazole (*5*; X=H, Y=NO_2), m.p. 168–170 °C, identical with the compound described below. The mixture of *7*; (Y=H) and (*8*) upon preparative TLC (ethyl acetate–hexane |1:4|) gave 7 mg (8 %) of the bis(2-phenyltriazol-4-yl) ether (*5*; X=Y=H), m.p. 76 °C, identical with the material described previously[1] and 2 mg (1 %) 4-methoxy-5-nitro-2-phenyltriazole (*8*), m.p. 125–127 °C, identical with the material described below.

In the same manner, (1; X=H) (0.15 g), silver nitrite[7] (0.28 g), and acetonitrile (1.5 ml) gave the products listed in Table 1.

4-Hydroxy-5-methyl-2-phenyl-1,2,3-triazole (4; X=Me) was prepared analogous to 4-hydroxy-2-phenyltriazole (4; X=H) by (i) treatment of the 1-

methoxytriazolium tetrafluoroborate (*1*; X=Me) with aqueous sodium hydroxide.[1] Yield 53 %. (In addition, 34 % of bis(4-methyl-2-phenyltriazol-5-yl) ether (*5*; X=Y=Me), see below, was isolated); (ii) hydrolysis of 4-acetoxy-5-methyl-2-phenyltriazole.[1] Yield 95 %, m.p. 158–159 °C. (Lit.,[8] 140–142 °C). Anal. $C_9H_9N_3O$: C, H, N. ^{1}H NMR δ ($CDCl_3$) 10.82 broad (1H, s, exchangeable, OH), 7.85–7.65 and 7.55–7.1 (2H, m, and 3H, m, Ph), 2.33 (3H, s, Me). MS (70eV) 175 (100 %, M^+).

4-Hydroxy-2-phenyltriazole (4; X=H)[1] (0.25 g) and conc. nitric acid (3 ml) were stirred for 1 h at 0 °C. Dilution with water (30 ml), extraction with dichloromethane (3 × 10 ml), and removal of the dichloromethane furnished 0.32 g (100 %) of *4-hydroxy-5-nitro-2-phenyl-1,2,3-triazole* (*7*) as yellow crystals, m.p. 127–129 °C. Recrystallization (ether –hexane) did not raise the m.p. Anal. $C_8H_6N_4O_3$: C, H, N. ^{1}H NMR δ ($CDCl_3$) 8.1–7.95 and 7.6–7.4 (2H, m, and 3H, m, Ph), 5.16 broad (1H, s, exchangeable, OH). MS (70 eV) 206 (100 %, M^+).

Bis(4-methyl-2-phenyl-1,2,3-triazol-5-yl) ether (*5*; X=Y=Me) was prepared like bis(2-phenyltriazol-4-yl) ether (*5*; X=Y=H)[1] by treatment of the 1-methoxytriazolium salt (*1*; X=Me) with aqueous sodium hydrogen carbonate. Yield 62 %, m.p. 101 °C (from hexane). Anal. $C_{18}H_{16}N_6O$: C, H, N. ^{1}H NMR δ ($CDCl_3$) 8.0–7.8 and 7.55–7.15 (4H, m and 6H, m, 2 Ph), 2.36 (6H, s, 2 Me). MS (70 eV) 332 (54 %, M^+).

4-Hydroxy-5-nitro-2-phenyltriazole (*7*) (59 mg) and potassium hydroxide (16 mg) were stirred in methanol (1 ml) for 1 h. The methanol was removed and the residue was dried at 1.3 Pa over P_2O_5. Then 1-methoxy-2-phenyltriazolium tetrafluoroborate (*1*; X=H) (75 mg) and dry acetonitrile (0.75 ml) was added. After stirring for 1 day under dry nitrogen the solvent was removed. The residue was extracted with dichloromethane (5 × 2 ml), the solution was filtered through silica gel 0.05–2 mm (2 g), and the dichloromethane was removed to give a residue which was triturated with boiling ether (2 × 2 ml), decanting after cooling to –25 °C. The residue consisted of 70 mg (69 %) of *4-nitro-2-phenyl-5-(2-phenyl-1,2,3-triazol-4-oxy)triazole* (*5*; X=H, Y=NO_2), buff crystals, m.p. 168–171 °C. Recrystallization (ethyl acetate) did not raise the m.p. Anal. $C_{16}H_{11}N_7O_3$: C, H, N. ^{1}H NMR δ ($CDCl_3$) 8.15–7.9 and 7.6–7.3 (4H, m, and 6H, m, 2 Ph), 7.86 (1H, s, H-5). MS (70 eV) 349.091 (100 %, M^+) (Calc.: 349.092). UV (EtOAc) λ_{max} (ε): 273.7 (27000), 311.8 (11000), 355.9 (4000).

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Isomerization of D-Glucose with Glucose-isomerase. A Mechanistic Study

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The isomerization of D-glucose to D-fructose by the enzyme glucose-isomerase (E.C. 5.3.1.5) is shown to proceed without participation of solvent molecules for a soluble as well as an immobilized form of the enzyme. The stereospecificity of the enzymatic reaction has been determined using D-glucose labeled with deuterium in the 1- or 2-position, respectively. Both are isomerized to D-fructose labeled with deuterium at C-1, the former with the 1*S* configuration and the latter with the 1*R* configuration. The reaction has been followed by ^{2}H and ^{13}C NMR spectroscopy; from the results it is concluded that both α-D-glucopyranose and β-D-fructofuranose are substrates for the enzyme. In addition, the substrate specificity for the immobilized enzyme has been investigated and it has been shown that 3-, 5- and 6-deoxy-D-glucose together with 3-*O*- and 6-*O*-methyl-D-glucose are substrates for the enzyme, whereas 4-deoxy- and 4-*O*-methyl-D-glucose cannot be isomerized.

The isomerization of D-glucose to a mixture of D-glucose and D-fructose can be accomplished enzymatically. For technical reasons the water soluble glucose-isomerase (SGI) is often transformed (*e.g.* by crosslinking with glutaric aldehyde) into an immobilized form (IGI). In this form, work-up procedures are simplified and the enzyme can be used in a continuous process. In the present paper, we have investigated the mechanism of the isomerization using both SGI and IGI (performing the isomerization in H_2O and D_2O) and using ^{2}H-labeled derivatives of D-glucose as substrates. The results obtained are compared with studies previously reported by Rose *et al.*[1,2] for similar enzymes. A detailed discussion of the reaction mechanism of glucose-isomerase has been given by Dyson and Noltmann[3] and by Alworth in two reviews.[4,5] The substrate specificity has furthermore been studied with IGI in order to gain further insight into the reaction mechanism and in order to investigate the possibility of using immobilized enzymes in synthetic carbohydrate chemistry. Finally, it has been determined by ^{13}C NMR spectroscopy, which anomeric form of D-glucose and D-fructose functions as the substrate for the soluble form of the enzyme.

RESULTS AND DISCUSSION

Equilibrium and solvent exchange of the isomerization. Treatment of D-glucose in water (60 %) at pH 8.5 and 65 °C for 3 h with IGI led to a mixture of 55 % D-glucose and 45 % of D-fructose as seen from a ^{13}C NMR spectrum of the reaction mixture. Similar results were obtained when D-fructose was used as a substrate in the reaction. If the experiment was conducted in heavy water (D_2O) it was not possible by ^{13}C NMR spectroscopy to detect incorporation of deuterium atoms in the products during the first 2–3 h of isomerization. ^{13}C NMR spectroscopy, however, is a fairly insensitive method for the measurement of deuterium incorporation below ~10 %, and hence ^{2}H NMR spectra of the reaction mixture were measured. This method is a very sensitive probe for the detection of even very small amounts of deuterium.[6] The results are shown in Table 1. Treatment of D-glucose with IGI at pD 8.5 in D_2O for 1 and 24 h results in 7 and 40 % incorporation of deuterium, respectively, whereas treatment of D-glucose or D-fructose under the same reaction conditions, but without added IGI, results in deuterium incorporation of 7 and 15 %, respectively. These results indicate that some solvent exchange occurs due to the basic conditions used for optimum

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Table 1. Solvent exchange of D-glucose and D-fructose under different reaction conditions at 65 °C.

Substrate	Enzyme	Time/h	pH	Incorporation of deuterium in products/%[a]
D-Glucose	IGI	1	8.5	7
D-Glucose	IGI	24	8.5	40
D-Glucose	IGI	24	7.0	21
D-Glucose	SGI	24	7.0	2
D-Glucose		24	8.5	7
D-Fructose		24	8.5	15

[a] Measured by ^2H NMR spectroscopy relative to internal acetone-d_6.

enzyme activity. D-Glucose was therefore isomerized in D_2O at neutral pD with SGI and IGI. ^2H NMR spectra showed a deuterium incorporation of approximately 21 % in the experiment using IGI and practically no incorporation using SGI. This indicates that SGI reacts through a mechanism without exchange with solvent molecules even under equilibrating reaction conditions. The incorporation in the experiment with IGI is most likely caused by the base added in the immobilization process.* These results are in accord with results published by Rose *et al.*[7] for D-xylose isomerase from *Lactobacillus brevis*, but differ from results using glucose-6-phosphate-isomerase which under equilibrating conditions exchange with the solvent.[8,9]

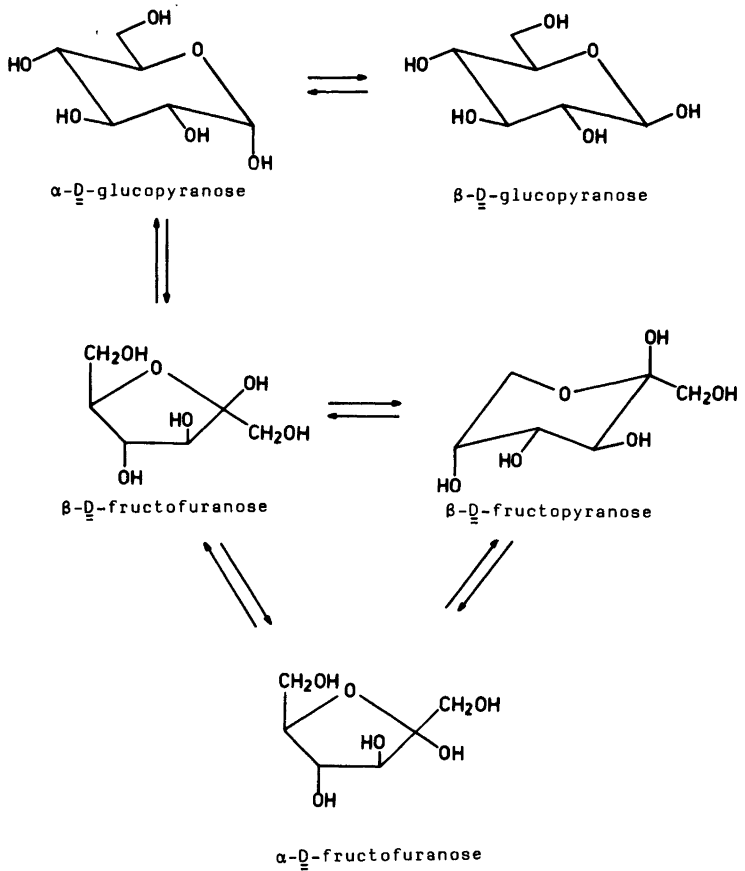
Stereochemistry of the isomerization reaction. (1-$^2H_1$)-D-Glucose (*1*) was synthesized by sodium amalgam reduction of δ-D-gluconolactone in D_2O as described.[10] Isomerization of *1* with SGI at pH 7.0 and at 65 °C in H_2O led to a mixture of (1-$^2H_1$)-D-glucose (*1*) and (1-$^2H_1$)-D-fructose (*2*) without loss of deuterium in agreement with the results described above. The reaction was followed by ^2H NMR spectroscopy, but it was not possible to detect any other intermediates than those shown in Scheme 1.

A complete separation of D-glucose from D-fructose by ion exchange column chromatography is not possible for preparative uses, but the products can be separated as their diisopropylidene derivatives *3a* and *4a*. Treatment of the crude product from the isomerization of *1*, adsorbed on silica gel, with acetone and concentrated sulfuric acid, gives a mixture of the two diisopropylidene derivatives *3a* and *4a* in 60 % yield from which *4a* can be crystallized in 7 % yield. Alternatively, *3a* and *4a* can be converted into the acetates *3b* and *4b* which can be separated by chromatography on silica gel raising the yield of *4b* to ~20 %. The ^1H NMR spectrum of *4b* showed no resonances centered at δ 3.82, typical for H-1 in the unlabeled compound.

(2-$^2H_1$)-D-Glucose (*5*) was synthesized from D-glucose- or D-fructose-6-phosphate.[11] Incubation of either of these compounds in D_2O with glucose-6-phosphate-isomerase[8] under equilibrating conditions gives a 9:1 mixture of (2-$^2H_1$)-D-glucose-6-phosphate and (1-$^2H_1$-*R*)-D-fructose-6-phosphate. (2-$^2H_1$)-D-glucose can be isolated after dephosphorylation with alkaline phosphatase by crystallization in 64 % overall yield. When (2-$^2H_1$)-D-glucose (*5*) is isomerized with SGI and IGI, similar results are obtained as described above. In this case ^2H NMR spectra of the reaction mixture using SGI do not allow a separate identification of the individual signals from the components in the mixture due to overlap of the signals which all resonate between δ 4.0 and 4.5. (The natural line width of the deuterium signal is ~45 Hz measured at 41.43 MHz).

Separation of the products is performed through the diisopropylidene derivatives *7a* and *8a* as described above. A ^1H NMR spectrum of *7b* shows no H-1 proton resonating at δ 3.95. Fig. 1A presents the 270 MHz ^1H NMR spectrum of 3-*O*-acetyl-1,2-4,5-di-*O*-isopropylidene-β-D-fructopyranose, whereas Fig. 1B portrays a $^1H-\{^1H\}$-difference n.O.e. experiment[12] with irradiation of H-3; the result shows that the H-1 proton resonating at higher field is close to H-3. Inspection of a Dreiding model indicates that the *pro-S* proton at C-1 of the fructose derivative is closer to H-3. This suggests that the proton resonating at 3.82 ppm is the *pro-S* proton and that the proton resonating at 3.95 ppm

*Information provided by Novo A/S, Copenhagen, Denmark.

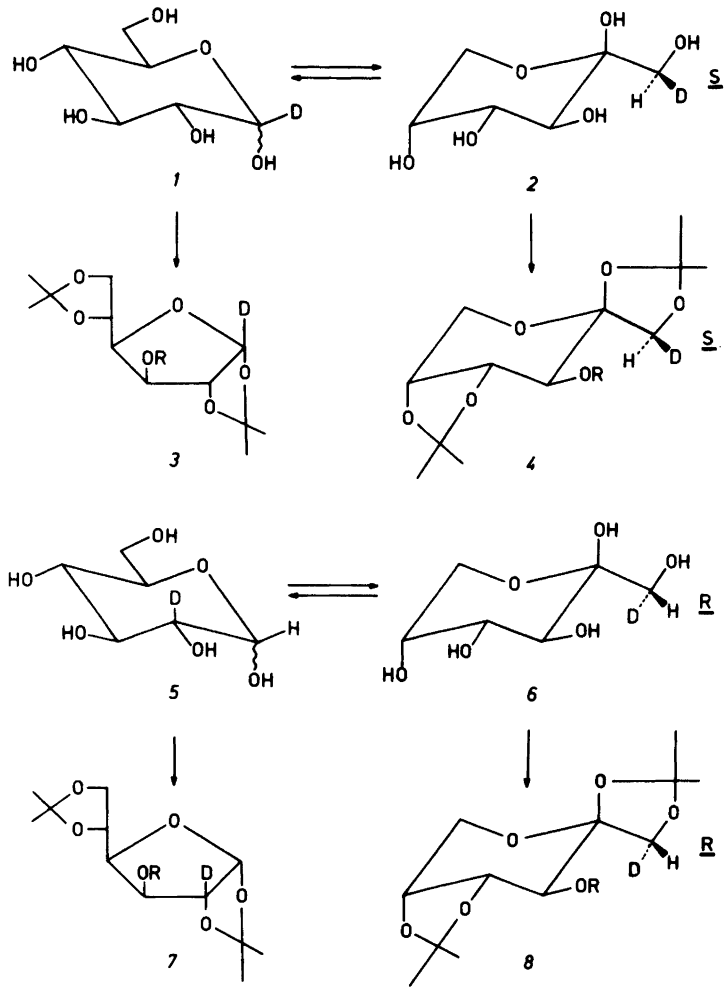


Scheme 1.

is the *pro-R* proton. Similar experiments were conducted with IGI and (1-$^2H_1$) and (2-$^2H_1$)-D-glucose as substrates and with the same results. Thus the enzyme [both in the soluble (SGI) and immobilized (IGI) form] transfers the 1-*pro-R* proton of D-fructose to the 2-position of D-glucose during the isomerization in a complete stereospecific fashion without incorporation of solvent molecules even under equilibrating conditions and with the same stereochemical course as that described for glucose-6-phosphate-isomerase.[13]

Substrate specificity of glucose-isomerase. Subsequent monosaccharides were synthesized in order to study the substrate specificity towards IGI; the results are presented in Table 2. It is seen that minor modifications at C-3 of D-glucose to 3-deoxy- or 3-*O*-methyl-D-glucose do not influence the enzymatic reaction very much. Both of these compounds as well as 3-*O*-methyl-D-fructose are substrates for the enzyme, whereas inversion of the hydroxy group at C-3 to D-allose arrests the reaction. Similarly, the 6-position can be modified to 6-deoxy-, 6-*O*-methyl-, 6-thio-D-glucose [14] or removed completely (D-xylose) without interfering with the substrate character. However, the 4-position appears to be very important for the enzymatic reaction because 4-deoxy-, 4-*O*-methyl- and 4,6-di-*O*-methyl-D-glucose or D-galactose cannot be isomerized by the enzyme. Finally, 5-deoxy-D-glucose appears to be a substrate for the enzyme, but the results must be interpreted with care because this compound is very base labile and rearranges easily to 5-deoxy-D-fructose under basic conditions.

Anomer specificity of glucose-isomerase. The anomerization of D-glucose and D-fructose is fast at the optimum temperature for isomerization with glucose-isomerase.[15] This fact makes it



a; R=H

b; R=Ac

difficult to obtained the information about the anomeric specificity of the enzyme. A computer simulation of the reactions outlined in Scheme 1, assuming pseudo first order kinetics with rate constants fitted to experimental data, has therefore been carried out.[16,17] This shows that deviations from equilibrium concentrations of anomers on the substrate side of the reaction is too small to be detected experimentally. On the other hand, the simulation reveals that it is possible within the first 10 min of the reaction to detect a build-up of the anomer released in the enzymatic reaction. The product concentration during this period is, however, very small and measurements, which are carried out by ^{13}C NMR spectroscopy shall therefore be interpretated with care. The optimum time for experimental proof of the anomeric specifity is expected to be 5–7 min after the start of the reaction. Fig. 2B shows a ^{13}C NMR spectrum of a reaction mixture 5 min after addition of SGI to a 3M solution of mutarotated D-fructose in D_2O. It is seen that the concentration of the α-anomer of D-glucose is significantly higher than that prevailing at equilibrium, Fig. 2A. Furthermore the results from a series of ^{13}C NMR measurements, which are presented in Table 3, show a concentration of

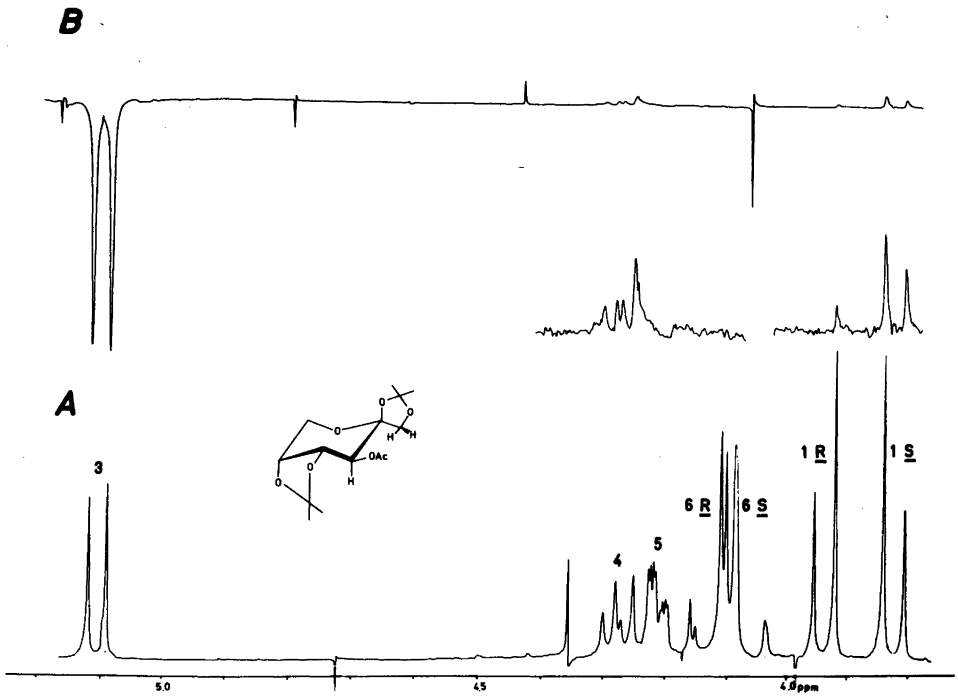


Fig. 1. Partial 270 MHz ^{1}H NMR spectrum of 3-*O*-acetyl-1,2-4,5-di-*O*-isopropylidene-β-D-fructopyranose in deuteriochloroform. A. Showing the normal spectrum with the assignments indicated above the resonances. B. Difference n.O.e. experiment with saturation of H-3, showing the enhancements of H-4 and H-1 (*pro-S*).

Table 2. Isomerization of different substrates with immobilized glucose-isomerase.

Substrate	pH	Conversion/ %[a]
D-Glucose	8.5	43.9
L-Glucose	8.5	0
D-Mannose	8.5	0
D-Allose	8.5	0
D-Galactose	7.3	0
D-Xylose	8.5	20
3-Deoxy-D-*ribo*-hexose	8.5	38
4-Deoxy-D-*xylo*-hexose	8.5	0
5-Deoxy-D-*xylo*-hexose	7.2	64[b]
6-Deoxy-D-glucose	8.5	15
3-*O*-Methyl-D-glucose	7.1	11[c]
3-*O*-Methyl-D-fructose	8.5	6[d]
4-*O*-Methyl-D-glucose	7.3	0
6-*O*-Methyl-D-glucose	7.5	21
4,6-Di-*O*-methyl-D-glucose	8.5	0
5-Thio-D-glucose	8.5	0
6-Thio-D-glucose	8.5	100[e]

[a] Determined by ^{13}C NMR spectroscopy on the reaction mixtures after incubation for 24 h at 65 °C. [b] Incubation for 40 h gave 100% conversion. Incubation at pH 8.5 for 72 h without enzyme also gave 100% conversion, but under these conditions unknown decomposition products were formed. [c] Incubation for 96 h gave 22% conversion. [d] Incubation for 192 h gave 25% conversion, which indicates that equilibrium is not obtained. [e] Ref. 14.

α-D-glucopyranose larger than that present at equilibrium in the spectra obtained within the first 15 min of the reaction. Based on these results α-D-glucopyranose is thus the substrate for glucose-isomerase. Similar experiments show that β-D-fructofuranose is released, when a solution of mutarotated D-glucose is used as substrate, Table 3. These findings are in accord with results obtained by Schray and Rose[2] and Feather *et al.*[18] for D-xylose-isomerase isolated from *Streptomyces sp.*

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter.

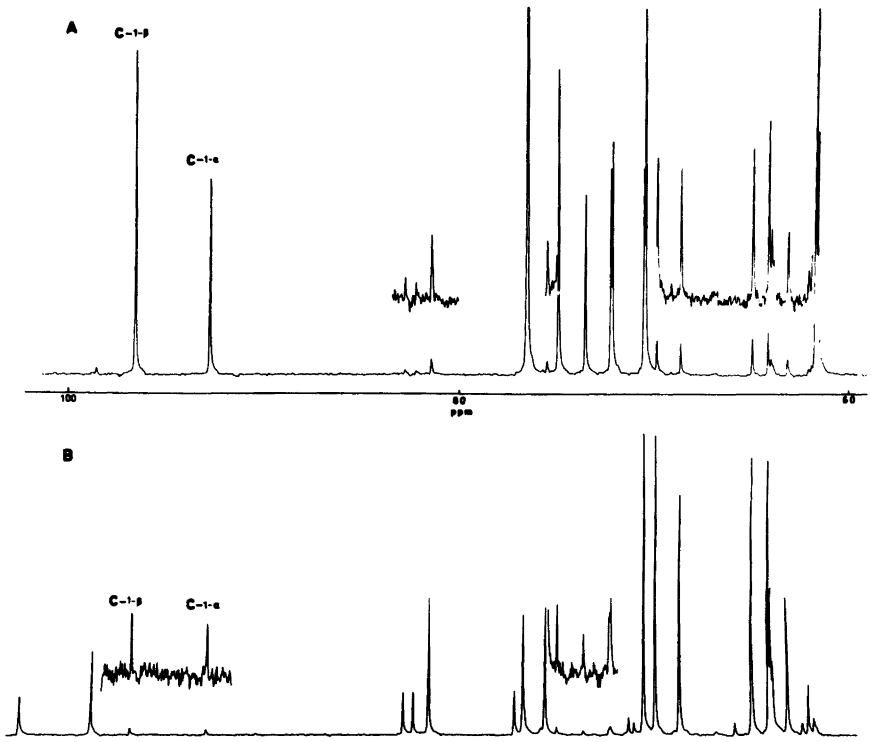


Fig. 2. A. 67.89 MHz ^{13}C NMR spectrum of mutarotated D-glucose in D_2O at 65 °C after incubation with SGI for 10 min. B. 67.89 MHz ^{13}C NMR spectrum of mutarotated D-fructose in D_2O at 65 °C after incubation with SGI for 5 min. The anomeric signals for the α- and β-D-glucopyranoses are indicated C-1-α and C-1-β, respectively.

Table 3. Isomerization of D-glucose and D-fructose with soluble glucose-isomerase at 65 °C.

	D-Fructose		D-Glucose	
Time (min)	α-Glucose/ Total glucose	β-Fructofuranose/ Total fructose	α-Glucose/ Total glucose	β-Fructofuranose/ Total fructose
0	–	–	–	–
5	0.50	0.29	0.40	0.38
10	0.48	0.31	0.40	0.36
14	0.43	0.30	0.43	0.33
18	0.40	0.30	0.40	0.33
23	0.41	0.29	0.42	0.32
28	0.40	0.29	0.42	0.30
38	0.40	0.29	0.41	0.30
48	0.39	0.29	0.42	0.30
58	0.40	0.30	0.41	0.29
73	0.40	0.31	–	–
108	0.41	0.30	0.41	0.30

^1H, ^{13}C and ^2H NMR spectra were obtained on Bruker WH-90, HX-90 and HX-270 NMR instruments. Preparative thin-layer chromatography was performed on silica gel PF_{254} (Merck) on 1 mm layers and compounds were detected by charring with a hot wire. The immobilized glucose-isomerase (IGI), Sweetzyme Q, and purified soluble form (SGI) of the same enzyme (E.C. 5.3.1.5) were obtained from Novo A/S, Copenhagen, Denmark. Glucose-6-phosphate-isomerase (E.C. 5.3.1.8) and alkaline phosphatase (E.C. 3.1.3.1) were from Boehringer Mannheim AG.

^{13}C NMR kinetic measurements. Kinetic experiments were carried out on 3M solutions (3 ml) of mutarotated substrates in D_2O (pD ~ 7) at 65 °C in a 10 mm tube measuring the ^{13}C NMR signals on a Bruker HX-270 NMR instrument operating at 67.89 MHz. The enzyme (200 mg in 0.5 ml D_2O) was added and spectra obtained as soon as possible after temperature equilibrium was established. The accuracy of the integrated ^{13}C NMR signals is considered 10 %.

(1-$^2H_1$)-D-Glucose (1).[10] To a solution of δ-D-gluconolactone (4.07 g) in D_2O (30 ml) was added a small amount of 2M D_2SO_4 until pD 3–4 was obtained. The solution was cooled to 0 °C and sodium amalgam (4 %, 100 g), divided into two portions, was added, while the pD was kept between 3 and 4 with 2M D_2SO_4. When the consumption of acid stopped, the solution was decanted from the deposited mercury, diluted and treated with a mixed bed ion exchange resin (Amberlite MB-3) to remove inorganic salts and unreacted lactone. Evaporation of the water gave 2.01 g (49 %) of syrupy *1*. Crystallization from ethanol containing a small amount of methanol gave 1.60 g (39 %) of crystalline (1-$^2H_1$)-α-D-glucopyranose (*1*), m.p. 144–146 °C. The isotopic purity of the compound was checked by ^1H and ^{13}C NMR spectroscopy and proved to be better than 97 %.

(2-^2H)-D-Glucose (5).[11] D-Fructose-6-phosphate monosodium salt (2.0 g) was evaporated twice with D_2O (10 ml) and then dissolved in D_2O (10 ml) and pD adjusted to 7 with solid sodium carbonate (~375 mg). Glucose-6-phosphate-isomerase (400 μl, activity 2 mg/ml) was added and the reaction mixture left overnight at 37 °C. The reaction mixture was then heated to 100 °C in order to denature the enzyme, filtered through carbon and evaporated to dryness. The residue was dissolved in water (46 ml), tris buffer (12 ml, 1M tri-(hydroxymethyl)aminomethane) and alkaline phosphatase (60 μl) were added and the reaction mixture left for two days at 37 °C. The reaction mixture was then treated with a mixed bed ion exchange resin and the eluate concentrated. The residue was crystallized from ethanol containing a small amount of methanol and gave (2-$^2H_1$)-α-D-glucopyranose (*5*), 728 mg (57 %), m.p. 145 °C. The isotopic purity of the compound was checked by ^1H and ^{13}C NMR spectroscopy and proved to be better than 98 %.

Isomerization of (1-$^2H_1$)-D-glucose (1) with IGI and SGI. Compound *1* (0.3 g) was dissolved in water (1.5 ml), $MgSO_4$, H_2O (0.5 mg) and IGI (90 mg) were added and the reaction stirred for 4 h at 65 °C. The reaction mixture was filtered through carbon and evaporated leaving the crude product (286 mg). The residue was dissolved into methanol (10 ml) and silica gel (3 g) added, followed by evaporation to dryness. The silica gel was suspended in dry acetone (30 ml) and H_2SO_4 (25 μl) was added. The suspension was stirred at room temperature for 1.5 h, the silica gel filtered off, and the filtrate neutralized with sodium hydroxide in water (1 % solution). The neutral solution was evaporated to dryness and the residue partitioned between water and chloroform. The organic phase was washed twice with water, dried ($MgSO_4$), filtered and evaporated leaving 236 mg of product. Crystallization from ether–pentane gave 30 mg (7 %) of (1-$^2H_1$-*R*)-1,2-4,5-di-*O*-isopropylidene-β-D-fructopyranose (*4a*), m.p. 117–118 °C. The mother liquor consisted of *4a* and (1-$^2H_1$)-1,2-5,6-di-*O*-isopropylidene-α-D-glucofuranose (*3a*), as seen from a ^1H NMR spectrum. The products were characterized through their ^1H, ^2H and ^{13}C NMR parameters and the isotopic purity of the compounds proved to be better than 90 %. Alternatively, *3a* and *4a* could be separated by preparative thin-layer chromatography using ethylacetate–pentane (3:7) as eluant after acetylation with acetic anhydride in pyridine. Similar results were obtained when *1* (200 mg) was isomerized with SGI (20 mg) under the same reaction conditions as mentioned above. *4a* could be isolated (29 mg, 10 % yield), m.p. 117 °C. The purity of the compound was checked by ^1H and ^{13}C NMR spectroscopy and the isotopic purity proved to be better than 95 %.

Isomerization of (2-$^2H_1$)-D-glucose (5) with IGI and SGI. Compound *5* (302 mg) was treated with IGI (90 mg) as described above and *8a* could be crystallized out after work-up in 8 % (36 mg) yield, m.p. 113–115 °C. Penta-*O*-acetyl-(2-$^2H_1$)-α-D-glucopyranose could be isolated in 14 % (90 mg) yield from the mother liquor after acetolysis. The products were characterized through their ^1H, ^2H and ^{13}C NMR parameters and the isotopic purity proved to be better than 90 %. Similar results were obtained when SGI was used as enzyme and *8a* was isolated in 11 % yield. The isotopic purity was better than 95 % as seen from a ^1H NMR spectrum.

Isomerization with IGI or SGI. Substrate (150–500 mg) was dissolved in H_2O (or D_2O) (2 ml), IGI (or SGI) (100 mg) added, followed by pH or pD adjustment (if applicable) with sodium carbonate. The mixture was stirred at 65 °C for the time in-

dicated in Table 1 or 2. The reaction mixtures were analyzed by ^{13}C NMR spectroscopy after filtration through carbon. The integrals were considered to be accurate with 5 %.[19]

*Synthesis of substrates. 3-Deoxy-*D-ribo-*hexose.* Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside [20] (1.0 g) was hydrolyzed with HCl (0.1 M, 20 ml) at 100 °C for 48 h. The reaction mixture was neutralized with a mixed bed ion exchange resin and evaporated leaving 3-deoxy-D-*ribo*-hexose as a syrup, which was characterized through its ^{13}C NMR data.[21]

*4-Deoxy-*D-xylo-*hexose.* Methyl 4-deoxy-α-D-*xylo*-hexopyranoside (0.554 g)[22] was hydrolyzed and worked up as described above. The syrupy 4-deoxy-D-*xylo*-hexose was characterized through its ^{13}C NMR data (D_2O). β-anomer: 97.1 ppm (C-1), 73.3 (C-2), 71.3 (C-3), 35.1 (C-4), 76.9 (C-5), 64.5 (C-6). α-anomer: 93.6 ppm (C-1), 69.3 (C-2), 67.8 (C-3), 35.1 (C-4), 74.1 (C-5), 64.6 (C-6).

*5-Deoxy-*D-xylo-*hexose.* 5-Deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose[23] (180 mg) was hydrolyzed with acetic acid (50 %, 20 ml) at 100 °C for 1 h. The syrupy 5-deoxy-D-*xylo*-hexose was characterized through its ^{13}C NMR parameters (D_2O). α-anomer: 96.5 ppm (C-1), 77.0 (C-2,C-3), 81.6 (C-4), 31.9 (C-5), 59.5 (C-6). β-anomer: 102.5 ppm (C-1), 77.0 (C-2), 76.1 (C-3), 79.8 (C-4), 32.6 (C-5), 59.5 (C-6).

*4-O-Methyl-*D-*glucose.* 1.6-Anhydro-4-*O*-methyl-β-D-glucopyranose[24] (917 mg) was hydrolyzed with 1M H_2SO_4 (15 ml) at 100 °C for 4 h, filtered through carbon and neutralized with a mixed bed ion exchange resin. Evaporation gave 4-*O*-methyl-D-glucose as a syrup which was characterized through its ^{13}C NMR parameters.[25]

*6-O-Methyl-*D-*glucose.* Methylation of 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose (1.45 g) with methyl iodide (6 ml) in dimethylformamide (20 ml) using barium oxide as base at 80 °C for 3 h gave after work-up 0.91 g (60 %) of crystalline 3.5-*O*-benzylidene-1,2-*O*-isopropylidene-6-*O*-methyl-α-D-glucofuranose, m.p. 88–90 °C. Hydrolysis with 1M H_2SO_4 gave after neutralization with a mixed bed ion exchange resin and evaporation, 6-*O*-methyl-D-glucose (289 mg, 62 %), m.p. 139–143 °C. The product was further characterized through its ^{13}C NMR data.[25]

*3-O-Methyl-*D-*fructose.* 1,2-4,5-Di-*O*-isopropylidene-β-D-fructopyranose was methylated as described above in 70 % yield. Acidic hydrolysis of the syrup obtained gave 3-*O*-methyl-D-fructose also as a syrup, which was characterized through its ^{13}C NMR parameters (D_2O). β-anomer, pyranose: 64.7 ppm (C-1), 99.0 (C-2), 78.2 (C-3), 70.7 (C-4), 70.2 (C-5), 64.1 (C-6), 61.7 (OMe). α-anomer, furanose: 63.6 ppm (C-1), 104.8 (C-2), 92.0 (C-3), 74.7 (C-4), 81.9 (C-5), 61.7 (C-6), 58.9 (OMe). β-anomer, furanose: 64.5 ppm (C-1), 102.2 (C-2), 85.2 (C-3), 74.9 (C-4), 81.5 (C-5), 62.9 (C-6), 59.3 (OMe).

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Nickel-catalyzed Addition or Coupling Reactions of Grignard Reagents with Halopyrimidines

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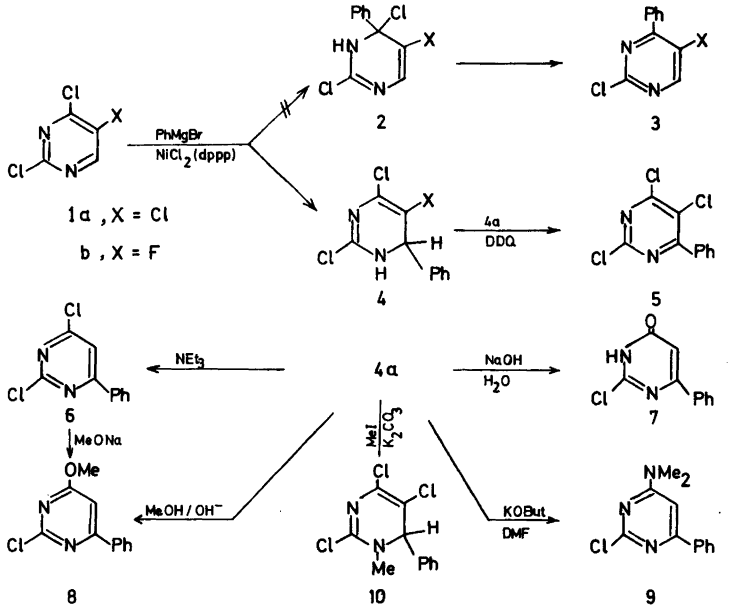
Phenylmagnesium bromide with $NiCl_2$(dppp) catalysis adds selectively to the unsubstituted position in 2,4-dichloro-5-chloro(fluoro)pyrimidine. X-Ray structure analysis of the hydrolyzed adduct shows an infinite chain by N1 – N3′ hydrogen bonding. The hydrolyzed adduct is aromatized by DDQ. With NEt_3, HCl elimination occurs whereby the 5-halogen atom is replaced by hydrogen; other basic nucleophiles give concurrent replacement of the chlorine in the 4-position. With the phenyl Grignard reagent and $NiCl_2$(dppp) exhaustive coupling results with 2,4-dichloro-6-phenylpyrimidine; with alkyl Grignard reagents and nickel catalysis selective coupling in the 4-position can be effected, but the reaction readily proceeds further to exhaustive coupling.

The coupling reaction between organometallic compounds and organic halides is now well recognized to be one of the most useful methods for the formation of $C-C$ bonds.[1] We are interested in the formation of $C-C$ bonds by coupling reactions to heterocyclic systems of the azine type. Herein we report on the reactivity of halopyrimidines[2,3] towards organonickel reagents as a route for the introduction of carbon substituents into the pyrimidine ring. The organonickel reagent was generated *in situ* as used by the addition of catalytic amounts of either dichlorobis(triphenylphosphine)nickel (II),[4] $NiCl_2(PPh_3)_2$, or dichloro-1,3-bis(diphenylphosphinopropane)nickel (II),[5] $NiCl_2$ (dppp), to a Grig-



Scheme 1.

0302-4369/83 $2.50

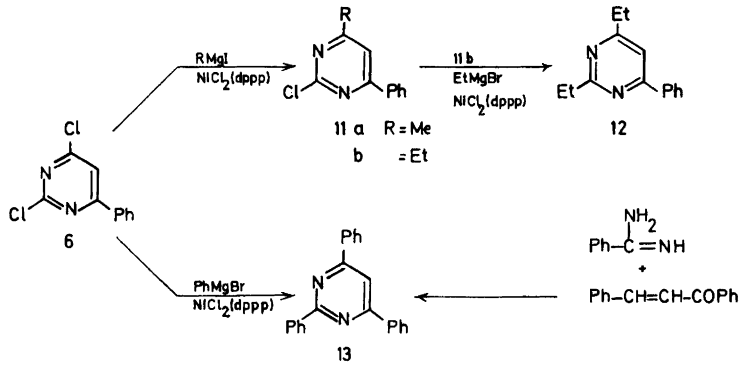
nard reagent. The latter nickel complex seems more efficient in our reactions and was most extensively investigated.

Phenylmagnesium bromide in the presence of either nickel complex, formed a 1:1 adduct (MS) with the trihalopyrimidines *1* rather than the expected 4-substitution product *3* as observed for alkyl Grignard reagents,[6] and also observed for the further Grignard reaction discussed below. The structures *2* and *4* must be considered for the adduct after hydrolysis. The latter (*4*) results from preferential addition of the organometallic reactants to unsubstituted carbon in electron deficient azine systems, a type of reaction presently being investigated.[7] The ^{1}H NMR signals from the heterocyclic ring in the adduct appear as a singlet at δ 5.2 (H-6) and as a broad singlet at 4.7 which rapidly fades out in deuterium oxide (*NH*). The ^{13}C NMR signals from the same moiety are found at 146.3 (C-2), 142.1 (C-4), 111.1 (C-5) and at 63.3 ppm (C-6), the latter having the line separation 144 Hz. Interpretation of these data is in accordance with an X-ray analysis (see below) which shows that the adduct has structure *4*. Chemically, the structure assignment has also been verified by dehydrogenation of the adduct from *1a* by means of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) which gave the phenylated trichloropyrimidine *5*; the latter has previously been described.[8]

When the adduct *4a* was heated with triethylamine in toluene, HCl elimination occurred. The product, which was isolated in 84 % yield, has been identified as compound *6*, which corresponds to chlorine-hydrogen exchange at C-5. Presumably the reaction is initiated by proton abstraction from the *NH* function in *4a*. The further reaction can be rationalized as reprotonation to the 5,6-dihydro-isomer which is the species undergoing HCl elimination to form *6*. The fluoride *4b* also gave *6* but only in 20 % yield presumably because HF elimination is more difficult. The structure *6* was assigned to the product by spectroscopy; ^{1}H NMR has H-5 at *ca.* δ 7.3 and ^{13}C NMR has the signals from the heterocyclic ring at 167.7, 162.9, 161.3 (C-2, C-4, C-6) and at 114.8 ppm (C-5), the latter having a line separation of 172 Hz which is consistent with a pyrimidine 5-position.[9] Chemically the assigned structure *6* has been verified by a further reaction with phenyl magnesium bromide with $NiCl_2$ (dppp) catalysis which gave 2,4,6-triphenylpyrimidine *13* (Scheme 2); for comparative purposes the latter was independently synthesized from benzylideneacetophenone and benzamidine.[10]

HCl elimination from *4a* also occurs in the presence of other bases. With aqueous sodium hydroxide or with alkaline methanol concurrent 4-substitution occurs with formation of the lactam *7* and the methoxy derivative *8*, respectively. Potassium *tert*-butoxide in DMF leads to the 4-dimethylamino derivative *9*. A further evidence for the structures assigned is available from the reaction of *6* with methanolic sodium methoxide; the chlorine in the more reactive 4-position is replaced with the formation of *8*. With methyl iodide and sodium carbonate in acetone the adduct *4a* is *N*-methylated without any elimination reaction occurring, the product being assigned structure *10*.

2,4-Dichloro-6-phenylpyrimidine (*6*) undergoes selective cross-coupling with primary alkyl Grignard reagents using $NiCl_2$ (dppp) catalysis to form the 4-alkyl derivatives *11*. This finding contrasts the behaviour of 2,4-dichloro-6-methylpyrimidine towards alkyl magnesium halide and catalysis in which case no selective cross-coupling could be



Scheme 2.

Table 1. Final fractional coordinates with estimated standard deviations. Hn are bonded to Cn. HNn are bonded to Nn.

ATOM	X	Y	Z
CL1	.32948(8)	-.15226(5)	.01931(2)
CL2	.26895(10)	-.34741(5)	.16026(3)
CL3	.06510(10)	-.21629(5)	.20078(3)
CL4	-.09966(8)	.03483(5)	.06193(2)
CL5	.43693(9)	.06440(5)	.04816(3)
CL6	.41605(8)	.26130(5)	.05694(2)
N1	.29265(26)	-.24011(15)	.09261(9)
N2	.17818(27)	-.11129(17)	.08462(9)
N3	.16996(27)	.06077(16)	.05555(9)
N4	.03083(28)	.17798(16)	.06547(9)
C1	.26049(32)	-.17100(19)	.07164(10)
C2	.23147(34)	-.25182(19)	.13419(10)
C3	.15028(33)	-.19717(19)	.15210(10)
C4	.12362(34)	-.11198(20)	.13098(10)
C5	.18823(33)	-.04312(20)	.16278(10)
C6	.10885(38)	.08024(22)	.19198(11)
C7	.16923(39)	.16218(22)	.22139(12)
C8	.30795(38)	.18214(22)	.22134(12)
C9	.38765(38)	.13843(23)	.19208(12)
C10	.32845(36)	-.02397(22)	.16355(12)
C11	.35464(31)	.09729(19)	.06072(10)
C12	.28312(33)	.11437(20)	.05536(10)
C13	.27557(32)	.19681(20)	.05983(10)
C14	.14146(34)	.24065(20)	.06661(11)
C15	.15232(32)	.29069(19)	.11236(11)
C16	.14504(40)	.25212(25)	.15527(12)
C17	.15855(44)	.29810(27)	.19664(14)
C18	.17976(42)	.38228(28)	.19565(16)
C19	.18795(42)	.42083(26)	.15315(17)
C20	.17251(38)	.37590(23)	.11128(14)
HN2	.1762(33)	-.0628(22)	.0685(11)
HN4	-.0573(39)	.1962(21)	.0719(12)
H4	.0254(32)	-.1043(18)	.1245(9)
H6	.0156(37)	-.0120(21)	.1919(11)
H7	.1138(34)	.0917(21)	.2400(11)
H8	.3447(36)	.1249(23)	.2411(12)
H9	.4914(37)	.0520(20)	.1909(11)
H10	.3839(35)	-.0545(21)	.1455(11)
H14	.1185(30)	.2756(19)	.0395(10)
H16	.1268(42)	.1934(27)	.1568(14)
H17	.1553(42)	.2699(26)	.2269(15)
H18	.1915(46)	.4160(28)	.2238(16)
H19	.1994(40)	.4773(26)	.1506(13)
H20	.1744(31)	.4025(20)	.0838(11)

achieved.[11] With two molar equivalents of the ethyl Grignard reagent, *6* gave the 2,4-dialkylated product *12* in 76 % yield. Using phenylmagnesium bromide and $NiCl_2$ (dppp) controlled stepwise substitution could not be effected; in the presence of less than two molar equivalents of the Grignard reagent, unreacted dichloride *6* and the triphenylpyrimidine *13* were the products.

X-Ray crystallographic analysis of 4. The crystals are monoclinic with cell dimensions $a=9.677(4)$ Å, $b=16.138(7)$ Å, $c=28.54(1)$ Å, $\beta=96.47(3)°$, space group $C2/c$, and $Z=16$ ($D_m=1.55$ gcm^{-3}, $D_X=1.56$ gcm^{-3}. The structure was solved by direct methods[12] and refined by full-matrix least squares technique[13,*] to an *R*-value of 3.7 % ($R_W=4.2$ %) for 3038 reflections measured on an automatic four-circle diffractometer at −150 °C. Final fractional coordinates and bond distances and angles with estimated standard deviations may be found in Table 1 and Table 2, respectively. Fig. 1 is a schematic drawing which shows the numbering of atoms and the hydrogen bonding system. The hydrogen bonds N2···N3 [2.900(3) Å] and N4···N1′ [2.844(3) Å] form infinite chains. The bond distances of Table 2 have normal values within estimated limits of errors. Bond angles are also normal except for those at C1 (128.3°) and C11 (128.5°), which are somewhat larger than expected.

Lists of thermal parameters and observed and calculated structure factors are available from P. Groth.

EXPERIMENTAL

^{1}H NMR data were recorded on a Varian A-60 spectrometer and the ^{13}C data on an FX-60 Fourier transform NMR spectrometer. The mass spectra were recorded on an MM70-70F VG Micromass spectrometer at 70 eV; the data are reported as MS [70 eV; *m/z* (% rel. int.)].

General procedure for the Grignard coupling reactions. The Grignard reagent (12 mmol) in ether (12 ml) was added with stirring over 5 min at 0 °C to a solution of $NiCl_2$ (dppp)[5] or$NiCl_2$ (PPh_3)[4] (0.2 mmol) and the halopyrimidine[2,3] *1* (10 mmol) in dry ether (100 ml) in an N_2-atmosphere. The nickel complex reacted at once with the Grignard reagent. When the addition was complete, the reaction mixture was removed from the cooling bath; after a few minutes an exothermic reaction ensued which turned the mixture dark yellow. The mixture was allowed to stand at room temperature overnight, it was then heated under reflux for 10 h, the cooled mixture cautiously hydrolyzed with 2 M HCl, the organic layer separated, the aqueous layer extracted with ether (3 × 25 ml), the combined ether solutions washed successively with water, saturated aqueous $NaHCO_3$ and water and the dried ($MgSO_4$) solution evaporated. The remaining product was further purified by recrystallization or chromatography.

6-Phenyl-2,4,5-trichloro-1,6-dihydropyrimidine 4a. *Method A.* From Mg (0.28 g, 0.012 gram atom) bromobenzene (1.8 g, 0.012 mol), $NiCl_2$(dppp) (108 mg, 0.02 mmol), and 2,4,5-trichloropyrimidine

* All programs used in the X-ray work (except those for phase determination) are included in this reference.

Table 2. Bond distances and angles with estimated standard deviations.

DISTANCE	(Å)	DISTANCE	(Å)
CL1 - C1	1.731(3)	CL2 - C2	1.735(3)
CL3 - C3	1.723(3)	CL4 - C11	1.737(3)
CL5 - C12	1.729(3)	CL6 - C13	1.724(3)
N1 - C1	1.289(4)	N1 - C2	1.399(4)
N2 - C1	1.331(4)	N2 - C4	1.480(4)
N3 - C11	1.287(4)	N3 - C12	1.398(4)
N4 - C11	1.334(4)	N4 - C14	1.473(4)
C2 - C3	1.324(4)	C3 - C4	1.514(4)
C4 - C5	1.525(4)	C5 - C6	1.387(5)
C5 - C10	1.392(5)	C6 - C7	1.392(5)
C7 - C8	1.375(5)	C8 - C9	1.398(5)
C9 - C10	1.381(5)	C12 - C13	1.341(5)
C13 - C14	1.512(4)	C14 - C15	1.530(4)
C15 - C16	1.384(5)	C15 - C20	1.392(5)
C16 - C17	1.389(5)	C17 - C18	1.376(6)
C18 - C19	1.375(6)	C19 - C20	1.392(6)

ANGLE	(°)	ANGLE	(°)
C1 - N1 - C2	114.1(3)	C1 - N2 - C4	122.0(3)
C11 - N3 - C12	114.1(3)	C11 - N4 - C14	122.7(3)
CL1 - C1 - N1	117.1(2)	CL1 - C1 - N2	114.6(2)
N1 - C1 - N2	128.3(3)	CL2 - C2 - N1	113.3(2)
CL2 - C2 - C3	122.2(2)	N1 - C2 - C3	124.4(3)
CL3 - C3 - C2	123.5(3)	CL3 - C3 - C4	114.1(2)
C2 - C3 - C4	122.3(3)	N2 - C4 - C3	107.5(3)
N2 - C4 - C5	111.3(3)	C3 - C4 - C5	112.7(3)
C4 - C5 - C6	120.5(3)	C4 - C5 - C10	120.4(3)
C6 - C5 - C10	119.0(3)	C5 - C6 - C7	120.1(3)
C6 - C7 - C8	120.7(3)	C7 - C8 - C9	119.4(3)
C8 - C9 - C10	119.9(3)	C5 - C10 - C9	120.8(3)
CL4 - C11 - N3	116.9(2)	CL4 - C11 - N4	114.6(2)
N3 - C11 - N4	128.5(3)	CL5 - C12 - N3	113.5(2)
CL5 - C12 - C13	122.2(3)	N3 - C12 - C13	124.3(3)
CL6 - C13 - C12	122.9(3)	CL6 - C13 - C14	114.7(2)
C12 - C13 - C14	122.3(3)	N4 - C14 - C13	108.0(3)
N4 - C14 - C15	111.2(3)	C13 - C14 - C15	112.3(3)
C14 - C15 - C16	120.8(3)	C14 - C15 - C20	120.0(3)
C16 - C15 - C20	119.1(3)	C15 - C16 - C17	120.2(4)
C16 - C17 - C18	120.7(4)	C17 - C18 - C19	119.3(4)
C18 - C19 - C20	120.7(4)	C15 - C20 - C19	119.9(4)

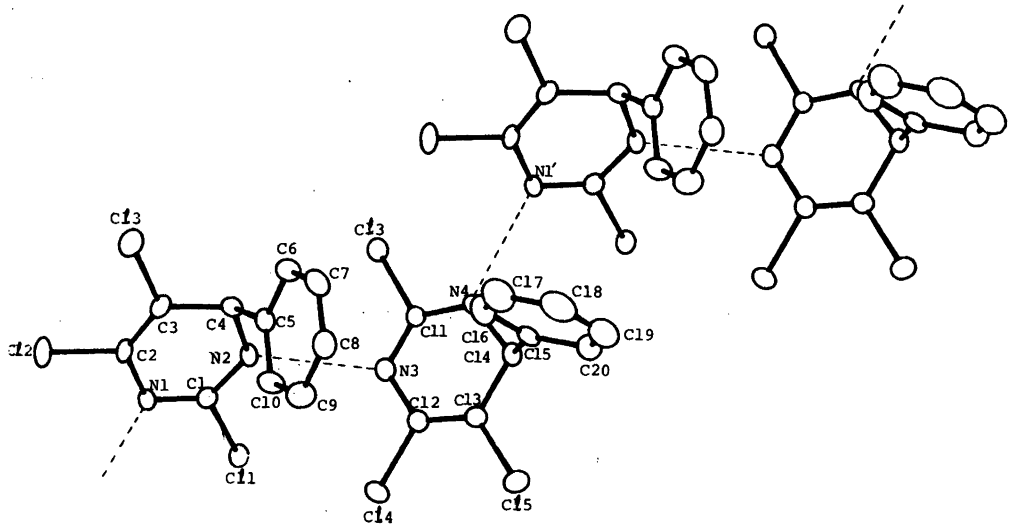


Fig. 1. Schematic drawing showing the numbering of atoms and the hydrogen bonding system.

(1.8 g, 0.01 mol). Colourless crystalline material; yield 82 %, m.p. 146 °C (MeOH). Anal. $C_{10}H_7Cl_3N_2$: C, H. ^{1}H NMR ($CDCl_3$): δ 4.7 (NH, exchanged in D_2O), 5.2 (H-6) and 7.3 (Ph). ^{13}C NMR (Me_2CO-d_6): δ146.3 (C-2), 142.1 (C-4); 135.3, 133.5, 130.1, 128.3 (Ph), 111.1 (C-5), 63.3 (C-6, $J_{C,H}$ 144 Hz). IR (KBr): 3100 cm^{-1} (NH).

Method B. The experimental conditions were the same as in method A except that the catalyst was changed; here was used $NiCl_2$ (PPh_3) (130 mg, 0.2 mol; yield of *4a* was 72 %.

2,4-Dichloro-5-fluoro-6-phenyl-1,6-dihydropyrimidine 4b. From Mg (0.28 g, 0.012 gram atom), bromobenzene (1.8 g, 0.012 mol), $NiCl_2(PPh_3)$ (130 mg, 0.2 mmol) and 2,4-dichloro-5-fluoropyrimidine (1.66 g, 0.01 mol). Colourless crystalline material; yield 66 %, m.p. 148 °C (CH_2Cl_2). Anal. $C_{10}H_7Cl_2FN_2$: C, H. ^{1}H NMR (DMSO-d_6): δ 3.4 (NH, exchanged in D_2O), 5.65 (H-6, $J_{H,F}$ 4.5 Hz) and 7.35 (Ph). IR (KBr): 3160 cm^{-1} (NH). MS: 246/244 (18/30, M), 245/243 (17/21), 211/209 (9/30), 169/167 (64/100) 133/131 (12/33), 78 (66), 77(18).

6-Phenyl-2,4,5-trichloropyrimidine 5. A solution of 6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine (1.3 g, 5 mmol) and DDQ (1.13 g) in dioxane (40 ml) was stirred at room temperature for 48 h. The hydroquinone was then filtered off, the filtrate evaporated at reduced pressure and the residual product purified by recrystallization; yield 45 %, m.p. 90 °C (MeOH).[8] ^{1}H NMR (DMSO-d_6): δ 7.6 (m, Ph).

2,4-Dichloro-6-phenylpyrimidine 6. A solution of 6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine (2.6 g, 10 mmol) and triethylamine (1.2 g, 12 mmol) in toluene (100 ml) was heated under reflux for 5 h. The cold reaction mixture was filtered, the filtrate evaporated at reduced pressure and the remaining product purified by preparative TLC (ether – light petroleum, 1:4); yield 84 %, m.p. 83 °C (heptane). Anal. $C_{10}H_6Cl_2N_2$: C, H. ^{1}H NMR (CCl_4): δ 7.3 (m, 4H; H-5 and *m,p*-Ph), 7.9 (m, 2H; *o*-Ph). ^{13}C NMR (CCl_4): δ 167.7 (C-2), 162.9 and 161.3 (C-4, C-6), 134.3, 132.2, 129.0, 127.5 (Ph), 114.8 (C-5, $J_{C,H}$ 172 Hz). MS: 226/224 (6/100, M), 191/189 (22/67), 164/162 (6/12), 154 (2), 128 (66), 127 (13), 77 (36). Compound *6* was also obtained in 20 % yield when the 5-fluoro analogue *4b* was used instead of *4a* in the above reaction.

2-Chloro-6-phenylpyrimidin-4-one 7. 1 M sodium hydroxide (10 ml) was added to a solution of 6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine (1,3 g, 5 mmol) in acetonitrile (30 ml) and the mixture heated at 80 °C for 2 h. The volume was then concentrated to *ca.* one fourth, the solution acidified (HCl) and the pricipitate purified by recrystallization; yellow crystalline material in 58 % yield, m.p. 185 °C (MeOH). Anal. $C_{10}H_7ClN_2O$: C, H. ^{1}H NMR (DMSO-d_6): δ 4.0 (NH, exchanged by D_2O), 7.03 (H-5), 7.4 and 7.9 (m, Ph) IR (KBr): 1648 cm^{-1} (CO). MS: 208/206 (31/100, M), 180/178 (5/15), 171 (34), 116 (16), 103 (36), 77 (18).

2-Chloro-4-methoxy-6-phenylpyrimidine 8. *Method A.* 1M sodium hydroxide (10 ml) was added to a solution of 6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine (1,3 g, 5 mmol) in methanol (20 ml) and the mixture heated under reflux for 3 h. The mixture was then concentrated to half its original volume and the precipitated product collected and recrystallized from methanol; colourless material in 86 % yield, m.p. 105 °C. Anal. $C_{11}H_9ClN_2O$: C, H. ^{1}H NMR ($CDCl_4$): δ 3.97 (OMe), 6.80 (H-5), 7.2 and 7.9 (m, Ph). MS: 222/220 (30/100, M), 221/219 (37/71), 191/189 (20/89), 155 (51), 128 (39), 102 (30), 77 (29).

Method B. A solution of 2,4-dichloro-6-phenylpyrimidine (1.13 g, 5 mmol) in methanol (15 ml) was added to a solution of sodium methoxide (from 0.13 g Na in 25 ml of MeOH). The mixture was heated to 40 °C and stirred at this temperature for 1 d. The mixture was then filtered, the filtrate evaporated, a little water added, the mixture extracted with ether, the solution evaporated and the product crystallized as above; yield 62 %.

2-Chloro-4-dimethylamino-6-phenylpyrimidine 9. A mixture prepared from potassium *tert*-butoxide (0.26 g, 2.2 mmol) and 6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine (0.5 g, 2 mmol) in DMF (30 ml) was heated at 140 °C for 3 h. The solvent was then distilled off at reduced pressure, the residue extracted with ether, the ether extracts washed with water, the dried ($MgSO_4$) ethereal solution evaporated and the residual material purified by TLC (Et_2O: pentane, 1:4); yellow crystalline material in 38 % yield, m.p. 122 °C (pentane). ^{1}H NMR (CCl_4): δ 3.30 (NMe_2), 6.8 (H-5), 7.4 and 7.9 (m, Ph). MS: 235/233 (26/72, M), 220/218 (25/75), 206/204 (30/89), 191/189 (4/6), 155 (30), 128 (89), 77 (31).

1-Methyl-6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine 10. A mixture of methyl iodide (1.0 g, 7 mmol), 6-phenyl-2,4,5-trichloro-1,6-dihydropyrimidine (1.3 g, 5 mmol) and potassium carbonate (0.69 g) in anhydrous acetone (100 ml) was heated under reflux for 24 h. The solvent was then distilled off, the residue triturated with water, and then recrystallized from methanol; colourless crystalline material in 64 % yield, m.p. 170 °C (dec.). Anal. $C_{11}H_9Cl_3N_2$: C, H. ^{1}H NMR (CCl_4): δ 2.93 (Me), 4.90 (H-6), 7.20 (Ph). MS: 276/274 (12/13, M), 241/239 (25/36), 203/201 (14/30), 199/197 (92/100), 128 (13), 77 (12).

2-Chloro-4-methyl-6-phenylpyrimidine 11a. Methylmagnesium iodide (6 mmol) in anhydrous ether (25 ml) was added at 0 °C to an ethereal suspension of 2,4-dichloro-6-phenylpyrimidine (1.1 g, 5 mmol) and $NiCl_2$(dppp) (54 mg, 0.1 mmol). The reaction mixture was treated in the same way as described

above for Grignard reactions; yield 49 %, m.p. 53 °C. ^{1}H NMR (CCl_4): δ 2.55 (Me), 7.4 (m, 4 H; H-5 and 3H, m, *p*-Ph), 7.9 (m, 2H, *o*-Ph). MS 206/204 (29/100, M), 191/189 (4/12), 169 (24), 141 (23), 129/127 (9/4), 77 (15).

2-Chloro-4-ethyl-6-phenylpyrimidine 11b was prepared as *11a* above using ethylmagnesium bromide. The product was purified by preparative TLC on silica (Et_2O–pentane, 1:4), yield 55 % of a colourless liquid. ^{1}H NMR (CCl_4): δ 1.33 and 2.77 (Et), 7.13 (H-5), 7.3 (m, 3H; m, *p*-Ph).

2,4-Diethyl-6-phenylpyrimidine 12 was prepared as above using 2 molar equivalents of ethylmagnesium bromide. The product was isolated after preparative TLC on silica (Et_2O – pentane); yield 76 % of a colourless liquid. ^{1}H NMR (CCl_4): δ 1.20 and 3.62 (Et), 6.70 (H-5), 7.2 and 7.8 (m, Ph).

2,4,6-Triphenylpyrimidine 13. Phenylmagnesium bromide, prepared from Mg (0.28 g, 0.012 gram atom) and bromobenzene (1.8 g, 0.012 mol), in anhydrous ether (25 ml) was gradually added to a solution of 2,4-dichloro-6-phenylpyrimidine (1.1 g, 5 mmol) and $NiCl_2$ (dppp) (54 mg, 0.1 mmol) in anhydrous ether (50 ml) at 0 °C. The reaction was worked up in the usual way and the product crystallized as white needles from heptane; yield 95 %, m.p. 190 °C.[10] Anal. $C_{22}H_{16}N_2$: C, H. ^{1}H NMR (CCl_4): δ 7.8 (H-5), 7.4, 8.2, 8.6 (3 × Ph). MS: 308 (100, M), 205 (55), 102 (65), 77 (14).

When one equivalent of the Grignard reagent was used the triphenylpyrimidine *13* and the dichloride starting material *6* were isolated from the reaction in 55 and 40 % yields, respectively.

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Synthesis and Isomerization Studies of 2-Alkenylthiopyrimidines and 2-Alkynylthiopyrimidines and Their *S*-Oxides

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Selective oxidations for the preparation of either sulfoxides or sulfones of 2-allyl, 2-propadienyl- and 2-propargylthio-5-chloropyrimidines are described. The 2-propargylsulfone as well as its sulfide is easily isomerized to the isomeric allenes. The unsaturated sulfides are conveniently prepared in a condensation reaction between 1,3-*bis*-*N*,*N*-dimethylamino-2-chlorotrimethinium perchlorate and the respective isothiourea.

Recently we reported syntheses and properties of some α-haloalkyl pyrimidine sulfides and their oxides.[1] Herein we describe studies of propargyl and alkenyl 2-pyrimidine sulfides and of their reactivity towards oxidizing agents.

2-Alkylthiopyrimidines can be prepared by *S*-alkylation of 2-mercaptopyrimidines,[2a] by the reaction of alkylmercaptide salts with pyrimidines containing mobile 2-substituents,[2b] or by the condensation of a three-carbon unit with *S*-alkylisothioureas.[2c] Thus, 2-propargylthiopyrimidine *4a* is formed in good yield from the 2-mercaptopyrimidine *1* and propargyl bromide under basic conditions. The *S*-vinyl derivative *4d* was prepared in a Michael reaction between *1* and the activated triple bond in ethynyl methyl ketone, this reaction being analogous to those we have reported for pyridine-2-thiones and activated acetylenic compounds.[3] Compound *4d* was obtained as a 1:1 stereoisomer mixture.

Since the route to the mercaptide starting material *1* is relatively lengthy,[4] condensation reactions between isothioureas and the trimethinium salt *2* have been explored as an alternative route to the preparation of *4*. The propargyl isothiourea *3a* can be prepared by alkylation of thiourea with propargyl bromide in ethanol.[5] We find, however, that if the reaction at 80 °C is left for more than 5–10 min increasing amounts of the isomeric propadienylisothiourea *3c* are formed. In 1,2-dimethoxyethane solution, however, no isomerization was observed when the reaction mixture was kept at 50 °C for 24 h, the yield being in excess of 90 %.

The trimethinium salt *2* is equivalent to 2-chloromalondialdehyde. It is readily available and is an excellent reactant in the formation of 5-chloropyrimidines by condensation reactions.[6,7] Thus both the propargyl- and the allyl-isothiourea *3* underwent condensation with the trimethinium salt *2* under basic conditions to form *4*. Heating is to be avoided in the case of the propargylisothiourea, because of its ready isomerization with formation of the 2-propadienylthiopyrimidine *4c*. Only the latter isomer together with decomposition products was formed in the presence of excess base. The ready base-catalyzed isomerization of propargyl sulfides has been rationalized by good stabilization of the intermediate carbanion by the ability of sulfur to expand its outer electron shell from eight to ten electrons.[8] The deprotonation rate is much higher for propargyl than for propadienyl thioethers,[9] and hence it should be possible to obtain relatively pure propadienylthioethers. This proved to be the case for the propadienyl thioether *4c* which was readily prepared on treatment of the propargyl thioether *4a* with 0.1 equivalent of *tert*-butoxide in *tert*-butanol. The crude product of the isomer *4c* also contained some ring-opened substance formulated as the isomer *5* (NMR; 15 %). With potassium *tert*-butoxide added to ethanol, however, there was no detectable isomerization of *4a* to *4c* after the solution had been kept at 60 °C for 2 days. This is notable since phenyl propargyl sulfide is rapidly isomerized (30 min.; 89 %) under

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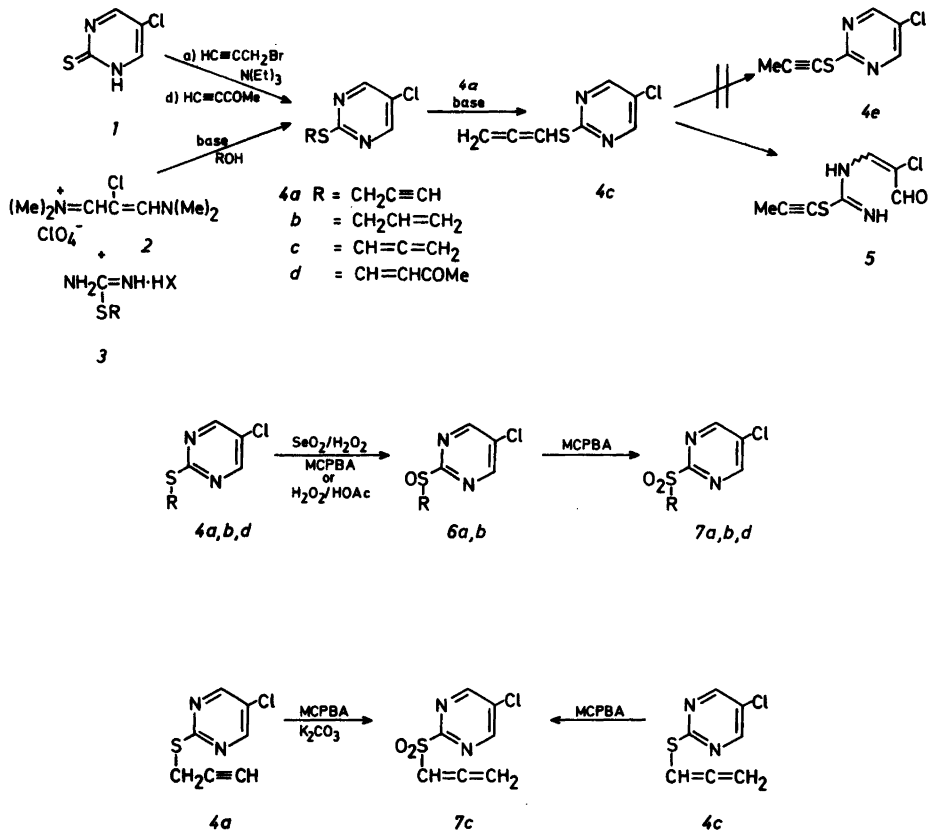

milder conditions.[10] This difference, perhaps in the opposite direction of what was to be expected on electronic grounds, may be rationalized by involving the lone pair of electrons on the nitrogen atoms; charge repulsion by the electrons will reduce the rate of proton abstraction by the approaching base. Support for this view can be found in the arguments used to explain that nucleophilic substitution in the pyrimidine 2-position is slower than in the 4-position.[11,12]

In no case did the isomerization reaction proceed to the 1-propynyl sulfide *4e* in contrast to phenyl propargyl sulfide which is readily isomerized to phenyl 1-propynyl sulfide.[10]

Acetylenic sulfoxides and sulfones can be prepared by oxidation of the corresponding sulfides.[13,14] In the case of *4a* formation of its sulfoxide *6a* was very slow when using sodium metaperiodate in methanol; selective oxidation with *m*-chloroperbenzoic acid (MCPBA) was difficult to achieve. The cleanest oxidation was observed by the use of selenium dioxide in hydrogen peroxide when the sulfoxide *6a* was isolated in satisfactory yield (63 %).

In the oxidation of the propargyl sulfide *4a* to sulfone using MCPBA, either the propargyl sulfone *7a* or the propadienyl sulfone *7c* could be isolated depending on the isolation procedure; neutralization of the reaction mixture with 1 M sodium bicarbonate gave the propargyl sulfone *7a* whereas treatment with saturated potassium carbonate solution gave the propadienyl sulfone *7c*, and with 1 M sodium carbonate solution a mixture of the isomers was obtained. Very ready base-catalyzed isomerization is also what has been reported for aryl propargyl sulfones,[13] and it has been shown that aryl propadienyl sulfones are thermodynamically more stable than their acetylenic isomers.[15] The propadienyl sulfone *7c* can also be prepared by direct oxidation of the allene *4c* using MCPBA.

Selective sulfoxide formation from the allyl sulfide *4b* was readily achieved using hydrogen peroxide in acetic acid as reported for simple allyl phenyl

sulfides.[16] For the selective oxidation to the sulfone *7b*, MCPBA rather than hydrogen peroxide in acetic acid[17] was the preferred reagent.

The *S*-vinyl derivative *4d* can also be selectively *S*-oxidized. Thus its 1:1 stereoisomer mixture with MCPBA was converted to the sulfone *7d* and the (*Z*)- and (*E*)-isomers were separated by preparative TLC.

EXPERIMENTAL

2-Propargylthiouronium bromide 3a. A mixture of propargylbromide (1.19 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in dimethoxyethane (20 ml) was heated at 50 °C for 15 h. The solvent was distilled off and the residue washed with ether. The product was recrystallized from acetone–pentane; yield 1.85 g (95 %), m.p. 124 °C.[10]

5-Chloro-2-propargylthiopyrimidine 4a. *Method A.* Potassium *tert*-butoxide (4.48 g, 40 mmol) in abs. ethanol (40 ml) was added dropwise over 10 min. to a mixture of 2-propargylthiouronium bromide (3.92 g, 20 mmol) and 1,3-bis-*N*,*N*-dimethylamino-2-chlorotrimethinium perchlorate[18] (5.33 g, 20 mmol) in abs. ethanol (80 ml). The mixture was stirred for 48 h at room temperature before the solvent was distilled off. The residue was washed with water, extracted into chloroform, dried ($MgSO_4$) and evaporated. The product was recrystallized from methanol–water; yield 2.44 g (66 %), m.p. 66 °C. Anal. $C_7H_5ClN_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.16 (HC, t, *J* 2 Hz), 3.88 ($\equiv CCH_2$-, d, *J* 2 Hz), 8.50 (H-4, H-6). IR (KBr): 3300 ($\equiv$CH), 1550 cm^{-1} (pyrimidine). MS[70 eV, *m/z* (% rel. int.)]: 186/184 (33/100, M), 150(33), 114(28), 71(30), 39(56).

Method B. A mixture of 5-chloropyrimidine-2-thione (0.73 g, 5 mmol) and triethylamine (0.70 ml, 5 mmol) was stirred together in dichloromethane (40 ml) for 5 min before propargyl bromide (0.71 g, 6 mmol) was added. The mixture was stirred at room temperature for 1 h and subsequently the solvent was evaporated. The residue was triturated with water (20 ml), and the solid recrystallized from methanol; yield: 0.80 g (87 %).

2-Allylthio-5-chloropyrimidine 4b. 1,3-Bis-*N*,*N*-dimethylamino-2-chlorotrimethinium perchlorate[18] (8.0 g, 31 mmol) and 2-allylthiouronium bromide[19] (6.90 g, 35 mmol) were dissolved in methanol (80 ml) and methanolic 1.67 M sodium methoxide (35 mmol) was added. The mixture was stirred at room temperature for 30 min before additional sodium methoxide solution (19 ml; 31 mmol) was added. The mixture was then heated under reflux for 2.5 h, the solvent distilled off, water (100 ml) added to the residue, the mixture extracted with chloroform, the dried ($MgSO_4$) chloroform solution evaporated, and the residue distilled; yield 5.0 g (86 %), b.p. 62–64 °C/0.1 mmHg. Anal. $C_7H_7ClN_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 3.76 (CH_2-S), 5.0–6.4 (vinyl), 8.40 (H-4, H-6). IR (film): 1560 and 1530 cm^{-1} (pyrimidine). MS[70 eV, *m/z* (% rel. int.)]: 188/186 (9/25, M) 173(38), 171(100), 155(16), 153(46), 118(22), 114(21).

5-Chloro-2-propadienylthiopyrimidine 4c. Potassium *tert*-butoxide (44 mg, 0.4 mmol) was added to a solution of 5-chloro-2-propargylthiopyrimidine (0.74 g, 4 mmol) in *tert*-butanol. The mixture was stirred at room temperature for 90 min before water (100 ml) was added and the solution was extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated and the crude product purified on a silica gel column (chloroform – light petroleum; 1:1); yield 0.40 g (54 %), m.p. 32–34 °C. ^{1}H NMR ($CDCl_3$): δ 5.00 (H_2C=, *J* 6 Hz), 6.45 (=CH-S, *J* 6 Hz), 8.36 (H-4, H-6). IR (KBr): 1940 (allene), 1550 and 1520 cm^{-1} pyrimidine). MS[70 eV, *m/z* (% rel. int.)]: 154 (10, M), 149(1), 114(2), 71(4), 70(2), 43(15). The product was chromatographically homogeneous and was directly oxidized to the corresponding sulfone because it appeared to decompose (colouration) on storage.

5-Chloro-2-(3-oxobuten-1-yl)thiopyrimidine 4d. 3-Butyn-2-one[20] (0.34 g, 5 mmol) in chloroform (25 ml) was added dropwise over 10 min at room temperature to a stirred suspension of 5-chloropyrimidine-2-thione (0.66 g, 4.5 mmol) in chloroform (25 ml). The mixture was stirred for an additional 10 min before the solvent was evaporated. The residue was crystallized from methanol; yield 0.70 g (72 %), m.p. 89 °C. Anal. $C_8H_7ClN_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.20 (Me-(*Z*)), 2.23 (Me-(*E*)), 6.52 (Hα, d, *J* 18 Hz (*E*)), 6.58 (Hα, d, *J* = 10 Hz (*Z*)), 8.43 (Hβ, d, *J* 10 Hz) (*Z*)), 8.57 (Hβ, d, *J* 18 Hz (*E*)), 8.62 (H-4, H-6); (*E*)/(*Z*) = 1:1. IR (KBr): 1660 cm^{-1} (CO). MS[70 eV, *m/z* (% rel. int.)] 216/214 (2/6, M), 199(4), 173(37), 171(100).

1-(2-Chloro-3-oxo-1-propenyl)-2-(1-propynyl)-isothiourea 5. Potassium *tert*-butoxide (0.11 g, 1 mmol) was added to a solution of 5-chloro-2-propargylthiopyrimidine (0.18 g, 1 mmol) in *tert*-butanol (10 ml). The mixture was stirred at room temperature for 2 h, neutralized with acetic acid, water (30 ml) added and the mixture extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated and the residue purified on a silica gel column (chloroform); yield 0.18 g (89 %), m.p. 182 °C. Anal. $C_7H_7ClN_2OS$: C, H. ^{1}H NMR ($CDCl_3$–acetone-d_6): δ 2.40 (Me), 6.40 (s, 1 H), 8.20 (s, 1 H), 9.28 (CHO). IR (KBr): 3200 and 3100 (NH), 1660 cm^{-1} (HC=O). MS[70 eV, *m/z* (% rel. int.)]: 204/202 (10/29, M), 185(9), 167(23), 140(10), 139(100), 114(12), 99(12), 72(19), 71(30).

5-Chloro-2-propargylsulfinylpyrimidine 6a. A mix-

ture of selenium dioxide (0.45 g, 4 mmol) and 35 % hydrogen peroxide (0.40 g, 4 mmol) in water (2.5 ml) was added to a solution of 5-chloro-2-propargylthiopyrimidine (0.78 g, 4 mmol) in methanol (10 ml). The mixture was stirred at room temperature for 18 h before water (50 ml) saturated with sodium chloride was added, and the mixture was subsequently extracted with chloroform (3 × 20 ml). The dried ($MgSO_4$) chloroform solution was evaporated and the residue recrystallized from chloroform–light petroleum; yield 0.50 g (63 %), m.p. 92 °C. Anal. $C_7H_5ClN_2OS$: C, H. ^1H NMR ($CDCl_3$): δ 2.28 (HC≡, t, J 2 Hz), 3.87 and 4.09 (–CH_2-SO, J15 Hz), 8.85 (H-4, H-6), IR (KBr): 3235 (HC≡), 2110 and 2100 cm^{-1} (–C≡C–). Ms[70 eV, m/z (% rel. int.)]: 200 (13, M), 199(19), 173(33), 171(100), 146(25), 114(30), 113(31), 111(47).

2-Allylsulfinyl-5-chloropyrimidine 6b. 30 % Hydrogen peroxide (5.67 g, 50 mmol) was added to a solution of 2-allylthio-5-chloropyrimidine (1.87 g, 10 mmol) in acetic acid (15 ml) and the mixture stirred at room temperature for 24 h. The solution was concentrated at reduced pressure to a small volume, water (20 ml) added and the mixture extracted with chloroform. The chloroform solution was washed with aqueous K_2CO_3, the dried ($MgSO_4$) solution evaporated and the residue recrystallized from chloroform–light petroleum; yield 1.60 g (78 %), m.p. 82 °C. Anal. $C_7H_7ClN_2OS$: C, H. ^1H NMR ($CDCl_3$): δ 3.7–4.0 (CH_2-SO), 5.0–6.2 (vinyl), 8.86 (H-4, H-6). IR (KBr): 1550 (pyrimidine) 1060 cm^{-1} (SO). MS[70 eV, m/z (% rel. int.)]: 204/202 (2/5, M), 185(8), 171(4), 114(6), 41(100).

5-Chloro-2-propargylsulfonylpyrimidine 7a. A mixture of 5-chloro-2-propargylthiopyrimidine (0.35 g, 1.9 mmol) and 90 % *m*-chloroperbenzoic acid (0.94 g, 4.9 mmol) in chloroform (20 ml) was stirred together at room temperature for 24 h before chloroform (20 ml) was added and the solution washed with 1 M $NaHCO_3$ (2 × 30 ml). The dried ($MgSO_4$) chloroform solution was evaporated and the residue recrystallized from methanol–water; yield 0.30 g (73 %), m.p. 70 °C. Anal. $C_7H_5ClN_2O_2S$: C, H. ^1H NMR ($CDCl_3$): δ 2.36 (HC≡, t, J 2 Hz), 4.39 (CH_2-SO_2, d, J 2 Hz) 8.78 (H-4, H-6). IR (KBr): 3270 (HC≡), 1320 and 1120 cm^{-1} (SO_2). MS[70 eV, m/z (% rel. int.)]: 218/216 (4/10, M), 154 (16), 152(52), 127(15), 125(46), 117(38), 115(26), 114(15), 113(78), 90(28), 88(16), 86(45).

2-Allylsulfonyl-5-chloropyrimidine 7b. 90 % *m*-Chloroperbenzoic acid (1.14 g, 6 mmol) was added to a solution of 2-allylthio-5-chloropyrimidine (0.43 g, 2.3 mmol) in chloroform (10 ml) and the mixture stirred at 40 °C for 90 min. The cold reaction mixture was extracted with aqueous K_2CO_3, the chloroform solution dried ($MgSO_4$), the solution evaporated and the residue recrystallized from methanol; yield 0.35 g (70 %), m.p. 84 °C. Anal. $C_7H_7ClN_2O_2S$: C, H. ^1H NMR ($CDCl_3$): δ 4.24 (CH_2, J 7 Hz) 5.1–6.2 (vinyl), 8.90 (H-4, H-6). IR (KBr): 1550 (pyrimidine), 1330 and 1140 cm^{-1} (SO_2). MS[70 eV, m/z (% rel. int.)]: 155(30), 154(9), 153(82), 128(11), 113(9), 86(12), 53(17), 41(100).

5-Chloro-2-propadienylsulfonylpyrimidine 7c.

Method A. A mixture of 5-chloro-2-propadienylthiopyrimidine (0.21 g, 1.1 mmol) and 90 % *m*-chloroperbenzoic acid (0.49 g, 2.6 mmol) in chloroform was stirred together at room temperature for 24 h before washing with aqueous K_2CO_3. The dried ($MgSO_4$) chloroform solution was evaporated and the residue recrystallized from methanol; yield 0.22 g (92 %), m.p. 130 °C. Anal. $C_7H_5ClN_2O_2S$: C, H. ^1H NMR (acetone-d_6): δ 5.65 (H_2C d, J 6 Hz), 6.73 (=CH-SO_2, t, J 6 Hz), 9.04 (H-4, H-6). IR (KBr): 1960 and 1920 (allene), 1550 (pyrimidine), 1330 and 1140 cm^{-1} (SO_2). MS[70 eV, m/z (% rel. int.)]: 218/216 (3/7, M), 152(26), 125(29), 113(52), 86(39), 53(45), 39(100).

Method B. A mixture of 5-chloro-2-propargylthiopyrimidine (0.39 g, 1.9 mmol) and 90 % *m*-chloroperbenzoic acid (0.94 g, 4.9 mmol) was stirred together in chloroform (20 ml) at room temperature for 24 h before chloroform (10 ml) was added and the solution washed with *saturated* K_2CO_3 (2 × 20 ml). The dried ($MgSO_4$) chloroform solution was evaporated and the residue recrystallized from methanol; yield 0.27 g (66 %).

5-Chloro-2-(3-oxobuten-1-yl)sulfonylpyrimidine 7d 90 % *m*-Chloroperbenzoic acid (2.20 g, 11.5 mmol) in chloroform (10 ml) was added to a solution of 5-chloro-2-(3-oxobuten-1-yl)thiopyrimidine (1.10 g, 5 mmol) in chloroform (10 ml) and the mixture stirred at 40 °C for 2 h. The cold reaction mixture was extracted with aqueous $KHCO_3$ and the dried ($MgSO_4$) chloroform solution evaporated; yield 1.14 g (92 %). The (*E*)/(*Z*) isomers could be separated by thick layer chromatography [silica gel; $CHCl_3$: EtOA (1:1)]. (*E*): m.p. 117 °C (MeOH). Anal. $C_8H_7ClN_2O_3S$: C, H. ^1H NMR (acetone-d_6): δ 2.43 (Me), 7.12 and 7.68 (Hα, Hβ, d, J 16 Hz), 9.10 (H-4, H-6). IR (KBr): 1695 (CO), 1330 and 1140 cm^{-1} (SO_2). MS[70 eV, m/z (% rel. int.)]: 246 (5, M), 231(5), 203(40), 182(41), 167(32), 161(50), 114(100). (*Z*): m.p. 95 °C (MeOH). Anal. $C_8H_7ClN_2O_3S$: C, H. ^1H NMR (acetone-d_4): δ 2.32 (Me), 6.97 and 7.13 (Hα, Hβ, d, J 12 Hz), 9.10 (H-4, H-6). IR (KBr): 1700 (CO), 1320 and 1140 cm^{-1} (SO_2). MS[70 eV, m/z (% rel. int.)]: 246(17), M), 231(40), 203(14), 182(38), 167(94), 161(75), 114(100).

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The Crystal and Molecular Structures of 3-Methyl-5-amino-1,2-thiazole-sulfate

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The crystal and molecular structures of 3-methyl-5-aminoisothiazole·$\frac{1}{2}H_2SO_4$ have been determined at 121 K by X-ray crystallographic methods using 4837 reflections observed by counter methods. The crystals are monoclinic, space group $C2/c$ with unit cell dimensions $a=7.417(2)$ Å, $b=11.265(1)$ Å, $c=15.929(1)$ Å, $\beta=101.948(8)°$. The structure was refined to a conventional R-factor of 0.027. Estimated standard deviations are 0.0005 Å and 0.05° in bond lengths and angles when hydrogen atoms are not involved. Bond lengths corrected for thermal motion are given.

Isothiazole derivatives represent a relatively new type of compound[1] and have attracted much interest, mostly because of the various biological activities they apperar to have.[2–5] The isothiazole moiety has been studied by several authors employing X-ray crystallographic methods.[6] However, most studies have been performed on fused ring systems such as 1,2-benzisothiazole derivatives and only few on the free isothiazole ring.[7,8] Thus we present here an accurate X-ray crystallographic study of the protonized 5-amino-3-methylisothiazole molecule as found in crystals of the sulphate complex.

EXPERIMENTAL

The general experimental procedure and conditions are given in the table below. Cell parameters were determined by a least squares fit to the diffractometer settings for 15 general reflections. The standard deviations in the measured intensities were calculated as $\sigma(I)=|C_T+(0.02C_N)^2|^{\frac{1}{2}}$ where C_T is the total number of counts and C_N is the scan count minus the background count. The intensity data were corrected for Lorentz and polarization effects as well as for absorption effects. The variation in the intensities of the test reflections was less than 1.5% and no corrections were made on this basis.

Scattering factors used were those of Doyle and Turner[9] for the non-hydrogen atoms and of Stewart, Davidson and Simpson[10] for the hydrogen atoms. The density of the crystalline compound was measured by means of flotation. The conditions for reflection were found to be

hkl: $h+k=2n$
$0k0$: $k=2n$
$h0l$: $l=2n$

which indicates space group Cc or $C2/c$. The space group $C2/c$ was chosen on the basis of $N(z)$ statistics indicating a center of symmetry as well as on the density and cell volume indicating 8 asymmetric units in the cell.

Experimental conditions.

Instrument	SYNTEX $P\bar{1}$
Radiation	Graphite crystal monochromated Mo$K\alpha$ $\lambda=0.71069$ Å
Crystal dimensions/mm	$0.3\times0.25\times0.5$
Scanning mode	$\theta/2\theta$
Scan speed/°min^{-1}	2–4 depending on intensity
Scan range/°	$2\theta\alpha_1-0.8$ to $2\theta\alpha_2+1.4$ for $2.0<2\theta<65.0$ $2\theta\alpha_1-0.8$ to $2\theta\alpha_2+1.0$ for $65.0<2\theta<90.0$
Background counts	For 0.35 of scan time at scan limits
2θ range/°	$2.0<2\theta<90.0$
Temperature/K	121
Number of reflections meas.	5355

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Number of reflections $I>2.5\sigma(I)$	4837
Number of test reflections	3
Number of reflections measured between test reflections	57
Absorption coefficient/mm^{-1}	0.572
Trans min.	0.8507
Trans max.	0.9208

CRYSTAL DATA

3-Methyl-5-amino-1,2-thiazol-sulfate, $C_4SN_2H_7 \cdot \frac{1}{2}SO_4$, monoclinic; $a=7.417(2)$ Å, $b=11.265(1)$ Å, $c=15.929(1)$ Å, $\beta=101.948(8)°$, $V=1302.0(1)$ Å^3, $M=149.2$, space group: $C2/c$, $Z=8$, $F(000)=624$, $D_x=1.6$ g/cm^3, $D_o=1.66$ g/cm^3.

STRUCTURE DETERMINATION

The structure was solved by direct methods using the program assembly MULTAN,[11] the positions of all the non hydrogen atoms in the asymmetric unit being determined from subsequent Fourier analysis. The structure was refined by least squares methods to an R-factor of 0.10 using isotropic temperature factors. At this stage, the positions of all the hydrogen atoms were determined from a difference map and the structure refined to an R-factor of 0.0266 using anisotropic temperature factors for all the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms. The figure of merit: $s=[\Sigma W\Delta^2/(m-n)]^{1/2}$ for the final data was found to be 1.69. The positional parameters for the non-hydrogen atoms were corrected for librational effects according to the method of Schomaker and Trueblood.[12] The r.m.s. value of the ΔU's were found to be 4.02×10^{-4} Å^2 and 2.92×10^{-4} Å^2 for the isothiazole and sulfate moieties, respectively.

The final parameters are given in Tables 1 and 2. Tables of the observed and calculated structure factores are available from the authors.

Table 2. Fractional atomic coordinates and isotropic thermal parameters for the hydrogen atoms. Estimated standard deviations in parentheses.

Atom	x	y	z	B
H 1	0.293(3)	0.432(2)	0.126(1)	3.9(2)
H 2	0.525(3)	0.610(2)	0.173(1)	4.1(3)
H 3	0.349(2)	0.682(2)	0.168(1)	3.9(3)
H 4	0.503(3)	0.728(2)	0.115(1)	4.5(3)
H 5	0.356(2)	0.672(1)	−0.047(1)	3.6(2)
H 6	0.207(2)	0.561(1)	−0.191(1)	3.7(2)
H 7	0.109(2)	0.451(1)	−0.181(1)	3.7(2)

DESCRIPTION AND DISCUSSION

The labelling of the atoms is given in Fig. 1 whereas bond lengths and angles are given in Table 3. The S2−C3 bond length of 1.7352 Å is in good agreement with the mean value of such bond lengths as given in Ref. 6. The mean values of the N−S and N−C bonds are given to be 1.658(7) and 1.320(4) Å, respectively, whereas those distances in the present study are found to be 1.6828(5) and 1.3446(7) Å.

Table 1. Fractional atomic coordinates multiplied by 10^5 and thermal parameters multiplied by 10^4 for the non-hydrogen atoms in 3-methyl-5-amino-isothiazole-sulfate. The anisotropic temperature factor is given by $\exp-2\pi^2(U_{11}a^{*2}h^2+\cdots2U_{12}a^*b^*hk+\cdots)$. Estimated standard deviations are given in parentheses.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S2	17450(0)	40066(1)	−1094(0)	169(1)	118(1)	170(0)	17(0)	65(0)	−36(0)
N2	27766(2)	47357(1)	7812(1)	195(1)	209(1)	128(0)	43(0)	42(0)	45(0)
C1	31505(8)	60671(5)	−2459(4)	144(2)	132(2)	129(2)	3(2)	26(2)	−1(2)
C2	34675(8)	57927(6)	6218(3)	139(2)	195(3)	123(1)	37(2)	21(2)	−9(2)
C3	21590(6)	51714(4)	−7517(3)	133(2)	119(1)	119(1)	9(1)	31(1)	10(1)
N1	15478(6)	51471(4)	−15989(3)	215(2)	168(2)	118(1)	−34(1)	17(1)	0(1)
C4	44219(6)	65413(4)	13509(3)	186(1)	328(0)	161(1)	43(1)	0(1)	−81(1)
S1	50000	28289(5)	25000	135(1)	90(2)	119(1)	0(1)	32(1)	0(1)
O1	48419(6)	20958(4)	32432(3)	285(1)	269(1)	383(1)	116(1)	191(1)	224(1)
O2	33418(8)	35905(6)	22796(4)	357(2)	434(3)	175(1)	270(2)	117(1)	133(2)

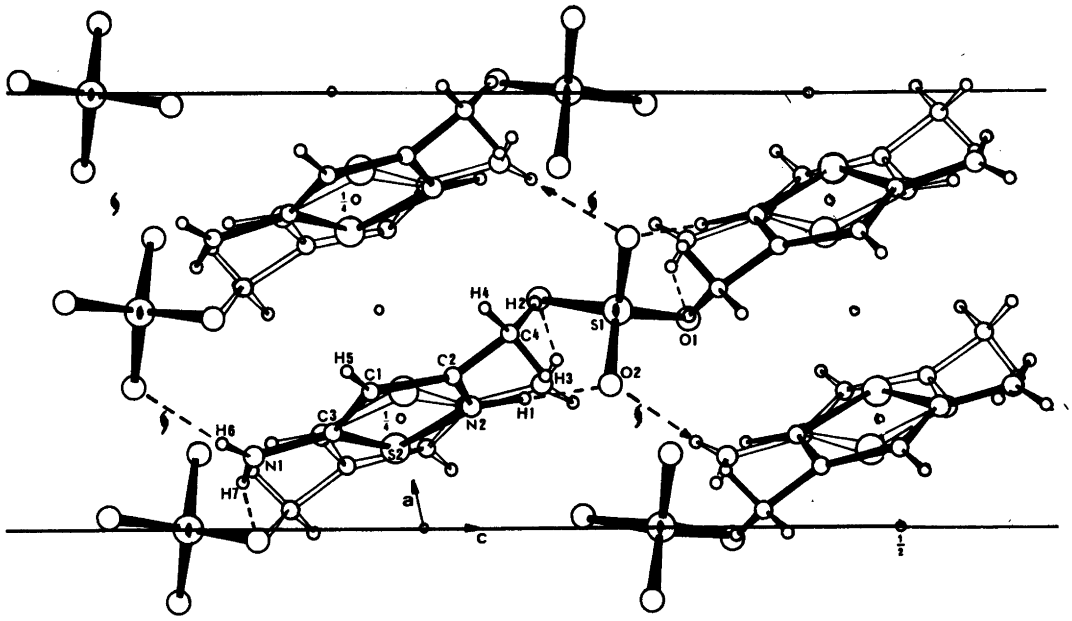


Fig. 1. Molecular packing in the crystals of 3-methyl-5-aminoisothiazole-sulfate as seen down the *b*-axis.

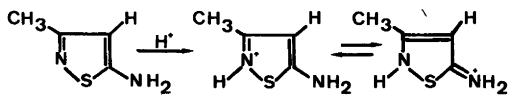
These elongations as well as the double bond character of the C3–N1 bond (1.3349(6) Å) are in accordance with the protonization of the N2-atom:

Table 3. Bond lengths and angles in 3-methyl-5-amino-isothiazole-sulfate. Estimated standard deviations given in parentheses.

Bond lengths (Å)		
	Uncorr.	Corrected
S2–N2	1.6802	1.6828(5)
S2–C3	1.7300	1.7352(4)
N2–C2	1.3413	1.3446(7)
C1–C2	1.3879	1.3916(6)
C1–C3	1.4010	1.4030(6)
C2–C4	1.4902	1.4924(7)
C3–N1	1.3319	1.3349(6)
S1–O1	1.4679	1.4839(4)
S1–O2	1.4809	1.5030(4)
N2–H1	0.88(2)	
C1–H5	0.90(2)	
N1–H6	0.86(2)	
N1–H7	0.83(2)	
C4–H2	0.91(2)	
C4–H3	0.99(2)	
C4–H4	1.03(2)	

Table 3. Continued.

Bond angles (°)			
O1–S1–O2	109.07(4)	S2–N2–H1	115(1)
O1–S1–O11	110.99(5)	C2–N2–H1	130(1)
O1–S1–O22	109.36(3)	C2–C1–H5	125(1)
O2–S1–O22	108.96(6)	C3–C1–H5	123(1)
		C2–C4–H2	110(1)
C3–S2–N2	91.36(3)	C2–C4–H3	108(1)
S2–N2–C2	113.46(3)	C2–C4–H4	112(1)
N2–C2–C1	113.26(4)	H2–C4–H3	106(2)
C2–C1–C3	111.84(4)	H2–C4–H4	112(2)
C1–C3–S2	110.02(3)	H3–C4–H4	108(2)
C2–C3–N1	121.31(3)	C3–N1–H6	118(1)
N2–C2–C4	119.50(5)	C3–N1–H7	117(1)
C1–C2–C4	127.22(5)	H6–N1–H7	118(2)
C1–C3–N1	128.67(4)		



The effect of the protonation on the N2 atom is furthermore apparent in the increase in the angle at N2 as well as the decrease in the neighbouring angles at S2 and C2. In general the situation may be compared to that in the crystal structure of 2-

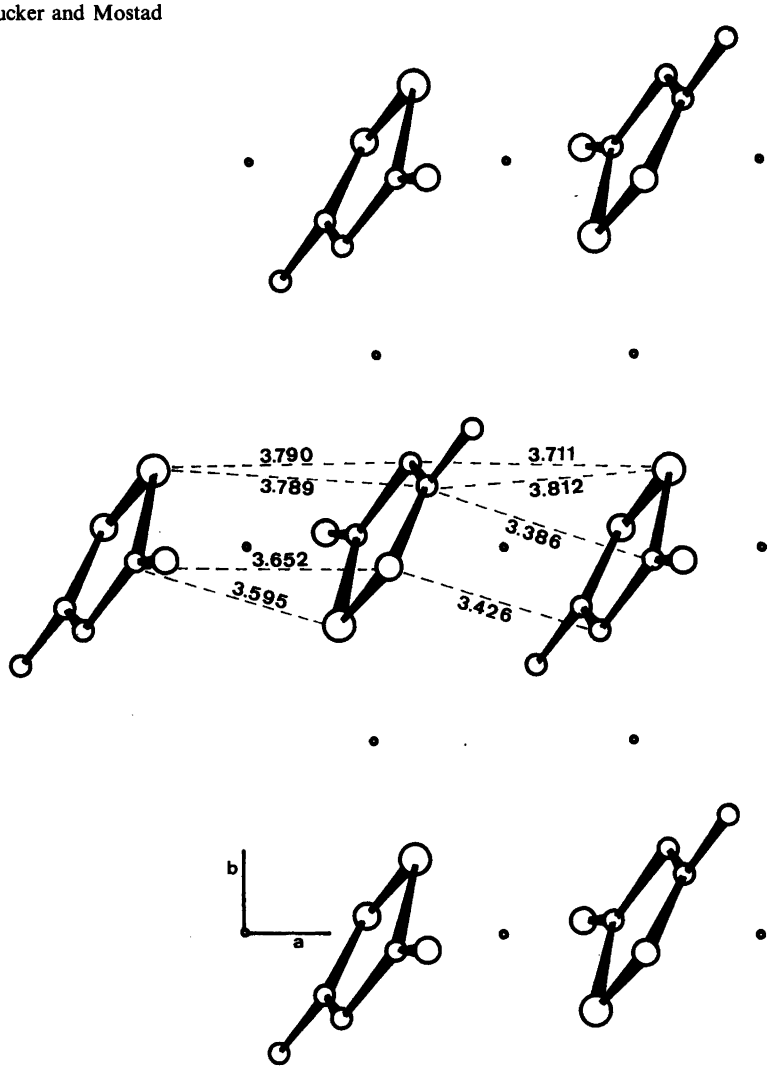


Fig. 2. Stacking of the 3-methyl-5-amino-isothiazole rings in crystals of the sulfate complex as seen down the *c*-axis.

aminopyrimidine·HCl.[13] The sum of the internal angles in the ring is 539.95° as compared to 540° in a planar pentagon. However, a least squares plane through the four ring atoms S2, N2, C2 and C1 indicates a significant envelope form. The atomic deviations from the least squares plane are given in Table 4. The asymmetry in the external angles at N2, C2 and C3 may well be explained by intramolecular non-bonded interactions. Thus the distances between the pairs of hydrogen atoms H1–H2, H1–H3, H4–H5 and H5–H6 are all close to 2.7 Å.

The conformation about the C2–C4 bond is such as to bring the H4 atom only slightly out of the ring

Table 4. Atomic deviations from a least squares plane through atoms S2, N2, C1 and C2.

Atom	Å
S2	−0.0011
N2	0.0052
C1	0.0037
C2	−0.0067
C3	0.0383
C4	−0.0014
N1	0.1088
H6	−0.137
H7	0.025

Table 5. Geometry in the hydrogen bonding system in crystals of 3-methyl-5-amino-isothiazole-sulfate.

D	H	A		D···A	H···A	∠D−H···A	∠H···A−S
N1	H7	O1	$(-\frac{1}{2}+x, \frac{1}{2}-y, -\frac{1}{2}+z)$	2.7991(6)	2.03(2)	153(2)	110.5(5)
N1	H6	O2	$(x, 1-y, -\frac{1}{2}+z)$	2.8094(6)	1.95(2)	170(2)	126.0(5)
N2	H1	O2	(x, y, z)	2.6483(6)	1.77(2)	175(2)	117.1(6)

plane, the C1−C2−C4−H4 angle being −8°. Similarily, both the hydrogen atoms H6 and H7 are close to the ring plane. As may be seen from Table 4, the N1 atom is somewhat above the ring plane due to the slight envelope form of the ring. Nor is the aminogroup strictly planar as the N1 atom is situated 0.14(1) Å above a least square plane through the atoms C3−H6−H7. The two torsional angles S2−C3−N1−H6 and S2−C3−N1−H7 are found to be −160(1) and −9(1)°, respectively.

Also in the sulfate ion there are significant variations in bond lengths and angles. The longest S−O bond is 1.5030 Å and involves the O2 atom which is engaged in two hydrogen bonds, whereas the shortest S−O distances of 1.4839 Å is to the oxygen atom O1 which is engaged in only one hydrogen bond. Finally the angle between the two short bonds (O1−S1−O11) is 110.99° as compared to the angle between the two long bonds (O2−S1−O22) of 108.96°.

The packing of the ions in the crystal as seen down the *b*-axis is depicted in Fig. 1 where also the hydrogen bonding is indicated. The geometry of the hydrogen bonding is given in Table 5.

Each isothiazole molecule is hydrogen bonded to three sulfate ions which in turn are connected to six different isothiazole moieties through such bonds. The crystals display a layered structure with all sulfate ions lying in planes containing the screw and rotation axes and the isothiazole molecules situated between these planes in stacked pairs related by the centers of symmetry; the shortest distance being 3.386 Å (C2−C3). The distance between the two ring planes is found to be 3.35 Å. The stacking of the rings in these layers is depicted in Fig. 2 where some of the distances are also indicated.

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Oxazoles in Diels-Alder Reactions. Transformation of The Adducts to Either Pyridines or Pyrroles

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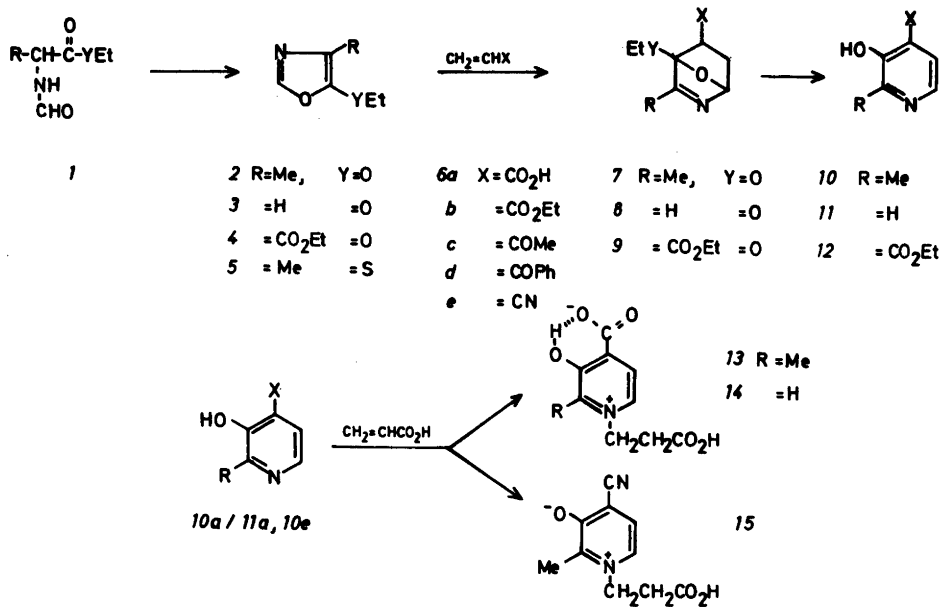
The cycloadducts between acrylic acid or acrylonitrile and 5-ethoxyoxazoles are converted as formed to 3-hydroxypyridines. The Diels-Alder adducts from ethyl acrylate, or methyl or phenyl vinyl ketone can be isolated. In ethanolic HCl the adducts are transformed into 3-hydroxypyridines; in aqueous HCl the products are acylpyrrole analogues. A 4-substituent in the oxazole affects the reaction. 4-Methyl-5-ethoxyoxazole reacts much faster than its 5-ethylthio analoque.

Adduct formation between 5-ethoxyoxazoles and dienophiles is a key step in an important synthetic route to substituted 3-hydroxypyridines.[1,2] The Diels-Alder adducts initially formed are unstable and are transformed further to pyridines. The positions of the substituents in the pyridine are consistent with strict regioselectivity in the adduct formation whereby C−2 of the oxazole forms the new C−C bond with the most electron deficient carbon atom. Hence the more electron attracting group from the ethene will be located in the 4- rather than in the 5-position in the pyridine.[3] In this report we describe work aimed at elucidating the effect of 4-substituents in 5-ethoxyoxazoles and 5-ethylthioxazoles on the course of the cycloaddition with electron deficient unsymmetrically substituted ethenes as well as ring-opening studies of the adducts.

5-Alkoxyoxazoles are commonly formed by cyclization of *N*-acylated amino acid esters.[1] The oxazoles are acid labile and the best yields are obtained using an acid anhydride as dehydrating agent in the presence of a base to neutralize the acid generated.[4,5] The time of heating may also be important for the outcome of the reaction. Thus in the cyclization of ethyl *N*-formylaminomalonate the yield of the *4*-ethoxycarbonyl derivate *4* was almost doubled (55 %) when the reaction time was reduced from the 6 h recommended[6] to 3 h. The 5-ethylthioxazole *5* is similarly prepared by cyclization of the corresponding thiol ester.[7]

The bicyclic Diels-Alder adducts are very sensitive to acid. In an acidic organic solvent the adduct will be transformed to pyridines. The acid instability also precludes isolation of Diels-Alder adducts in those cases where the dienophile carries an acid function (*e.g. 6a*). In the absence of an acidic function such as in ethyl acrylate, methyl vinyl ketone and phenyl vinyl ketone, the reaction with the 4-methyloxazole *2* does not proceed beyond the Diels-Alder adduct under mild conditions. With acrylonitrile, however, the reaction went all the way to the corresponding pyridine. The reaction times varied. The reaction with the acrylate *6b* was complete after 5 days, with the methyl ketone *6c* after *ca.* 20 h whereas the more polarizable phenyl ketone *6d* gave a rapid and highly exothermic reaction. The order of stability of the adducts was the reverse of their rate of formation. Both the ester *7b* and the methyl ketone *7c* could be distilled but the latter decomposed slowly on storage. Attempted distillation of the phenyl ketone adduct *7d* led to retro-Diels-Alder reaction. The *endo:exo* ratios of the adducts were *ca.* 3:1 (^{1}H NMR). The isomer assignments are based on the chemical shifts for H−5 which is found at lower field in the *endo* isomer than in the *exo* isomer (Scheme 2);[8,9] H−5 resonates as a quartet partially or fully resolved. H−1 appears as a doublet (*J* 4 Hz) by coupling with the *exo*-proton on C−6 in agreement with findings in related series.[8] Normal

0302-4369/83 $2.50



Scheme 1.

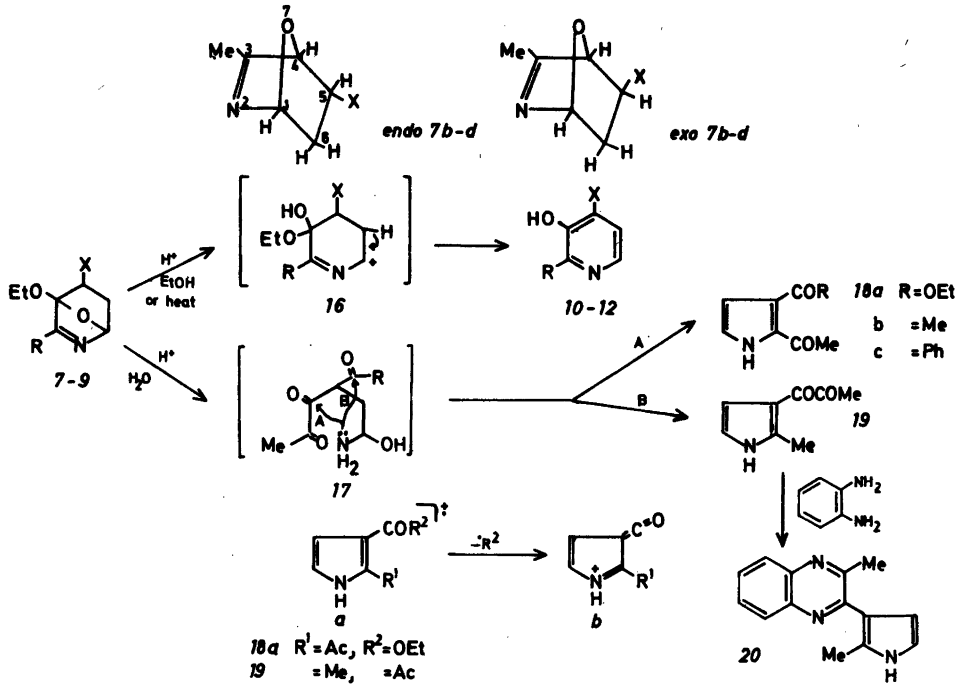
carbonyl absorptions in IR are found at 1735 (*7b*), 1710 (*7c*) and 1680 cm^{-1} (*7d*) with the C=N band at *ca.* 1630 cm^{-1}. The mass spectra are characterized by a weak molecular ion and dominated by ions corresponding to retro-Diels-Alder reactions which may occur either before electron impact as a thermal process, or after electron impact.

Treatment of the adducts with ethanolic HCl rapidly gave the corresponding 3-hydroxypyridines. In aqueous acids, however, the reaction takes another way as discussed below. In the case of the phenyl ketone *7d* its high reactivity demanded slow addition of the ethanolic acid. ^1H NMR confirms the structures assigned to the pyridines. Thus the vicinal protons on C–5 and C–6 are confirmed by the magnitude of their coupling, *J ca.* 5 Hz, and hence the carbonyl substituent is on C–4. The two 3-hydroxypyridines *10b* and *10c* have previously been prepared directly from the Diels-Alder reactants using acetic acid as solvent;[10,11] the overall yield in the two-step process described above is superior.

In the reaction between acrylonitrile and the 4-methyloxazole *2* the intermediate adduct *7e* was at once transformed to the pyridine *10e*; the overall rate of the reaction is therefore controlled by the time (4 h) required for adduct formation. Acrylic acid gives *10a* rapidly and exothermally. The 4-desmethyl oxazole *3* is much less reactive in the cycloaddition than is the 4-methyl analogue *2*, the reaction time being increased to 48 h; the yields of the pyridines were lower and in no case was the Diels-Alder adduct isolated. The relative reactivities of the dienophiles were the same as before. A further decrease in the reactivity of the oxazole was observed for the 4-ethoxycarbonyl derivative *4*; the latter reacts slowly with acrylic acid to form the pyridine *12a* and hardly at all with phenyl vinyl ketone.

It is notable that there is a very marked difference in the effects of the 5-ethoxy and 5-ethylthio substituents in *2* and *5*, respectively, on the reactivity of the oxazole. The former gives an immediate and exotherm reaction with acrylic acid to form the pyridine *10a* whereas the latter gave 20 % conversion to *10a* after 4 days; with phenyl vinyl ketone 10 % yield of the pyridine *10d* was obtained after 12 days whereas methyl vinyl ketone did not react with *5* under the usual conditions.

In the absence of a solvent the main product is a 2:1 adduct between acrylic acid and the oxazole *2* or *3*. The products have been identified as the



Scheme 2.

Michael adducts between the initially formed pyridine and a second molecule of acrylic acid, *viz.* *13* and *14*. These betaines are volatile in the mass spectrometer without decarboxylation. The chemical shifts in the ^1H and ^{13}C NMR spectra are consistent with pyridinium structures formulated. The size of the C–H couplings on C–2 and C–6 in *14*, $^1J_{CH}$ 189 and 193 Hz, respectively, are in good agreement with the value 193 Hz reported for *N*-methylpyridinium-3-olate.[12,13] The structures assigned were also proved chemically by heating the pyridines *10a* and *11a* with acrylic acid, which gave the adducts *13* and *14*. In the same way the 4-cyanopyridine *10e* gave the 3-pyridiniopropanoic acid *15*.

The pathway to pyridines *10–12* from the Diels-Alder adducts *7–9* can be rationalized in terms of an initial cleavage of the Cl–O bond (*16*; Scheme 2) which is succeeded by elimination of ethanol. In aqueous acids, however, the reaction takes another course; the products isolated have been identified as pyrroles. Thus the product from the 5-ethoxycarbonyl adduct *7b* has been assigned the structure 2-acetyl-3-ethoxycarbonylpyrrole *18a*. In IR the product showed absorption bands for NH at 3310, for ester carbonyl at 1710 and for keto carbonyl at 1640 cm^{-1}; the latter agrees well with the value 1626 cm^{-1} reported for 2-acetylpyrrole.[14] The ^1H NMR shifts at δ 6.77 and 6.94 are at higher fields than in pyridines but agree well with the values δ 6.62 (H–4) and 7.18 (H–5) reported for 2-formyl-3-ethoxycarbonylpyrrole.[15] In the mass spectrum the base peak is due to [M–OEt] which is rationalized as a favourable process *a*→*b* shown in Scheme 2.

For the 5-acetyl derivative *7c* in conc. HCl the transformation took another course. The base peak in the mass spectrum is due to [M–Ac] which again is rationalized as a favourable process *a*→*b* for the structure *19* assigned (pathway B; Scheme 2). In IR the CO bands are at 1620 and 1700 cm^{-1}; the former corresponds well to the absorption at *ca.* 1630 cm^{-1} for both 2- and 3-acetylpyrrole,[15,16] whereas the latter corresponds to the normal carbonyl band in 1,2-diketones. In ^1H NMR the two methyl groups appear as singlets at δ 2.42 and 2.55 whereas the

two protons of the pyrrole ring overlap at *ca.* δ 6.6. Finally the structure assigned was confirmed in a condensation reaction with *o*-phenylenediamine in which case the guinoxaline *20* was formed. In 1 M HCl the course of the transformation of *7c* is different, and the new product has been assigned structure *18b* corresponding to pathway A. Its mass spectrum is characterized by [M−Me] and [M−Me−CO] and the remainder of the spectroscopic evidence is in agreement with structure *18b*. In the concentration region between the dilute HCl and conc. HCl, mixtures of the two isomeric structures *18b* and *19* were formed. No such dependency on the acid strength for the ethoxycarbonyl derivative *7b* was observed and *18a* was the only pyrrole isolated irrespective of the strength of the acid solution. Similarly the 5-benzoyl adduct *7d* gave only one pyrrole irrespective of the acid strength. The assignment of structure *18c* follows from spectroscopy; CO bands in IR at 1640 and 1650 cm^{-1}, ^1H NMR signals at δ 6.35 (H−4) and 7.00 (H−5) and a mass spectrum characterized by [M−Ph] and [M−Ph−CO].

The formation of pyrroles from the Diels-Alder adducts in aqueous acid solution may be rationalized by a reaction involving hydrolytic cleavage of the N2−C3 bond in *7*. In the postulated acyclic intermediate *17* cyclization to a five-membered ring may take two courses; pathway A gives the 2,3-diacylpyrroles *18* whereas pathway B gives a 3-(1,2-dioxoalkyl)pyrrole *19*.

EXPERIMENTAL

Formation of cycloadducts. The dienophile *6* (0.01 mol) and one of the oxazoles *2*−*5* (0.01 mol) were mixed and the mixture stirred at room temperature or at the temperature given below for the individual compounds; the progress of the reaction was monitored by TLC.

5-Ethoxy-4-ethoxycarbonyloxazole 4.[6] Diethyl *N*-formylaminomalonate (12.0 g, 0.06 mol) was added dropwise to a vigorously stirred suspension of phosphorus pentoxide (4.0 g, 0.33 mol) in chloroform (200 ml) at room temperature. The mixture was stirred at this temperature for 30 min after the addition was completed, and was then heated under reflux for 3 h. The cold reaction mixture was hydrolyzed by slow addition of 10 % NaOH and ice, the chloroform solution collected from the two-phase system, the water solution extracted twice with chloroform, the combined chloroform solutions washed with a little water, the dried ($MgSO_4$) solution evaporated and the residue distilled; yield 55 %, b.p. 104 °C/0.6 mmHg.

3-Methyl-4-ethoxy-5-ethoxycarbonyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene 7b from 4-methyl-5-ethoxyoxazole and ethyl acrylate (4 h, 40 °C); yield 93 %, b.p. 78−80 °C/0.35 mmHg. Anal. $C_{11}H_{17}NO_3$: C, H. Isomer ratio *endo*−*exo* 3:1. ^1H NMR (CCl_4) for *endo* isomer: δ 1.27, 1.34 and 3.76 (OEt and the Me from OEt), 1.96 (3−Me), 2.89 (H−5, d), 5.47 (H−1, d, *J* 4 Hz), the rest unresolved. *exo* isomer: δ 1.24, 1.34 and 3.80, 4.04 (2 OEt), 2.01 (3−Me), 2.3 (m, 3H), 5.61 (H−1), d, *J* 4 Hz). IR (CCl_4): 1735 (CO) and 1630 cm^{-1} (C=N). MS[70 eV; *m/z* (% rel. int.)]: 227 (3, M), 181 (12), 135 (22), 127 (44), 107 (13), 99 (71), 71 (52), 55 (100).

3-Methyl-4-ethoxy-5-acetyl-7-oxa-2-azabicyclo-[2,2,1]-2-heptene 7c [8] from *5* and buten-2-one (20 h); yield 91 %, b.p. 62−64 °C/0.1 mmHg. Isomer ratio *endo*—*exo* 3:1. ^1H NMR (CCl_4) for *endo* isomer: δ 1.30 and 3.73 (OEt), 1.72 (H−6, 2 d), 1.85 (Me), 2.17 (Me), 2.1 (H−6, m), 3.06 (H−5, 2 d), 5.45 (H−1, d, *J* 3.5). *exo*-isomer: δ 1.10 and 3.78 (OEt), 2.03 (Me), 1.6−2.3 (3H), 2.13 (Me), 5.60 (H−1, d, *J* 3.5 Hz). IR (film): 1710 (CO), 1625 cm^{-1} (C=N). MS[70 eV; *m/z* (% rel. int.)]: 197 (1, M), 151 (9), 127 (45), 99 (66), 82 (11), 71 (48), 70 (47), 55 (100).

3-Methyl-4-ethoxy-5-benzoyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene 7d from *5* and 1-phenyl-2-propen-1-one (5 min) in an exothermic reaction; yield almost quantitative. Unstable liquid which largely underwent retro-Diels-Alder reaction on attempted distillation. Isomer ratio *endo*−*exo* 3:1. ^1H NMR (CCl_4) for *endo* isomer: δ 1.23 and 3.80 (OEt), 1.81 (H−6, 2 d), 2.02 (3−Me), 2.3 (H−6, m), 3.45 (H−5, 2 d), 5.68 (H−1, d, *J* 4 Hz), 7.5−8.0 (Ph). *Exo* isomer: δ 1.02 and 3.88 (OEt), 1.58, (H−6, 2 d), 2.22 (3−Me), 2.23 (H−6, H−5, m). 5.83 (H−1, d, *J* 4 Hz), 7.5−8 (Ph). IR (film): 1680 (CO), 1635 cm^{-1} (C=N). MS[70 eV; *m/z* (% rel. int.)]: 259 (1, M), 213 (6), 132 (52), 127 (50), 105 (100), 99 (51), 77 (43), 71 (36), 55 (86).

3-Hydroxypyridines 10 *from Diels-Alder adducts* 7. *2-Methyl-3-hydroxy-4-ethoxycarbonylpyridine 10b* [10] was obtained in 90 % yield when *6b* was treated with ethanolic HCl as described below; aromatization in acetic acid gave 67 % yield.

2-Methyl-3-hydroxy-4-acetylpyridine 10c [6] was obtained in 72 % yield when *6c* was treated with ethanolic HCl as described below; aromatization in acetic acid gave 25 % yield.

2-Methyl-3-hydroxy-4-benzoylpyridine 10d. Ethanolic HCl (saturated, 5 ml) was added

dropwise to a solution of 3-methyl-4-ethoxy-5-benzoyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene (2.59 g, 0.01 mol) in abs. ethanol (50 ml). After 10 min the solution was evaporated to dryness, the residual hydrochloride triturated with acetone, the insoluble material dissolved in water, the solution neutralized with sodium carbonate and the pyridine extracted into dichloromethane. Evaporation and distillation of the residue gave the title compound in 74 % yield, b.p. 130 °C/0.1 mmHg. Anal. $C_{13}H_{11}NO_2$: C, H. ^{1}H NMR (CCl_4): δ 2.47 (2−Me), 7.12 (H−5, J 5 Hz), 7.5 (Ph), 8.00 (H−6). IR (film): 1650 cm^{-1} (CO). MS[70 eV; *m/z* (% rel. int.)]: 213 (100, M), 212 (74), 136 (10), 135 (19), 115 (13), 108 (12), 107 (23), 105 (88).

Direct isolation of 3-hydroxypyridines from oxazoles. The oxazole and the dienophile were mixed directly together without solvent and the mixture was stirred at room temperature; the reaction time is given separately for each compound. The progress of the reactions was monitored by TLC.

2-Methyl-3-hydroxy-4-carboxypyridine[18] 10a. *Method A.* The reaction between 4-methyl-5-ethoxyoxazole *3* and acrylic acid was exothermic and was over as soon as the mixing was completed. The product was purified by recrystallization from water; yield 85 %.

Method B: When 4-methyl-5-ethylthioxazole and acrylic acid were mixed there was no heat evolution. The reaction mixture was worked up after 4 days; yield 20 %.

2-Methyl-3-hydroxy-4-cyanopyridine[10] *10e* was obtained in 85 % yield from *2* and acrylonitrile (4 h); purified by recrystallization from methanol.

3-Hydroxy-4-carboxypyridine[19] 11a. Equimolar amounts of 5-ethoxyoxazole *3* and acrylic acid were left in ethanolic solution for 48 h. Evaporation and recrystallization of the residue from water gave the title compound in 30 % yield.

3-Hydroxy-4-acetylpyridine 11c was formed from *3* and 3-buten-2-one (48 h) in 8 % after preparative GLC on a 15 % Apiezon L-column; MS molecular ion 137.0478; calc. for $C_7H_7NO_2$: 137.0477. ^{1}H NMR ($CDCl_3$): δ 2.71 (Me), 7.60 (H−5, J 5 Hz), 8.27 (H−6), J 5 Hz), 8.49 (H−2, J<1). IR (film):1660 cm^{-1} (CO). MS[70 eV; *m/z* (% rel. int.)]: 137 (69, M), 43 (100).

3-Hydroxy-4-benzoylpyridine[20] *11d* was formed from *3* and 1-phenyl-2-propen-1-one (48 h) and was isolated by thick-layer chromatography on silica gel (Merck PF 254; plates 20×40 cm^2, thickness 2 mm) using $CHCl_3$ for development. The desired product was extracted from the silica gel by dichloromethane, the solution evaporated and the residue crystallized from MeOH/Et_2O; yield 23 %, m.p. 105−106 °C. Anal. $C_{12}H_9NO_2$:C, H.

3-Hydroxy-4-cyanopyridine 11e from *3* and acrylonitrile (48 h). The reaction mixture was dissolved in ether; the title compound crystallized from this solution on slow concentration; yield 55 %, m.p. 166−167 °C. Anal. $C_6H_4N_2O$: C, H. ^{1}H NMR (acetone-d_6): δ 7.60 (H−5, J 5 Hz), 8.28 (H−6, J 5 Hz), 8.53 (H−2, J<1). IR (KBr) 2210 cm^{-1} (CN): MS[70 eV; *m/z* (% rel. int.)]: 120 (100, M), 93 (22), 92 (39), 65 (44), 64 (44), 52 (11).

2-Ethoxycarbonyl-3-hydroxy-4-carboxypyridine 12a from 4-ethoxycarbonyl-5-ethoxyoxazole and acrylic acid (48 h). The solid precipitate was recrystallized from ethanol; yield 34 %, m.p. 194 °C. Anal. $C_9H_9NO_5$: C, H. ^{1}H NMR (NaOD/D_2O):δ 1.14 and 3.62 (OEt), 7.05 (H−5, J 5 Hz), 7.43 (H−6, J 5 Hz). IR (KBr): 2550 (OH-acid), 1705 (CO-ester), 1665 cm^{-1} (CO-acid). MS[70 eV; *m/z* (% rel. int.)]: 211 (7, M), 167 (40), 166 (8), 148 (8), 139 (100), 121 (46), 120 (12), 93 (30).

1-(3-Propionic acid)-2-methyl-3-hydroxypyridinium-4-carboxylate 13. 2-Methyl-3-hydroxy-4-carboxypyridine (1.53 g, 0.01 mol) and acrylic acid (0.70 g, 0.01 mol) were heated together at 100 °C for 4 h. The solid was then filtered off and washed well with acetone; yield 63 %, m.p. 220 °C (decomp., water). Anal. $C_{10}H_{11}NO_5$:C, H. ^{1}H NMR (TFA): δ 2.92 (2−Me), 3.25 (2′−CH_2), 5.02 (3′−CH_2), 8.3 (H−5, H−6). IR (KBr):1700 (CO_2H), 1620 cm^{-1} (CO_2^-). MS[70 eV; *m/z* (% rel. int.)]: 225 (22, M), 153 (22), 136 (19), 135 (100), 108 (11), 107 (85), 79 (38), 55 (73).

1-(3-Propionic acid)-3-hydroxypyridinium-4-carboxylate 14. 5-Ethoxyoxazole (1.13 g, 0.01 mol) and acrylic acid (1.40 g, 0.02 mol) were stirred together at room temperature for 24 h. The reaction mixture was then triturated with acetone and the solid filtered off; yield 78 %, m.p. 230 °C (decomp., water). Anal. $C_9H_9NO_5$: C, H. ^{1}H NMR (TFA): δ 3.38 and 5.02 (prop.), 8.5 and 8.8 (3H, pyr.). IR (KBr):1685 and 1575 cm^{-1} (CO). MS [70 eV; *m/z* (% rel. int.)]: 211 (18, M), 139 (89), 122 (10), 121 (100), 94 (9), 93 (70), 72 (84), 66 (22), 65 (28), 55 (58).

1-(3-Propionic acid)-2-methyl-4-cyanopyridinium-3-olate 15. 2-Methyl-3-hydroxy-4-cyanopyridine (1.34 g, 0.01 mol) and acrylic acid (0.70 g, 0.01 mol) were heated together at 100 °C for 20 h. The cold reaction mixture was triturated with acetone and the solid filtered off; yield 47 %, m.p. 250 °C (decomp., water). Anal. $C_{10}H_{10}N_2O_3$: C, H. ^{1}H NMR (TFA): δ 2.98 (2-Me), 3.32 and 5.07 (prop.), 7.88 (H−5, J 5 Hz), 8.50 (H−6). IR (KBr) 2220 (CN), 1685 cm^{-1} (CO). MS[70 eV; *m/z* (% rel. int.)]: 206 (4,

M), 135 (8), 134 (100), 106 (23), 105 (90), 79 (30), 65 (13), 64 (22).

Formation of pyrroles 18, 19 *from the Diels-Alder adducts.* 18. The adducts 7 (0.01 mol) were added to 1 M HCl (20 ml), the mixture stirred for 15 min before extraction with ether. The ether solutions were evaporated and the residue subjected to thick-layer chromatography as above. The plates were developed with CH_2Cl_2–pentane (4:1) and thereafter with acetone. The compounds were extracted from the isolated silica gel bands by dichloromethane. *19*: Conc. HCl was used instead of 1 M HCl; the product was isolated as above.

2-Acetyl-3-ethoxycarbonylpyrrole 18a from 3-methyl-4-ethoxy-5-ethoxycarbonyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene in 63 % yield, m.p. 75–77 °C (benzene–hexane). Anal. $C_9H_{11}NO_3$: C, H. ^{1}H NMR ($CDCl_3$): δ 1.43 and 4.38 (OEt), 2.78 (Me), 6.77 (H–4), 6.94 (H–5). IR ($CHCl_3$): 3400 (NH), 1710 and 1640 cm^{-1} (CO). MS[70 eV; *m/z* (% rel. int.)]: 181 (71, M), 138 (35), 137 (10), 136 (100), 135 (64), 120 (51), 107 (11), 94 (49).

2,3-Diacetylpyrrole 18b from 3-methyl-4-ethoxy-5-acetyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene in 56 % yield, m.p. 117–118 °C (benzene–hexane). Anal. $C_8H_9NO_2$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.59 (Me), 2.71 (Me), 6.69 (H–4), 6.96 (H–5). IR ($CHCl_3$): 3420 (NH), 1670 and 1630 cm^{-1} (CO). MS[70 eV; *m/z* (% rel. int.)]: 151 (88, M), 136 (100), 94 (61), 79 (5), 67 (6), 52 (7).

2-Acetyl-3-benzoylpyrrole 18c from 3-methyl-4-ethoxy-5-benzoyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene in 42 % yield, m.p. 78–80 °C (benzene–hexane). Anal. $C_{13}H_{11}NO_2$: C, H. ^{1}H NMR (CCl_4): δ 2.43 (Me), 6.35 (H–4), 7.00 (H–5), 7.5–7.9 (Ph). IR ($CHCl_3$): 3400 (NH), 1650 and 1640 cm^{-1} (CO). MS[70 eV; *m/z* (% rel. int.)]: 214 (100, M), 198 (24), 184 (19), 136 (93), 122 (22), 105 (25), 94 (42), 77 (33).

2-Methyl-3-(1,2-dioxopropan-1-yl)pyrrole 19 from 3-methyl-4-ethoxy-5-acetyl-7-oxa-2-azabicyclo[2,2,1]-2-heptene in 46 % yield, m.p. 85–86 °C (benzene–hexane). Anal. $C_8H_{11}NO_2$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.42 (Me), 2.55 (Me), 6.55 (H–4), 6.60 (H–5). IR ($CHCl_3$): 3420 (NH), 1700 and 1620 cm^{-1} (CO) MS[70 eV; *m/z* (% rel. int.)]: 151 (10, M), 136 (5), 109 (9), 108 (100), 94 (6), 80 (16), 54 (25).

2-(2-Methylpyrrol-3-yl)-3-methylquinoxaline 20. A mixture of 2-methyl-3-(1,2-dioxopropan-1-yl)pyrrole (0.30 g, 0.002 mol) and *o*-phenylenediamine (0.22 g, 0.002 mol) in ethanol (20 ml) was heated under reflux for 2 h. The solvent was then evaporated and the residue triturated with ether; the title compound remained in 92 % yield, m.p. 159–160 °C (EtOH). Anal. $C_{14}H_{13}N_3$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.40 (Me), 2.83 (Me), 6.45 (H–4), 6.70 (H–5), 7.6 (2H-quin), 8.0 (2H-quin.), IR (KBr): 3220 (NH). MS[70 eV; *m/z* (% rel. int.)]: 223 (56, M), 222 (14), 209 (15), 208 (100), 182 (5), 181 (6), 105 (28).

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Reactions between Aldehydes and Polyhydric Alcohols. VI.* Conformational Analysis of Methyl Substituted *cis*-2,4,7,9-Tetraoxabicyclo[4.4.0]decanes

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Methyl substituted 2,4,7,9-tetraoxabicyclo-[4.4.0]decanes (*cis*-tetraoxadecalins) have been examined with respect to the so-called 'O-inside – H-inside' equilibrium. Axial 5,10-dimethyl substitution in the O-inside form is shown to produce an otherwise unobservable equilibrium. ΔH and ΔS for the conformational reaction are determined by temperature dependent NMR and a set of diastereomers have been equilibrated by means of acid catalysis and studied by GLC providing a ΔG for the isomerization. Observed solvent effects in the latter case produce a favouring of the O-inside form in polar solvents and correlate very well with results on similar systems. Molecular mechanics calculations have been applied to the system and are found to provide useful information in cases where the experimental data are scarce.

In 1955 Mills[2] realized the possible existence of an O-inside and an H-inside form of *cis*-2,4,7,9-tetraoxabicyclo[4.4.0]decane. He furthermore suggested a relative instability of the H-inside form due to H–H repulsion, a view also sustained by Lemieux and Howard.[3] In 2,4,7,9-tetraoxabicyclo[4.4.0]decane the conformational equilibrium between the two forms (corresponding to the enantiomers of *cis*-decalin) is shifted to the O-inside conformation by an unknown ΔG.[3] Attempts to force the equilibrium towards the H-inside by 1,6-dimethyl substitution (*2–O, 2–H*) failed as no *2–H* was observed[4] (ΔG>1.3 kcal mol^{-1}). This indicates the parent system to be, in fact, very biased towards the O-inside. Appropriate methyl substitution at the 5 and 10 positions (compounds *3–O* and *3–H*) would expectedly destabilize the O-inside and has indeed been shown to give a mixture of the conformers.[5] Further evidence of the possible existence of the H-inside conformation has appeared recently.[6,7] We now wish to report on the isolation of the isomeric compounds, *4–O* and *5–H*, which adopt

* For part V, see Ref. 1.

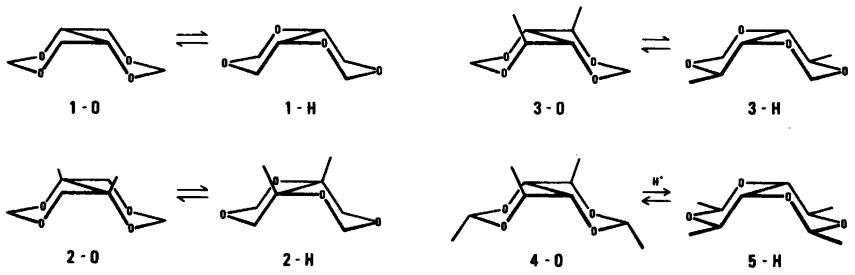


Fig. 1.

0302-4369/83 $2.50

the O-inside and H-inside conformations, respectively. Also a more detailed study of the conformational equilibrium *3–O*⇌*3–H* has been undertaken.

The compounds *3*, *4* and *5* were synthesized from D-mannitol in order to get the correct stereochemistry of the carbon frame, such that the methyl groups adopt axial positions in the O-inside conformation. By condensation with acetaldehyde it is possible to obtain the two forms as stable diastereomers (*4–O*, *5–H*). The interconversion of the two is, however still feasible *via* acid-catalyzed equilibration. From similar systems other isomers than the tetraoxadecalins have been found, either from the synthesis or during equilibration experiments.[8] In the present study no effort was made at isolating or identifying such isomers. During equilibration of the isomers *4–O* and *5–H*, only these two were observed.

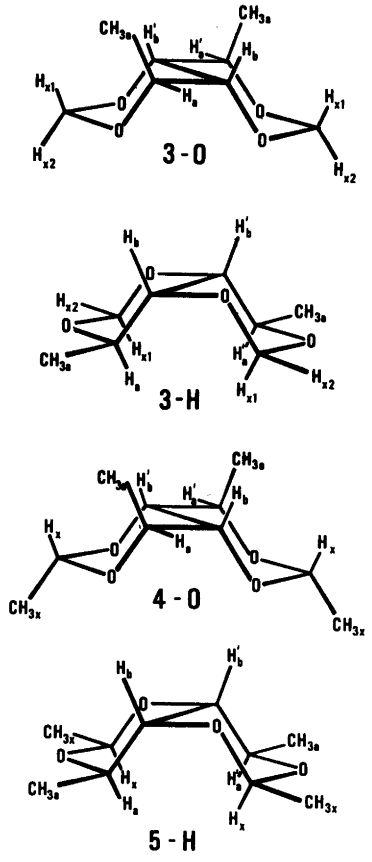


Fig. 2. Proton labelling for compounds *3–5*.

NMR ANALYSIS

The spectral data for the compounds *3*, *4–O* and *5–H* are summarized in Tables 1a, 1b and 1c. They provide fairly strong evidence that the *4–O* and *5–H* compounds are in fact O- and H-inside isomers. *E.g.*, the coupling constant J_{ab} (Fig. 2) is consistent with a *gauche* arrangement in *4–O* and *anti* in *5–H*. Furthermore the chemical shifts of H_a and H_b imply these protons to have the axial/equatorial positions shown. As far as compound *3* is concerned, most of the NMR parameters show values intermediate to those of the conformationally stable homologues *4–O* and *5–H*, indicating the presence of the H-inside isomer in the equilibrium *3–O*⇌*3–H*.

The structure of the H-inside isomer *5–H* was further affirmed by a nuclear Overhauser experiment on the 'inside' protons. Being close to each other one would expect an intensity increase in

Table 1a. NMR data for compound *4–O*.

	ppm		Hz
$\delta(H_{3a})$	1.35	$J(H_a,H_{3a})$	7.2
$\delta(H_{3x})$	1.37	$J(H_x,H_{3x})$	5.0
$\delta(H_b)$[a]	3.47[b]	$J(H_a,H_b)$	1.23[c]
$\delta(H_a)$	4.16[d]	$J(H_b,H_b)$	1.56[e]
$\delta(H_x)$	5.04	$J(H_a,H_b')$	–0.48

[a] The chemical shifts and coupling constants for H_a and H_b are obtained from a LAOCOON simulation. [b] Compare with 3.62 ppm in the parent compound, *cis*-tetraoxabicyclo[4.4.0]decane *1–O*. [c] *Ca.* 0.3 Hz in *cis*-2,4,7,9-tetraoxabicyclo[4.4.0]decane. [d] 4.15 ppm in *cis*-2,4,7,9-tetraoxabicyclo[4.4.0]decane. [e] *Ca.* 0.0 Hz in *cis*-2,4,7,9-tetraoxabicyclo[4.4.0]decane.

Table 1b. NMR data of compound *5-H*.

	ppm		Hz
$\delta(H_{3a})$	1.33	$J(H_a,H_{3a})$	5.8
$\delta(H_{3x})$	1.27	$J(H_x,H_{3x})$	4.9
$\delta(H_b)$[a]	3.78	$J(H_a,H_b)$	10.61
$\delta(H_a)$	4.31	$J(H_b,H_b)$	6.23
$\delta(H_x)$	4.94	$J(H_a,H_b')$	–0.54

[a] Chemical shifts and coupling constants of H_a and H_b were obtained from a LAOCOON spectrum simulation.

Table 1c. NMR data of compound *3* at room temperature.[a]

	ppm		Hz
$\delta(H_{3a})$	1.33	$J(H_a,H_{3a})$	6.6
$\delta(H_b)$[b]	3.65	$J(H_a,H_b)$	6.49
$\delta(H_a)$	4.22	$J(H_b,H'_b)$	4.04
$\delta(H_{x1})$	4.79	$J(H_a,H'_b)$	-0.56
$\delta(H_{x2})$	4.81	$J(H_{x1},H_{x2})$	-6.6[c]

[a] Values reported in Ref. 5 are: $\delta(H_{3a})=1.34$, $\delta(H_b)=3.65$, $\delta(H_a)=4.22$, $\delta(H_{x1})=\delta(H_{x2})=4.83$ ppm and $J(H_a,H_b)=5.9$ Hz. [b] Chemical shifts and coupling constants of H_a and H_b were obtained from a LAOCOON spectrum simulation. [c] Sign of coupling constant assumed.

Table 1d. Chemical shifts (ppm) of *3–O* and *3–H* at -100 °C. Errors are estimated at 0.05 ppm.

	3–O	*3–H*
$\delta(H_{3a})$	1.33[a]	
$\delta(H_b)$	3.55	3.81
$\delta(H_a)$	4.12	4.30
$\delta(H_{x1})$	5.04	4.72
$\delta(H_{x2})$	4.88	4.85

[a] Overlapping of peaks.

Table 1e. Temperature dependence of coupling constants (Hz) in *3*. Estimated error in coupling constants 0.01 Hz.

	$J(H_a,H_b)$	$J(H_b,H'_b)$	$JH_a,H'_b)$
3 (293 K)	6.49_3	4.03_7	-0.55_5
3 (303 K)	6.75_7	4.28_3	-0.55_9
3 (313 K)	6.91_4	4.33_4	-0.54_7
3 (323 K)	7.09_7	4.44_4	-0.52_0
3 (333 K)	7.20_0	4.63_5	-0.54_7
4–O	1.22_7	1.56_0	-0.47_7
5–H	10.61_3	6.22_7	-0.54_1

one signal (*e.g.* H_a) upon irradiation of the other (H_x).[9] Increases of 18.8 (±1.0) % (H_a) and 19.4 (±1.0) % (H_x) relative to internal $CHCl_3$ standard were observed. The proof of the structure seems convincing.[10] However, we do not feel that any quantitative determinations of the H–H distances should be carried out, as extensive coupling is involved in the system. Finally, it may be mentioned that the O-inside does not show any nuclear Overhauser effect.

The conformational equilibrium *3–O*⇌*3–H* is, as expected, fast on the NMR time scale at room temperature, showing only 5 chemical shifts. Profound changes in the spectrum occur on cooling, however. At -20 °C the AB quartet of the O–CH_2–O groups narrows to a singlet, but spreads out again upon further cooling. Below -80 °C all lines broaden and finally at -100 °C split into two broad signals of relative intensities *ca.* 3.7 (±0.5):1. Assigned to the O-inside and H-inside, respectively (see discussion), this corresponds to a ΔG_{173} for the above reaction (*O* to *H*) of *ca.* +0.5 kcal mol^{-1}. The NMR data given in Table 1d agree well with the assignment of the conformers when compared to Tables 1a and 1b.

To obtain a more accurate ΔG value (at room temperature) the coupling between H_a and H_b was used as a measure of the equilibrium. The dihedral angle between the two protons being *gauche* in the O-inside and *anti* in the H-inside would expectedly give rise to a marked difference in $^3J_{ab}$. Due to excessive line widths the respective couplings could not be determined from the spectrum of the two conformers at -100 °C. Therefore, *4–O* and *5–H* were used as model systems, the influence of the (equatorial) 3- and 8-methyl groups on ring geometry and J_{ab} being ignored. Thus the O-inside was assigned a J_{ab} of 1.23 (±0.05) Hz and the H-inside 10.61 (±0.05) Hz. Measuring J_{ab} at temperatures ranging from 20 to 60 °C (Table 1e) then gave the equilibrium constants from which the following data were derived:

$$\Delta G_{298} = -0.18\ (\pm 0.02)\ \text{kcal mol}^{-1}$$
$$\Delta H = +1.5\ (\pm 0.3)\ \text{kcal mol}^{-1}$$
$$\Delta S = +5.6\ (\pm 1.0)\ \text{cal mol}^{-1}\ \text{K}^{-1}$$

At -100 °C this gives a ΔG_{173}= +0.52 (±0.13) kcal mol^{-1}, in good agreement with the values found above. The large positive ΔS values for the reaction *3–O*→*3–H* could indicate that the H-form is in fact a chair/twist-boat conformation. However, as the $^2J(OCH_2O)$ of -6.6 Hz is quite normal[11] and the compound further shows characteristics similar to the symmetrical and non-twisted *5–H*, we conclude that the twist-boat conformation is not present to any appreciable extent.

Table 2. Equilibrium constants and derived ΔG values for the equilibrium *4–O*⇄5–H in 4 different solvents.

Solvent	[*5–H*]/[*4–O*]	ΔG_{298} (kcal mol^{-1})
CCl_4	14.3±0.7	–1.57±0.03
C_6H_6	7.9±0.4	–1.22±0.02
$CHCl_3$	3.0±0.2	–0.65±0.02
CH_3CN	1.8±0.1	–0.35±0.02

ACID-CATALYZED EQUILIBRATION

The tetramethyl compounds *4–O* and *5–H* are conformationally stable but can be interconverted by means of an acidic catalyst. The 3- and 8-methyl groups are equatorial and would hardly influence the equilibrium much compared to the compound *3* derived from formaldehyde. The equilibration has been carried out in 4 different solvents at 25.0 °C and the equilibrium constants were determined by GLC. The results are shown in Table 2.

The sizable solvent dependence reflects the more polar character of the O-inside (dipole moment of 3.7 D as calculated by molecular mechanics; the H-inside has one of 2.0 D). Quantitatively, it was somewhat surprising to find that the difference in "effective polarity" between CCl_4 and benzene is larger than that between $CHCl_3$ and acetonitrile. In a study of solvent effects on acetal conformations Eliel *et al.*[12] obtained similar results, however. Fig. 3 illustrates this fact as a plot of ΔG (O-inside→H-inside) of this study *versus* ΔG (OMe_{ax}→OMe_{eq})

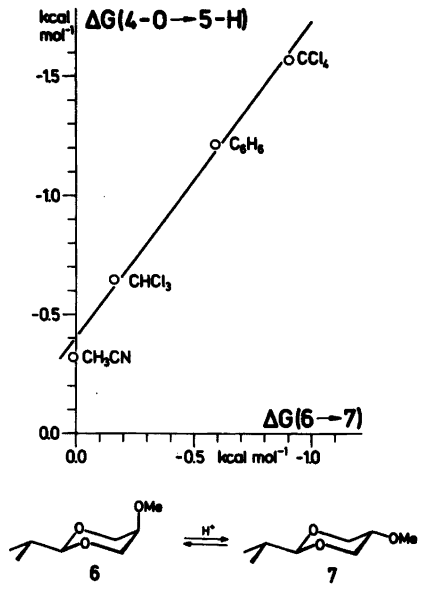


Fig. 3. Plot of observed ΔG for (*4*→*5*) in different solvents *versus* ΔG for the similar equilibrium in 5-methoxy-1,3-dioxane (*6*→*7*).[12]

for the acid catalyzed equilibration of 2-isopropyl-5-methoxy-1,3-dioxane.[12] The correlation coefficient is 0.997, slope=1.33 and intercept = 0.40. A slope greater than unity is not surprising since the bicyclic system contains a larger number of polar bonds.

Neither from the syntheses nor from the equilibration reactions have any other isomers been isolated. Formation of isomeric compounds

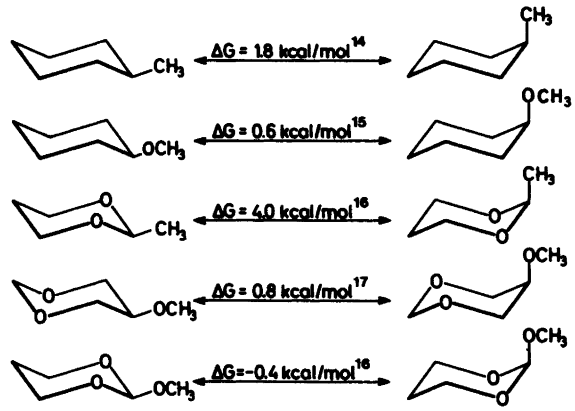
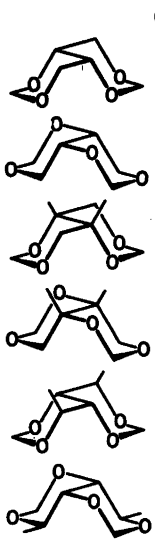


Fig. 4. Model compounds for *gauche* butane interaction terms.



C-C-C-C	C-C-C-O	C-O-C-C	O-C-C-O	C-O-C-O	Energy (kcal mol^{-1})	ΔG(O→H) (kcal mol^{-1})
0	4	4	3	4	9.6	3.7
1	4	6	0	4	13.3	
3	4	6	3	4	16.3	-0.9
2	8	6	0	4	15.4	
2	4	6	3	4	15.4	-1.5
1	6	6	0	4	13.9	

Fig. 5. Schematic enumeration of *gauche* butane interaction terms in compounds *1–O* to *3–H*.

might have been anticipated[4,8] and may well be present in the by-products of the aldehyde condensations. Whether the isomeric bisdioxolanes are energetically unfavourable compared to the bicyclo[4.4.0]decane systems cannot be judged, since the reaction leading to interconversion of *4–O* and *5–H* may not provide a route to 5-membered rings.

FORCE FIELD CALCULATIONS

An estimate of the energy difference between O-inside and H-inside in the parent 2,4,7,9-tetraoxabicyclo[4.4.0]decane system has been made by applying the method of counting *gauche* butane interactions[13] as outlined in Figs. 4 and 5. The increments for CCCC, CCCO, CCOC, COCO and OCCO have been taken from A-values of methylcyclohexane,[14] methoxycyclohexane,[15] 2-methyl-1,3-dioxane,[16] 5-methoxy-1,3-dioxane[17] and 2-methoxy-1,3-dioxane.[16] As shown in Fig. 5 the trend *1* to *2* to *3* is reproduced but the calculated energies are rather poor, *e.g.* $\Delta G = -0.9$ kcal mol^{-1} for *2–O→2–H* for which no *2–H* has been observed experimentally.[4] Whether this is due to lack of transferability from cyclohexane/1,3-dioxane model compounds or it is due to non-additivity of these contributions we cannot conclude, but the method seems to be of limited value especially when force field calculations are available.

In order to gain further insight into the O-inside/H-inside problem, we have performed force field calculations on the compounds *1–O–5–H* (Fig. 1) using Allinger's molecular mechanics programs MM1 and MM2.[18,19] Of particular interest in this case is the geometry of the minimum energy conformations, the dipole moments and the relative enthalpy contents. The MM2 program is a somewhat modified version of MM1, but with respect to the geometries of *1–O – 5–H* very little difference is observed.

When assuming tetrahedral angles around all carbon and oxygen atoms one would expect the *trans*-annular H–H repulsions in the H-inside forms to be quite severe.[2] According to the force field calculations, however, the decalin skeleton is sufficiently flexible to allow inter-annullar H–H distances of 2.3–2.5 Å in the H-inside forms. The calculated dipole moments (O-inside; 3.7–3.8 D, H-inside: 1.9–2.1 D) agree with the measured values[3,4] for *1* and *2* if these are assumed to exist predominantly as O-inside. Dipole moments are, however, rather insensitive to minor changes in geometry. One geometrical parameter of importance in connection with the NMR studies is the H_a–C–C–H_b torsion angle (Fig. 2). According to the force field calculations this angle varies less

Table 3. Enthalpies of the reaction O-inside⇌H-inside as calculated by the MM1/MM2 force field method. A dielectric constant of 2.2 was employed. Values in parentheses are the experimentally determined ones.

1–O⇌1–H	ΔH=1.97/5.45 kcal mol^{-1} ($\Delta G \gg 0$)[a]
2–O⇌2–H	ΔH=1.39/2.76 kcal mol^{-1} ($\Delta G \gg 0$)[b]
3–O⇌3–H	ΔH= –0.84/+0.84 kcal mol^{-1} (ΔH= +1.5)[c] (ΔG= –0.18)[c]
4–O⇌5–H	ΔH= –0.75/+0.86 kcal mol^{-1} (ΔG= –0.65)[d]

[a] Estimated from dipole moment in benzene at 25 °C (Ref. 3). [b] Estimated from dipole moment in benzene at 25 °C (Ref. 4). [c] From NMR data in $CDCl_3$ solution (this work). [d] From GLC determination in $CHCl_3$ solution (this work).

than 0.4° when going from *3–O* to *4–O* and from *3–H* to *5–H*, thus justifying the use of *4–O* and *5–H* as model compounds for *3–O* and *3–H* in the NMR experiment.

When turning to the energies (strictly speaking: enthalpies) of these compounds, substantial differences between MM1 and MM2 are found (Table 3). Quantitatively, they show the same trend regarding the ΔH (O-inside→H-inside), that is, 1,6-dimethyl substitution (bridgehead positions) lowers the energy difference and 5,10 (axial in O-inside) even more so. We conclude that MM2 results are superior to those of MM1 and in fairly good agreement with the limited experimental data available. Finally, the difficulty for the force field in calculating energies of polar molecules well should be mentioned as a probable source of error.[20] The above-mentioned results were obtained with the original parameters of the program and a dielectric constant of 2.2 was employed in the dipole–dipole interaction term. Higher dielectric constants favour the O-inside forms and, in fact, at ε=10.0, ΔH=1.37 kcal mol^{-1} for *3–O/3–H* matches the experimental value of 1.5 kcal mol^{-1}.

DISCUSSION

Having shown that the H-inside form can be produced by appropriate methyl substitution, that is, by destabilization of the O-inside form, it could be tempting to make an estimate of the energy difference between the unsubstituted O-inside and H-inside forms. To this end one could apply purely numerical methods as above, which gives 3.7:1.97:5.45 kcal mol^{-1} from *gauche* butane counting, MM1 and MM2, respectively. These numbers could possibly be refined by assuming constant errors, *i.e.* the calculated value for *3–O→3–H* is set equal to the experimental one (in $CDCl_3$). With these corrections (+3.0:+2.34:+0.66) one obtains ΔH (*1–O→1–H*)=6.7:4.3:6.1 kcal mol^{-1} from the same three methods. Regardless of which of these numbers is the most correct, it does mean that the H-inside form of *cis*-2,4,7,9-tetraoxabicyclo[4.4.0]decane is inherently very unstable, and even trapping of thermally excited molecules (as that of twist-cyclohexane)[21] would hardly enable the observation of this conformation.

There remain a few points to be considered. The substantial solvent effect indicates part of the O-inside/H-inside energy difference to be of electrostatic character and, furthermore, confirms the value of Eliel's scale of solvent polarity [12] as applied to polyoxa compounds. When considering the free energy difference between the dimethyl system (*3–O/3–H*) and the tetramethyl (*4–O/5–H*) of *ca.* 0.5 kcal mol^{-1} this difference could be attributed to buttressing effects. However, the force field calculations do not reproduce this effect.

It has been questioned[22] whether the O-inside isomer *3–O* really is the most stable conformation at low temperature. We find the proton spectrum at –100 °C quite convincing, but one might argue that the presence of the O-inside could be caused by the freon solvent which is known to influence conformations of crown ethers.[23] However, since the equilibrium constants derived from the coupling constants in $CDCl_3$ at ambient temperature and above yield ΔH and ΔS in agreement with the low temperature experiment, any special effect of the freon solvent must be minor, and the low temperature conformation can be stated as being O-inside.

EXPERIMENTAL

The NMR spectra were recorded on a Bruker HX 270 MHz instrument, mass spectra on an

AEI MS-902 and the gas chromatograph was a Hewlett Packard 5840A.

Preparations. 1,6-Dichloro-2*S*,3*S*,4*S*,5*S*-hexantetraol (*8*) was prepared from D-mannitol.[24]

5,10-Dichloromethyl-2,4,7,9-tetraoxabicyclo-[4.4.0]decane (*9*). 2.19 g of *8* (10 mmol) were mixed with 0.60 g trioxane (6.7 mmol) and 20 mg *p*TsOH (0.12 mmol) in 25 ml $CHCl_3$ and heated to 50 °C for 25 h, cooled and stirred for 4 h with 1 g $NaHCO_3$. Filtration and evaporation of solvent yielded 2.5 g of grayish material. Two recrystallizations from absolute ethanol gave 1.49 g (61 %) with m.p. 158 °C (lit. 156 °C).[24]

cis- and *trans*-5,10-dichloromethyl-3,8-dimethyl-2,4,7,9-tetraoxabicyclo[4.4.0]decane (*10, 11*). 5.48 g of *8* (25 mmol) were mixed with 39.6 g of CH_3CHO (0.90 mol, freshly distilled), 30 ml of benzene and 50 mg of *p*TsOH (0.3 mmol) and stirred for 24 h at 25 °C. Then 1 g of $NaHCO_3$ was added and stirred for 2 h. Filtration and evaporation of solvent gave a sirupy white mass, which upon suction filtration and washing with 2×5 ml ether yielded 1.7 g of a white solid (m.p. 140–150 °C). Preparative TLC (silica, $2\times CH_2Cl_2$) yielded two fractions (rf. 0.4 and 0.2). Recrystallization from absolute ethanol gave 690 mg (10.2 %) of *10* (*cis*) m.p. 156 °C (lit. 160 °C) and 315 mg (4.6 %) of *11* (*trans*.) m.p. 196 °C (lit. 195 °C).[25]

5,10-Dimethyl-2,4,7,9-tetraoxabicyclo[4.4.0]-decane (*3*) and 3,5,8,10-tetramethyl-2,4,7,9-tetraoxabicyclo[4.4.0]decane (*cis* and *trans*) (*4* and *5*). The compounds were prepared from the corresponding dichloro compounds (*9, 10* and *11*) by adding these (2 mmol) to a suspension of $LiAlH_4$ (10 mmol) in 75 ml THF and refluxing for 24 h. After successive addition of 1.76 g EtOAc (20 mmol), 380 mg H_2O (21 mmol), 380 mg 4 N NaOH (1.5 mmol) and 1140 mg H_2O (63 mmol),[26] the suspension was filtered on celite and the residue extracted in a Soxhlet extractor for 48 h with ether. Subsequently the combined ether and THF solutions were evaporated to yield a yellow waxy solid. Recrystallization from isopentane yielded 188 mg (54 %) of *3*, m.p. 54 °C (lit. 60 °C),[27] 242 mg (60 %) of *4*, m.p. 74 °C and 226 mg (56 %) of *5*, m.p. 195 °C. All melting points are corrected.

NMR experiments. The spectra were run on samples containing about 10 % (by weight) of the compound in question dissolved in $CDCl_3$. An exception is the spectrum of *3* at low temperature which was run in a mixture of CD_2Cl_2 and CF_2Cl_2 (3:1 by volume). The nuclear Overhauser experiment was carried out with a thoroughly degassed sample containing 75 mg (0.37 mmol) *3* plus 0.75 mmol $CHCl_3$ as internal standard in $CDCl_3$. Decoupling power was just sufficient to delete any H_x (H_a) signal (gated decoupling). The spectrum simulations were performed with a modified LAOCOON program.[28]

Acid-equilibration. Ca. 2 mg of the tetramethyl compound (*4–O* or *5–H*) (*ca.* 10 μmol) were dissolved in 100 μl of solvent to which were added 0.1 μl BF_3–Et_2O (1 μmol) and 0.5 mg of naphthalene (4 μmol) as internal standard. The vessel was closed under N_2 and kept in a thermostated bath at 25.0 (±0.2) °C. After 2–3 days the equilibrium was established and 0.5 μl of the sample was injected on a 10 % polypropyleneglycol on Chromosorb W 60/80 column (180 cm, 4.0 mm Ø, flow=15 ml/min). Several runs were made on each sample. The same composition was obtained when starting from either isomer, thus assuring complete equilibration. The ratios *H/O* as calculated from the integrated GLC peaks were multiplied by a response factor of 0.951 to give the equilibrium constant with a reproducibility of ±4 % corresponding to standard errors on ΔG of *ca.* 0.02 kcal mol^{-1}. By comparison with the naphthalene peak in each run it was ascertained that no loss of *4* or *5* had occurred during the equilibration.

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Reduction of Organofluorine Compounds with Lithium Triethylborohydride

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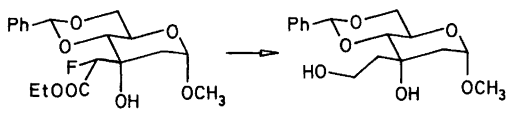
The reductive cleavage of the C–F bond with lithium triethylborohydride is strongly retarded by steric hindrance but is accelerated by adjacent hydroxyl groups in the organofluorine compound (Table 1). The effect of the hydroxyl group is strongest when it is tertiary, and compounds having such hydroxyl groups are evidently reduced exclusively *via* epoxides.

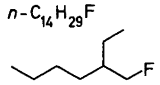
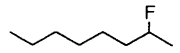
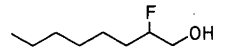
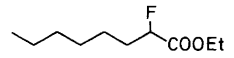
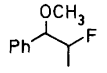
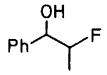
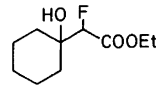
Lithium triethylborohydride is an efficient reducing agent which for many purposes is superior to lithium aluminium hydride.[1,2] Several functional groups have been reduced with lithium triethylborohydride,[1,2] but there seems to be only one example in the literature of a reductive cleavage of a carbon–fluorine bond.[3] We found that the reduction *1*→*2* occurred with remarkable ease and *2* was isolated in a 56 % yield, whereas treatment of *1* with lithium aluminium hydride gave only traces of *2*.[3] These results prompted us to undertake a more extensive study of the reduction of organofluorine compounds with lithium triethylborohydride, including both an investigation of structure–reactivity relationships and a mechanistic study.

Eight organofluorine compounds (*3*–*10*) were synthesized and their reduction with lithium triethylborohydride in THF (22 or 65 °C) followed by GLC. The relative rates of reduction of the alkylfluorides *3*–*5* (Table 1) demonstrate a pronounced sensitivity to steric effects. Thus, while the primary alkylfluoride *3* was completely (>99.5 %) reduced in one hour at 65 °C, the



1 *2*

Table 1. Approximate relative rate constants for the reductive cleavage of the C–F bond in compounds *3*–*10* with lithium triethylborohydride. The reductions were performed at two different temperatures (see Experimental). Compounds *8* and *9* are mixtures of diastereomers and each compound therefore gives two relative rate constants.

	Compound	k_{rel}
3	n-$C_{14}H_{29}F$	1
4		0.08
5		0.0009
6		0.005
7		0.005
8		0.0003 0.0006
9		0.04 0.13
10		14

0302-4369/83 $2.50

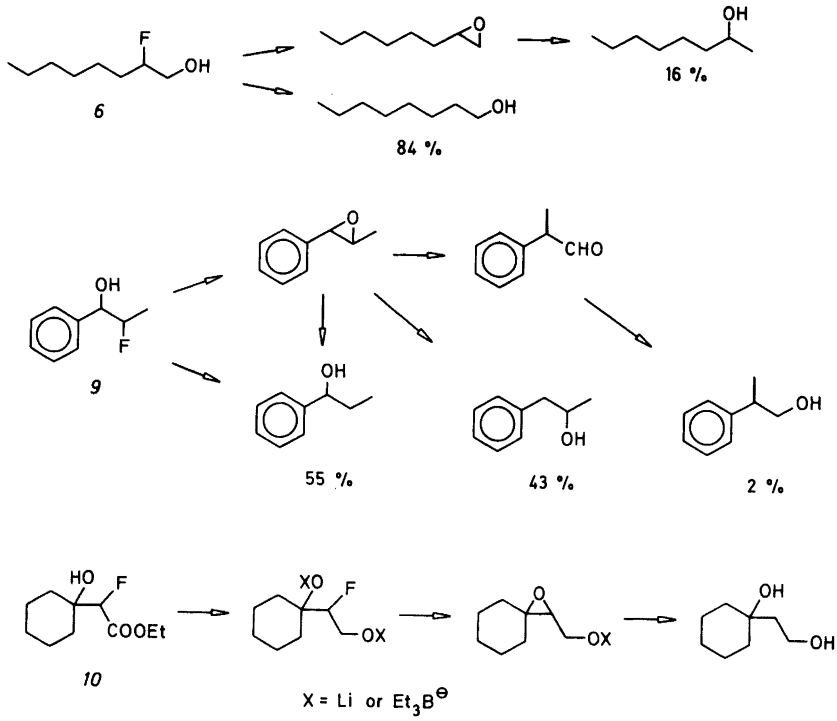


Fig. 1. The structures of the products obtained on reduction of *6*, *9* and *10* with lithium triethylborohydride and the relative rates of reduction (Table 1) strongly indicate the intermediacy of the epoxides shown.

secondary fluoride *5* was only reduced to about 3 % under these conditions. After 24 h (65 °C), 42 % of *5* had been reduced and, since competing reactions such as eliminations were negligible, it should also be possible to obtain high yields in the reduction of secondary alkyl fluorides.

Further inspection of Table 1 shows that an adjacent hydroxyl group, or a functional group which is reduced to a hydroxyl group during the initial stage of the reaction, accelerates the reductive cleavage of the carbon–fluorine bond. This increase in reaction rate seems at least in part to be due to the formation of epoxide intermediates. The fact that mixtures of isomeric products are obtained in the reductions of *6* and *9* is strong evidence in favour of such intermediates (Fig. 1). Since 1,2-epoxybutane[1] and 1,2-epoxyoctane[8] are reduced to 2-alkanols exclusively, one may conclude that 16 % of *6* is reduced *via* an epoxide. An adjacent methoxy group has an effect on the reduction rate which is opposite to that of the hydroxyl group (*cf.* Table 1, compound *8*), probably due to the increased steric hindrance.

Compound *9* is a 53:47 mixture of diastereomers, 9_{major} and 9_{minor}. On reduction with lithium triethylborohydride, it yields a mixture of three compounds (Fig. 1). Since the yield of 1-phenyl-2-propanol is 43 %, one may conclude that at least 43 % of *9* must be reduced *via* an epoxide. By studying the composition of the reduction mixture as a function of reaction time, we found that 9_{major} was reduced about three times faster than 9_{minor} and that 1-phenyl-1-propanol was formed faster than 1-phenyl-2-propanol. Reduction of *trans*-1-phenyl-1,2-epoxypropane with lithium triethylborodeuteride yields[4] deuteriated analogues of 1-phenyl-1-propanol and 1-phenyl-2-propanol in the approximate ratio 1:9; reduction of the corresponding *cis* epoxide with the protium reagent gives[8] the approximate ratio 11:1. These results indicate that the main precursor to 1-phenyl-2-propanol is the *trans* epoxide which in turn should be formed from (1*RS*,2*SR*)-

9. For the minor product 2-phenyl-1-propanol (≈2 %) a reduction pathway involving an epoxide to aldehyde rearrangement may be envisaged. An analogous epoxide to ketone rearrangement followed by reduction is a formally possible route to 1-phenyl-2-propanol. It cannot, however, be the major route since reduction with lithium triethylborodeuteride leads to a monodeuteriated 1-phenyl-2-propanol which has its deuteron at C−1 rather than at C−2 (^{1}H NMR).

Of the organofluorine compounds in Table 1, compound *10*, which is structurally similar to *1*, has the most reactive C−F bond. The only product detected on reduction of *10*, 1-(2-hydroxyethyl)-cyclohexanol, was isolated in a 78 % yield. Thus, the reactivity increases in going from *6* to *9* to *10*. These three compounds all have a secondary fluorine and an adjacent hydroxyl group which is primary, secondary or tertiary, respectively. We interpret the increased reactivity as being due to an increasing ease of formation of an epoxide intermediate (*cf.* Ref. 5). The high reactivity of *10* indicates that it is the tertiary rather than the primary hydroxyl group which is involved in the epoxide formation. Both mono- and trisubstituted epoxides are reduced exclusively by attack at the least substituted carbon.[1] Therefore, the structure of the single product from *10* is a further indication of the intermediacy of the trisubstituted epoxide (Fig. 1).

Besides the direct displacement of fluorine by hydride and the reduction *via* epoxide, a third, minor mechanism may also be operating. After reduction and oxidative work-up using alkaline hydrogen peroxide, significant amounts of 2-butanol were detected (GLC) in the crude reaction products obtained from *6*, *7*, *9* and *10*, but not in those from *3*, *4*, *5*, and *8* (< 1 %). On reduction of *6* with lithium triethylborodeuteride, deuteriated analogues of 1-octanol and 2-octanol were obtained in 77 and 23 % yields, respectively; the molar ratio 2-butanol:1-octanol was 0.19:1. The deuterium contents of 1-octanol and 2-octanol, as found by mass spectra of *o*-(methylamino)-benzoates,[6] were approximately 90 and 95 %, respectively. In view of the four times excess of reagent and a deuterium isotope effect of presumably normal magnitude, it seems not unreasonable to obtain as much as 5 % of nondeuteriated 2-octanol. On the other hand, the percentage of nondeuteriated 1-octanol was unexpectedly high. A reduction mechanism consistent with the formation of both 2-butanol and nondeuteriated 1-octanol is shown in Fig. 2. However, if this kind of reaction occurs, it seems to be responsible for only about 25 % (*i.e.* 5/19) of the total formation of 2-butanol. An intermolecular hydride-transfer accompanied by a rearrangement of the same kind as that in Fig. 2 occurs *e.g.* in reductions with lithium 9,9-dibutyl-9-borabicyclo[3.3.1]nonanate.[7]

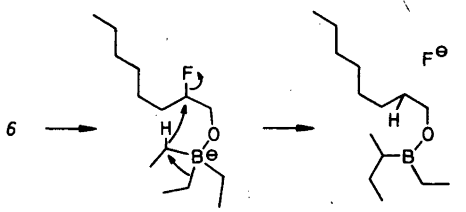


Fig. 2. Hypothetical minor reaction pathway which accounts for the formation of both 2-butanol and nondeuteriated 1-octanol in the reduction of *6* with lithium triethylborodeuteride.

A fourth mechanistic possibility is the elimination of fluoride ion by a hydride shift from the adjacent hydroxyl-bearing carbon. Such a shift occurs *e.g.* in the reduction of methyl 4,6-*O*-benzylidene-2-*O*-(4-methylbenzenesulfonyl)-α-D-mannopyranoside to methyl 4,6-*O*-benzylidene-[3-^{2}H$_1$]-α-D-*ribo*-hexopyranoside with lithium triethylborodeuteride,[8] and in the reactions between fluorohydrins and Grignard reagents.[9] However, reduction of *9* with lithium triethylborodeuteride leads to a monodeuteriated 1-phenyl-1-propanol in which H-1 integrated for 1.0 H and one may therefore conclude that the hydride shift mechanism plays no significant role in this reduction.

Even though no reaction intermediates or isomeric products were found in the reduction of *1*, the product distribution and the reactivity in the series *6*, *9* and *10* indicates that the reduction of *1* with lithium triethylborohydride occurs *via* a trisubstituted epoxide analogous to the reaction pathway shown for *10* in Fig. 1.

EXPERIMENTAL

THF was distilled over $LiAlH_4$ before use. Lithium triethylborohydride and lithium triethylborodeuteride were obtained (Aldrich) as 1 M solutions in THF. Reductions were carried out under an atmosphere of nitrogen. Analytical

GLC was performed on a Hewlett-Packard 5830 A gas chromatograph (electronic integration) equipped with an SP 2100 or an FFAP fused silica capillary column (25 m × 0.2 mm). The latter column was used for the determination of 2-butanol. GLC-MS was performed on a Varian MAT 311 instrument; ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL JNM-FX 100 instrument.

1-Fluorotetradecane (*3*) was obtained as a commercial product.

2-Ethyl-1-fluorohexane (*4*) was prepared from 2-ethylhexyl tosylate largely as described for 1-fluorooctane[10] (20 h, 60–65 °C). After work-up, the crude reaction product was treated with bromine to a persistent colour in order to convert the alkene(s) into dibromide(s). Subsequent distillation (70–71 °C, ≈ 12 kPa) gave *4* in a 16 % yield and in a purity of 99 % (GLC). ^1H NMR: δ 4.35 (2 H, d of d, J_{HF} 47.0 Hz, J_{HH} 6.0 Hz), 1.5–0.7 (15 H, m). ^{13}C NMR: 85.9 (d, J_{CF} 168.5 Hz), 40.7 (d, J_{CF} 18.3 Hz), 30.0 (d, J_{CF} 6.1 Hz), 29.3, 23.4 (d, J_{CF} 6.1 Hz), 23.2, 14.0 and 11.1 ppm.

2-Fluorooctane (*5*) was prepared from 2-octanol using the method of Olah and Watkins.[11] As in the synthesis of *4*, the crude product was treated with bromine. The b.p. and ^1H NMR spectrum of *5* were as previously described;[12] GLC purity: 99.7 %.

2-Fluoro-1-octanol (*6*). 1,2-Epoxyoctane (19.2 g), prepared from 1-octene and *m*-chloroperbenzoic acid, was dissolved in methylene chloride and allowed to react with 42 % hydrogen fluoride in pyridine[13] (22 °C, 43 h). Work-up, including washing with aqueous sodium carbonate, afforded a crude mixture (8.0 g) containing 49 % (GLC) of *6*. Separation of the component on silica gel (light petroleum–ethyl acetate; 5:1) followed by distillation (67.5–69 °C, 0.3 kPa) gave *6* in a purity of 99.2 % (GLC). Lit.[14] b.p. 98–100 °C/12 Torr. ^1H NMR: $\delta \approx 4.5$ (1 H, d of m, $J_{HF} \approx 47$ Hz), 3.7 (2 H, broad d, $J_{HF} \approx 27$ Hz), 2.1 (1 H, broad s), 2–0.7 (m, 13 H). ^{13}C NMR: 94.8 (d, J_{CF} 168.5 Hz), 64,9 (d, J_{CF} 22.0 Hz), 31.7, 31.0 (d, J_{CF} 20.8 Hz), 29.1, 24.9 (d, J_{CF} 4.9 Hz), 22.6 and 14.0 ppm.

Ethyl 2-fluorooctanoate (*7*) was prepared from the corresponding α-bromo ester and potassium fluoride in acetamide.[15] After work-up and treatment of the crude reaction product with bromine, *7* was distilled at 60–61.5 °C, 80 Pa (31 %, GLC purity 88 %); ^1H NMR: 4.3–4.0 (m, 3 H), 2.1–0.7 (m, 19 H), ^{13}C NMR: 170.0 (d, J_{CF} 23.8 Hz), 89.0 (d, J_{CF} 183.7 Hz), 61.2, 32.4 (d, J_{CF} 20.8 Hz), 31.5, 28.7, 24.3 (d, J_{CF} 3.1 Hz), 22.5, 14.1, and 13.9 ppm.

2-Fluoro-1-methoxy-1-phenylpropane (*8*) was prepared from *9*, silver(I) oxide and methyl iodide;[16] B.p. 30 °C, 0.1 kPa; 90 % yield. GLC purity 97 %; diastereomeric ratio 53:47. ^{13}C NMR: 138.1, 137.9, 128.5–127.7 (six signals), 92.0 (d, J_{CF} 173.3 Hz), 91.6 (d, J_{CF} 175.2 Hz), 86.7, 86.1, 85.8, 85.2 (four signals from C–1), 56.9, 56.8, 17.4, 16.5, 16.3 and 15.4 ppm, the latter four signals from C–3.

2-Fluoro-1-phenyl-1-propanol (*9*) was prepared by reduction of the corresponding ketone[17] with excess sodium borohydride in methanol (0–23 °C, 1 h); b.p. 76–77 °C, 27 Pa; diastereomeric ratio 53:47; GLC purity 96.3 %. The ^1H NMR spectrum was in accord with the structure.

Ethyl 2-fluoro-2-(1-hydroxycyclohexyl)-ethanoate (*10*) was prepared using a modification[3] of a published procedure.[18] After distillation it showed a GLC purity of 99 %; ^{13}C NMR: 168.4 (d, J_{CF} 24.4 Hz), 93.4 (d, J_{CF} 190.4 Hz), 72.4 (d, J_{CF} 19.5 Hz), 61.7, 32.8 (d, J_{CF} 2.4 Hz), 32.7 (d, J_{CF} 3.7 Hz), 25.4, 21.2, 21.1, 14.2 ppm.

Determination of reduction rates for 3–10. The organofluorine compound (1 mmol) was allowed to react with lithium triethylborohydride in THF (22 or 65 °C). Nonane or tetradecane (for the reduction of *10*) was used as internal standard. The amounts of reagent and THF were chosen so that a 0.8 M solution (4 mmol) of the reagent was left for reduction of the C–F bond after reaction with hydroxyl and ester groups. In the reductions of *7* and *10* the concentrations were, however, 0.7 and 0.6 M, respectively. Three to six aliquots (0.2 ml each) were withdrawn with a syringe at reaction times between 0.5 and 28 h and quenched and oxidized with aqueous NaOH (1 M, 0.5 ml)+aqueous H_2O_2 (35 %, 0.5 ml). After 30 min at 65 °C, the mixture was cooled, the organic components extracted with methylene chloride (3×1 ml) and the combined extracts dried ($MgSO_4$) and analyzed by GLC.

Compounds *3* and *10* were reduced at 22 °C, compounds *5–8* at 65 °C, and compounds *4* and *9* at both temperatures. The reductions were treated as pseudo first order reactions and the rate constant was divided by the concentration of the reducing agent left after its reaction with the hydroxyl and ester groups. Points corresponding to high or low conversions of the starting material were deleted and a mean rate constant was calculated from the remaining points. Second order rate constants were also calculated but the differences between these k_{rel} values and the values in Table 1 were insignificant. The largest difference was found for *9* which showed nearly doubled values of k_{rel}.

The structures of the reduction products were confirmed by comparison with authentic samples

(capillary column GLC and MS). One of the products isolated and characterized by NMR was *1-(2-hydroxyethyl)-cyclohexanol.* Compound *10* (1 mmol) was allowed to react with lithium triethylborohydride (7 mmol) in THF (65 °C, 10 min). After work-up as described above, the crude reaction product was purified on a silica gel column (ethyl acetate) and the diol was obtained as a colourless oil in a 78 % yield; GLC purity 98 %; ^{13}C NMR: 71.4, 57.9, 40.7, 36.7, 24.9 and 21.3 ppm.

Reduction of 6 *with lithium triethylborodeuteride.* A mixture of *6* (2 mmol) and lithium triethylborodeuteride (10 mmol) in THF (10 ml) was heated under reflux (43 h). Work-up as described above gave a mixture of 1-octanol (42.2 %), 2-octanol (12.3 %) and *6* (45.4 %). For the conversion into *o*-(methylamino)-benzoates, a sample of the above mixture (20 mg) and *N*-methyl-isatoic anhydride (50 mg) was heated (115 °C, 20 h) in pyridine (1 ml) and triethylamine (5 drops) in a sealed tube. After evaporation of the solvents, the products were analyzed by GLC-MS using an OV-225 packed column. The deuterium contents were calculated from the intensities in the molecular ion groups and are only approximate because of the isotope separation on GLC.

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Letter

3-*N*-Acyl Uridines: Preparation and Properties of a New Class of Uracil Protecting Group

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It has been clearly shown by Reese and Ubasawa [1] that the protection of the aglycone in uridine is necessary to be able to carry out the chemical synthesis of oligoribonucleotides containing uridine moieties.

Thus, Reese and his co-workers have introduced a 4-*O*-aryl protective group [3] for the uracil moiety of the oligoribonucleotide synthesis. Similarly, Hata *et.al* [4] have proposed a 3-*N*-2,2,2-trichloro-*t*-butyloxycarbonyl group to protect the imide function during their seven-step synthesis of 2′-*O*-methyluridine. In the present work, we propose a more convenient set of N^3-acyl protecting groups, as in *2a* to *2e*, which may be prepared in high yields by a "one-pot" synthesis as outlined in Scheme 1. The general procedure for the "one-pot" synthesis of such N^3-protected acyl derivatives, (*2a*) to (*2e*), involved trimethylsilylation [5] of uridine in dry pyridine solution which is followed by the addition of acyl chlorides *in situ* and then hydrolysis. Standard work-up and purification by column chromatography on silinized silica gel (MeOH–H_2O mixture in the mobile phase) gave pure 3-*N*-protected acyl uridines *2a* to *2e* in 70, 55, 60, 50 and 50 % yields, respectively. To our knowledge, the present preparation of the above N^3-acyl uridines constitutes their first report in the literature.

It was then interesting to explore the relative stabilities of these N^3-acyl groups in 3-*N*-acyluridines, *2a* to *2e*, under a variety of basic conditions to evaluate their possible use in total chemical synthesis of tRNA molecules in conjuction with other base-labile groups on the pentose sugar and phosphotriester moieties. Table 1 describes the relative rates of removal of the acyl groups from the corresponding 3-*N*-acyl derivatives. Thus it becomes readily clear from experiments 2, 3 and 5 in Table 1 that most of these acyl groups may be used in conjunction with other sugar and phosphate protecting groups which are removable either by hydrazine hydrate (0.5 M) in pyridine–acetic acid 3:2 v/v (*e.g.* levulinyl [6]) or by fluoride ions (*e.g.* *t*-butyldimethyl silyl [7] and

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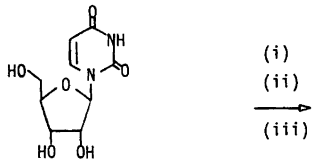

1

2a Ar = Ph
2b Ar = p-tolyl
2c Ar = o-tolyl
2d Ar = p-anisyl
2e Ar = mesityl

Scheme 1. (i), Me_3SiCl; (ii), ArCOCl; (iii), H_2O.

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Table 1. The relative rates of hydrolyses of acyl groups from N^3-acyl uridines *2a–2e*.

Expt. No.	Reagents	$t_{1/2}$ (min) of hydrolyses; Ar=				
		Ph	*o*-Tolyl	Mesityl	*p*-Anisyl	*p*-Tolyl
1	Morpholine (5 eq.) THF–H_2O (98:2 v/v)	30	70	[a]	120	270
2	$N_2H_4 \cdot H_2O$ (0.5 M) in pyridine: AcOH (3:2 v/v)	[c]	[d]	[a]	[e]	[e]
3	n-$Bn_4N^+F^-$ (1 M) in dry THF	[a]	[a]	[a]	[a]	[a]
4	Aq. NH_3 (d.0.9) in dioxan (1:1 v/v)	9	20	720	30	13
5	Et_3N (20 eq.) in dry pyridine (10 ml/mmol)	[a]	[a]	[a]	[a]	[a]
6	2.5 M NH_3 in dry CH_3OH	6	25	[b]	37	15

[a] Stable for 8 h. [b] Stable for 1 h. [c] <*ca.* 10 % degradation in 1 h. [d] <*ca.* 5 % degradation in 1 h. [e] <*ca.* 1 % degradation in 1 h.

1,1,3,3-tetraisopropyldisiloxane-1,3-diyl [8]) or by a non-nucleophilic base like triethylamine (*e.g.* 2-phenylsulfonylethoxycarbonyl,[10] 2-(4-chlorophenyl)-sulfonylethoxycarbonyl,[11] fluoren-9-ylmethoxycarbonyl,[12] 2-phenylsulfonylethyl [13] and fluoren-9-methyl [14]).

However, it seems unlikely that the 3-*N*-mesityl uridine would find any useful synthetic application as a protecting group in view of its relatively high stability even under the condition of experiment 4 in Table 1.

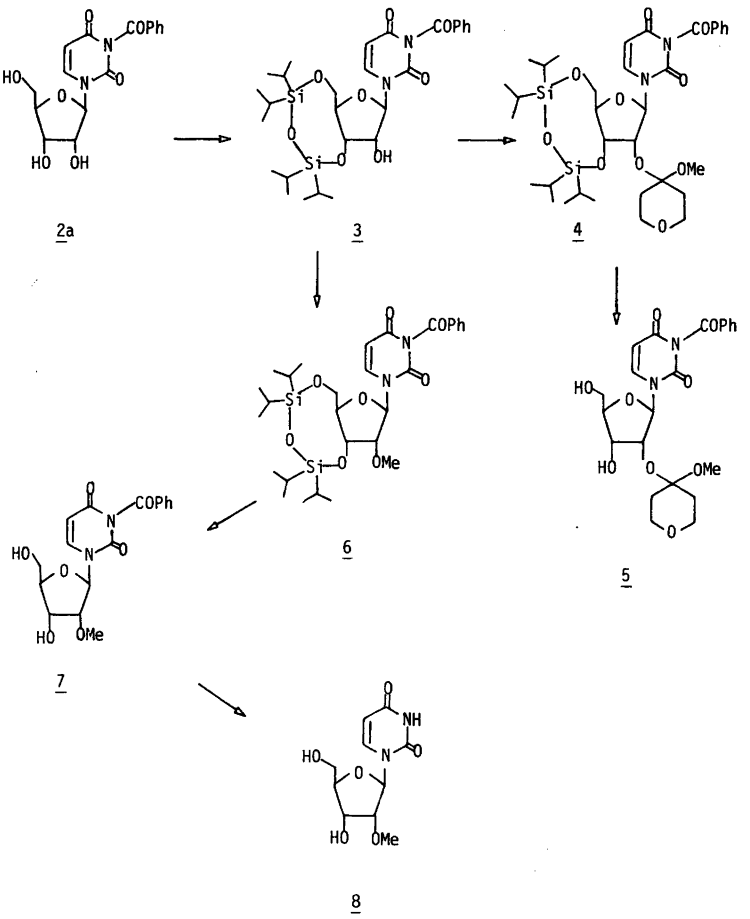
After carefully evaluating the stabilities of the above N^3-acyl groups under a variety of conditions, we are convinced that the 3-*N*-benzoyluridine itself would fulfill the requirements of a 3-*N*-protected building block of uridine [3] in an actual chemical synthesis. Thus, we have synthesized: (a) 3-*N*-benzoyl-2′-*O*-(4-methoxytetrahydropyranyl)-uridine (*5*), a crucial building block for oligoribonucleotide synthesis, and (b) 2′-*O*-methyl uridine (*8*) which has been prepared recently by a Japanese group [4] in seven steps in 29 % overall yield. The outline for the syntheses of *5* and *8* is shown in Scheme 2.

The common intermediate *3* for the synthesis of the above target compounds was prepared in 77 % yield by the treatment of *2a* with a slight excess of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDSiCl_2) in dry pyridine solution following a procedure reported in the literature.[8] The 4-methoxytetrahydropyranyl group [9] was then introduced at 2′-position of *3*, by acid catalysis, to give *4*, which was isolated as a crude mixture (*ca.* 95 % purity by TLC; 10 % MeOH–$CHCl_3$ mixture). The crude mixture was subsequently treated with n-$Bu_4N^+F^-$ in dry tetrahydrofuran ([F^-]≈0.01 M; 2.2 equiv.) for 5 min at 20 °C, to remove the TIPDSi group, to obtain *5*, in 82 % overall yield from *2a*. The ^{1}H NMR absorptions confirmed the structure of *5*. This is currently in use in our laboratory for the synthesis of tRNA fragments.

The procedure for the preparation of *8* was as follows: *3* was methylated with methyl iodide (15 equiv.) in dry acetone solution (10 ml/mmol) in the presence of silver oxide (50 equiv.).[2a] Thus, *6* was obtained in 86 % yield as a white powder. The TIPDSi group was then removed from *6* with n-tetrabutylammonium fluoride, as described above, to give *7* in 90 % yield. Finally, the N^3-benzoyl group was removed by an excess of 5 M ammonia solution in dry methanol to give 2′-*O*-methyl uridine (*8*) in 92 % yield (overall yield from *2a* was 58 %). The identity of all new compounds has been established by ^{1}H NMR, UV and element analysis.

Thus, the present work offers a set of convenient 3-*N*-protected uridine derivatives which are easily accessible in high yields and useful for further chemical transformations.

Experimental. All new compounds have been characterized by ^{1}H NMR, UV and element analysis. ^{1}H NMR spectra were measured at 90 MHz with a Jeol FX 900 spectrometer. UV



Scheme 2.

absorption spectra were measured with a Cecil Ce 545 double beam scanning spectrometer.

Compound 2a. UV (water): λ_{max} 260 nm (pH 2); 260 nm (pH 7); 260 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.17 (*d*, 8.2 Hz, 1H), H-6; 8.0 (*m*, 1H), 1- and 5-H of 3-*N*-benzoyl group; 7.68 (*m*, 3H), 2-, 3- and 4-H of 3-*N*-benzoyl group; 5.93 (*d*, 8.2 Hz, 1H), H-5; 5.77 (*d*, 3.9 Hz, 1H), H-1′; 4.06 (*m*, 3H), H-2′, -3′ and -4′; 3.65 (*m*, 2H), H-5′.

Compound 2b. UV (water): λ_{max} 260 nm (pH 2); 260 nm (pH 7); 260 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.13 (*d*, 8.2 Hz, 1H), H-6; 7.86 (*d*, 9 Hz, 1H), *ortho* protons adjacent to >C=O of 3-*N*-4-tolyl group; 7.41 (*d*, 9 Hz, 1H), *meta* protons of 3-*N*-4-tolyl group; 5.93 (d, 8.2 Hz, 1H), H-5;5.77 (*d*, 4.3 Hz, 1H), H-1′; 4.05 (*m*, 3H), H-2′, -3′ and -4′; 3.64 (*m*, 2H), H-5′.

Compound 2c. UV (water): λ_{max} 270 nm (pH 2); 270 nm (pH 7); 270 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.14 (*d*, 8.2 Hz, 1H), H-6; 7.7–7.29 (*m*, 4H), *o*-tolyl group; 5.75 (*d*, 4.2 Hz, 1H), H-1′; 5.93 (*d*, 8.2 Hz, 1H), H-5; 4.05 (*m*, 3H), H-2′, -3′ and -4′; 3.62 (*m*, 2H), H-5′.

Compound 2d. UV (water): λ_{max} 280 and 298 nm (pH 2); 282 and 298 nm (pH 7) 278 and 298 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.15 (*d*, 8.2 Hz, 1H), H-6; 7.92 (*d*, 8.1 Hz, 1H), *ortho* protons adjacent to >C=O of 3-*N*-*p*-anisyl group; 7.1 (*d*, 8.1 Hz, 1H), *meta* protons of 3-*N*-*p*-anisyl group; 5.75 (*d*, 4.3 Hz, 1H), H-1′; (*m*, 3H), 4.06 (*m*, 3H), H-2′, -3′ and -4′; 3.64 (*m*, 2H), 5′-CH_2.

Compound 2e. UV (water): λ_{max} 274 nm (pH 2); 278 nm (pH 7); 280 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.07 (*d*, 8.2 Hz, 1H), H-6;

6.97 (*s*, 2H), aromatic protons of 3-*N*-mesityl group; 5.85 (*d*, 8.2 Hz, 1H), H-5; 5.75 (*d*, 4.3 Hz, 1H), H-1′; 3.97 (*m*, 3H), H-2′, -3′ and -4′; 3.64 (*m*, 2H), H-5′.

Compound 3. ^{1}H NMR ($CDCl_3$+D_2O): δ 7.92 (*d*, 8.2 Hz, 1H), H-6; 7.84–7.4 (*m*, 5 H), 3-*N*-benzoyl group; 5.80 (*d*, 8.2 Hz, 1H), H-5; 5.69 (*d*, 5.0 Hz, 1H), H-1′; 4.28 (*m*, 1H), H-3′; 4.22 (*m*, 4 H), H-2′, -4′ and -5′; 1.1 (*m*, 28 H), tetraisopropyl groups.

Compound 5. ^{1}H NMR (DMSO-d_6+D_2O): δ 8.20 (*d*, 9 Hz, 1 H), H-6; 8.05–7.56 (*m*, 5H), 3-*N*-benzoyl group; 6.06 (*d*, 9 Hz, 1 H), H-5; 5.97 (*d*, 5 Hz, 1 H), H-1′; 4.40 (*m*, 1H), H-2′; 3.95 (*m*, 2H), H-3′ and -4′; 3.82 (*m*, 2H), 5′-CH_2; 3.54 (*m*, 4H), 2- and 6-protons of 4-methoxytetrahydropyranyl group (MTHP); 3.28 (*s*, 3H), –O*C*H_3 of 4-MTHP group; 1.68 (*m*, 4H), 3- and 5-protons of 4-MTHP group. This compound upon debenzoylation with 5*M* NH_3 in MeOH gave 2′-*O*-(4-methoxytetrahydropyranyl)uridine as the sole product.

Compound 6. ^{1}H NMR ($CDCl_3$); δ 8.0 (*d*, 8.5 Hz, 1 H), H-6; 7.93–7.4 (*m*, 5 H), 3-*N*-benzoyl group; 5.83 (*d*, 8.5 Hz, 1H), H-5; 5.79 (*d*, 4 Hz, 1 H) H-1′; 4.18–4.05 (*m*, 3H), H-2′, -3′ and -4′; 3.78 (*m*, 2H), H-5′; 3.60 (*s*, 3H), 2′-O-*C*H_3; 1.15 (*m*, 28H).

Compound 7. ^{1}H NMR (DMSO-d_6+D_2O): δ 8.20 (*d*, 9 Hz, 1 H), H-6; 8.02–7.85 (*m*, 2H) and 7.80–7.5 (*m*, 3H) are 3-*N*-benzoyl protons; 5.93 (*d*, 9 Hz, 1H), H-5; 5.87 (*d*, 4.6 Hz, 1 H), H-1′; 4.19 (*dd*, 4.6 andd 3.8 Hz, 1 H), H-2′; 3.87 (*m*, 2H), H-3′ and -4′; 3.64 (*m*, 2H), 5′-CH_2; 3.38 (*s*, 3H) 2′-O–*C*H_3.

Compound 8. ^{1}H NMR (DMSO-d_6+D_2O): δ 7.92 (*d*, 8.1 Hz, 1 H), H-6; 5.83 (*d*, 5 Hz, 1 H), H-1′; 5.63 (*d*, 8.1 Hz, 1 H), H-5; 4.09 (*m*, 1 H), H-2′; 3.82 ((*m*, 4H), H-3′, -4′ and -5′; 3.35 (*s*, 3H), 2′-O–CH_3.

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Short Communications

Preparation of 2-Aryl-3-oxetanols

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The literature reports describe only a few reactions in which formation of 3-oxetanols is observed. These compounds are formed in the Norrish type II photoreactions of ketones,[1–11] in the reduction of oxetanones[12] or oxetanyl acetates,[13] in the cleavage of ether linkage of some substituted oxetanes,[14,15] in the photoaddition of acetone to its enol at low temperature,[16] in the chlorination of some β,γ-unsaturated α-hydroxy ketones[17] and in the ring closure of derivatives of substituted 1,2,3-triols.[18]

In this work we have synthesized 3-acetoxy-2-aryloxetanes and 2-acetoxy-4-aryloxetanes from vinyl acetate and aromatic aldehydes through a photochemical reaction. The basic hydrolysis of the oxetanyl acetate mixture gave a mixture of *cis*- and *trans*-2-aryl-3-oxetanols. The major product in each case was *cis*-2-aryl-3-oxetanol, which was purified by crystallization and identified by spectroscopic methods.

The photolyses of benzaldehyde or *o*-chlorobenzaldehyde with vinyl acetate were carried out in benzene solution in a photochemical reactor equipped with a high pressure mercury lamp. In both of the reactions a mixture of four oxetanyl acetates *1–4* (R=$COCH_3$, Scheme 1) was formed. The mixtures were analyzed by gas chromatography and ^1H NMR spectrometry. The product ratio was 61:16:5:18 for *1a*, *2a*, *3a* and *4a* and 60:18:5:17 for *1b*, *2b*, *3b* and *4b*. The overall oxetanyl acetate yields were 23 % for the compounds *a* and 37 % for the compounds *b*. The isomers *1*, *2*, *3* and *4* were isolated by preparative gas chromatography. The structures of the isolated compounds were established by means of ^1H and ^{13}C NMR, infrared and mass spectra. The NMR data of oxetanes are presented in the Tables 1–3.

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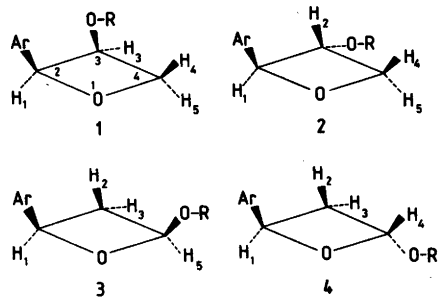

Scheme 1. a, Ar=phenyl, R=$COCH_3$; *b*, Ar=*o*-chlorophenyl, R=$COCH_3$; *c*, Ar=phenyl, R=H; *d*, Ar=*o*-chlorophenyl, R=H.

The NMR signal of the methyl protons of the acetyl group was diagnostic in the identification of the pairs of *cis*- and *trans*-3-acetoxy-2-aryloxetanes (compounds *1a*, *2a* and *1b*, *2b*). The signal of the methyl protons is at a higher field by 0.44 ppm in compound *1a* than in compound *2a* and at a higher field by 0.35 ppm in *1b* than in *2b* in $CDCl_3$ solution. The chemical shifts of the protons of the carbon atom three of the oxetane ring are characteristic for the 2-acetoxy-4-aryloxetanes (compounds *3a*, *3b*, *4a* and *4b*), because these signals are at high field compared with the other possible methylene protons (chemical shifts about 3 ppm).

Hydrolysis of the mixture of oxetanyl acetates in aqueous sodium hydroxide solution gave a mixture of *cis*- and *trans*-2-aryl-3-oxetanols. Only the major product, *cis*-2-aryl-3-oxetanol (*1c* and *1d*), was separated in a pure form from the mixture. The acetates prepared from the purified oxetanols *1c* and *1d* were identical with the compounds *1a* and *1b*, respectively.

Experimental. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions at 100 MHz and 25.06 MHz, respectively, on a Jeol JNM-FX100 instrument. The IR spectra were recorded on a Perkin-Elmer 457 spectrometer and the mass spectra on a Hitachi Perkin-Elmer RMU 6E mass spectrometer. The photolyses were performed in a one-litre photoreactor (manufac-

Table 1. The ^{1}H NMR chemical shifts (δ, ppm)[a] and coupling constants (J_{HH}, Hz) of oxetanes *1–4* in $CDCl_3$.

Compound	H-1	H-2	H-3	H-4	H-5	CH_3
1a	5.96 d $J_{1,3}$=6.5	–	5.74 m	4.71 dd $J_{4,5}$=7.4 $J_{3,4}$=5.1	5.01 dd $J_{4,5}$=7.4 $J_{3,5}$=6.1	1.69 s
2a	5.65 d $J_{1,2}$=5.1	5.23 m	–	4.88 dd $J_{4,5}$=7.3 $J_{2,4}$=6.6	4.62 dd $J_{4,5}$=7.3 $J_{2,5}$=5.7	2.13 s
1b	6.22 d $J_{1,3}$=6.3	–	5.88 m	4.56 dd $J_{4,5}$=7.7 $J_{3,4}$=4.6	5.07 dd $J_{4,5}$=7.7 $J_{3,5}$=6.0	1.80 s
2b	6.02 d $J_{1,2}$=5.1	5.30 m	–	4.87 dd $J_{4,5}$=7.4 $J_{2,4}$=6.5	4.61 dd $J_{4,5}$=7.4 $J_{2,5}$=5.6	2.15 s
3a	5.50 t	2.72 m	3.33 m	–	6.57 dd	2.16 s
4a	5.88 t $J_{1,2}$=7.3 $J_{1,3}$=7.3	2.92 m	3.00 m	6.57 dd $J_{2,4}$=5.3 $J_{3,4}$=3.8	–	2.17 s
3b	5.79 t $J_{1,2}$=6.8 $J_{1,3}$=6.8	2.43 m	3.49 m	–	6.56 dd $J_{2,5}$=4.3 $J_{3,5}$=5.6	2.12 s
4b	6.07 t $J_{1,2}$=7.2 $J_{1,3}$=7.2	2.79 m	3.15 m	6.51 dd $J_{2,4}$=3.4 $J_{3,4}$=5.7	–	2.19 s
1c	5.77 d $J_{1,3}$=5.6	–	4.8 m	4.40 m	4.8 m	–
1d	5.96 d $J_{1,3}$=5.6	–	4.8 m	4.35 m	4.8 m	–

[a]s=singlet, d=doublet, dd=doublet of doublets, m=multiplet, t=triplet.

Table 2. The ^{13}C NMR chemical shifts (ppm) of some oxetanes in $CDCl_3$.

Compound	C-2	C-3	C-4	CH_3
1a	87.0	69.5	74.5	20.2
1b	83.7	68.8	75.7	20.4
1c	88.6	67.5	79.3	–
1d	86.6	67.5	78.5	–

Table 3. $^1J_{C-H}$ coupling constants (Hz) of oxetanols in $CDCl_3$.

Compound	$J_{H_1C_2}$	$J_{H_3C_3}$	$J_{H_4C_4}$	$J_{H_5C_4}$
1c	152.7	156.3	150.4	150.4
1d	153.8	156.9	150.7	150.7

tured by Otto Fritz GmbH, Germany) equipped with a high-pressure Hanau 700-W mercury lamp (TQ 718).

Photolysis of o-chlorobenzaldehyde and vinyl acetate. 57.4 g (0.41 mol) of *o*-chlorobenzaldehyde and 54.0 g (0.63 mol) of vinyl acetate in 700 cm^3 of benzene were irradiated through a pyrex filter at ambient temperature in a photoreactor for 24 h. After evaporation of the solvent, the residue was distilled in vacuum (b.p. about 170 °C/10Pa). The yield of the mixture of oxenates *1b*, *2b*, *3b* and *4b* was 34 g (37 % based on *o*-chlorobenzaldehyde). The compounds of the mixture were separated by preparative gas chromatography (1.2 m long glass column Silicone Gum Rubber SE-30 as a stationary phase, temperature programme from 120 to 140 °C) for structural elucidation.

The ^1H NMR spectra of the isomeric oxetanes are presented in Table 1. The mass and IR spectral data of the compounds *1b* and *4b* are presented in the following list. The mass and IR spectra of the compounds *2b* and *3b* are highly similar to the spectra of the compounds *1b* and *4b*, respectively.

cis-*3-Acetoxy-2-(2-chlorophenyl)oxetane* (1b). Mass spectrum: *m/z* 228 (0.1 %, M), 226 (0.3, M), 185 (3), 183 (9), 156 (1), 154 (4), 143 (4), 141 (12), 139 (2), 131 (1), 127 (3), 125 (9), 91 (1), 89 (4), 86 (15), 77 (4), 75 (3), 73 (2), 44 (4), 43 (100); *m** 183 → 141, obs 108,5, calc. 108.6.

IR spectrum (cm^{-1} in CCl_4): 3060 (w), 2950 (m), 2880 (m), 1745 (s), 1470 (m), 1440 (s), 1370 (s), 1360 (m), 1230 (s), 1145 (m), 1105 (s), 1055 (s), 1035 (m), 980 (s), 940 (m), 915 (s), 690 (m), 630 (w).

trans-*2-Acetoxy-4-(2-chlorophenyl)oxetane* (4b). Mass spectrum: *m/z* 228 (0.1 %, M), 226 (0.3, M), 185 (2), 183 (5), 169 (1), 168 (6), 167 (4), 166 (18), 143 (8), 142 (2), 141 (28), 140 (6), 139 (18), 138 (14), 137 (2), 131 (20), 127 (1), 125 (3), 113 (3), 111 (3), 104 (2), 103 (22), 102 (4), 91 (3), 89 (3), 86 (3), 77 (16), 76 (3), 75 (6), 74 (2), 63 (2), 51 (8), 50 (4), 44 (6), 43 (100).

IR spectrum (cm^{-1} in CCl_4): 3050 (w), 3010 (w), 2960 (w), 2910 (w), 1755 (s), 1470 (m), 1435 (m), 1375 (m), 1360 (m), 1340 (w), 1220 (s), 1145 (m), 1120 (m), 1070 (m), 1055 (m), 1030 (m), 995 (s), 960 (s), 680 (w).

Photolysis of benzaldehyde and vinyl acetate. Photolysis was performed as described above in the case of *o*-chlorobenzaldehyde. The time of irradiation was 48 h and the yield of isomeric oxetanes was 23 % of the theory. The ^1H NMR data of the separated oxetanes are presented in Table 1. The IR and mass spectral data of the isomeric oxetanes *1a*, *2a*, *3a* and *4a* are similar to the data of the compounds *1b*, *2b*, *3b* and *4b*, respectively.

cis-*2-(2-Chlorophenyl)-3-oxetanol* (1d). 34 g of the mixture of the oxetanes *1b*, *2b*, *3b* and *4b* were dissolved into 200 cm^3 of methanol. Into this solution potassium hydroxide solution (25 g KOH, 100 cm^3 H_2O and 200 cm^3 methanol) was added in drops for 1 h. Methanol was evaporated in a rotary evaporator and 500 cm^3 of water was added. The organic compounds were extracted by methylene chloride. The combined extracts were washed with water and the solvent was evaporated. The residue was distilled in vacuum (b.p. *ca.* 120 °C/5 Pa). The solid distillate, which was a mixture of oxetanols, *o*-chlorocinnamaldehyde and other compounds, was crystallized several times from diethyl ether. The recrystallized product (m.p. 88–89 °C) weighed 8.2 g. On the basis of the spectroscopic properties, the product was oxetanol *1d*. Acetylation of the pure product yielded an oxetanyl acetate which was identical to the compound *1b*. The ^1H and ^{13}C NMR spectra of compound *1d* are presented in the Tables 1–3.

Mass spectrum: *m/z* 186 (<0.1 %, M), 184 (0.1, M), 156 (3), 154 (9), 143 (27), 142 (7), 141 (100), 140 (5), 139 (32), 127 (5), 125 (15), 119 (10), 115 (6), 114 (3), 113 (20), 112 (5), 111 (13), 105 (5), 91 (23), 90 (6), 89 (19), 77 (62), 76 (9), 75 (14), 74 (4), 65 (5), 63 (15), 51 (18), 50 (16), 44 (47), 43 (24); *m** 143→115, obs. 92.4, calc. 92.5; 141→113, obs. 90.5, calc. 90.6; 139→111, obs. 88.6, calc. 88.6; 113→77, obs. 52.5, calc. 52.5; 115→77, obs. 51.4, calc. 51.6.

IR spectrum (cm^{-1} in CCl_4): 3595 (s), 3440 (m), 3060 (w), 2950 (s), 2870 (s), 1465 (s), 1400 (m), 1330 (m), 1260 (m), 1200 (s), 1170 (s), 1050 (s), 1030 (s), 990 (s), 880 (m), 685 (m).

cis-*2-Phenyl-3-oxetanol* (1c). 5 g of the mixture of the oxetanyl acetates *1a*, *2a*, *3a* and *4a* were hydrolyzed as described above. Recrystallization of the crude product from diethyl ether gave 1.4 g of oxetanol *1c*, which has a melting point at 69–70 °C. ^1H and ^{13}C NMR spectra of the compound *1c* are described in the Tables 1–3.

Mass spectrum: *m/z* 150 (1 %, M), 120 (16), 119 (2), 108 (7), 107 (100), 106 (4), 105 (32), 92 (10), 91 (62), 90 (4), 89 (8), 79 (55), 78 (9), 77 (40), 65 (14), 63 (9), 51 (21), 50 (10), 44 (26), 43 (14).

IR spectrum (cm^{-1} in CCl_4): 3560 (s), 3420 (m), 3025 (m), 3010 (m), 2950 (s), 2870 (s), 1490 (m), 1450 (m), 1400 (m), 1330 (w), 1300 (w), 1200 (m), 1135 (s), 1070 (w), 1025 (m), 980 (s), 875 (s), 700 (s).

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3-Acyl-1-aryltriazenes. Preparation and Structure

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Acylaryltriazenes were first synthesized by Alfred Bertho in 1927 from benzoyl azide and phenylmagnesium bromide.[1] Since then only a few acylaryltriazenes have been mentioned.[2-4] Oddo and Algerino [2] prepared acetylphenyltriazene from acetamide and benzenediazonium chloride and described the product as an example of *besonders schöner fall von mesohydrischer isomerie* with a proposed bridge-bonded hydrogen atom. Ito and Fukuyama [3] prepared benzoylphenyltriazenes from nitrosobenzene and benzohydrazide.

Formyl-*p*-chloro- and formyl-*p*-bromophenyltriazene, as precursors for diazoisocyanides,[4] were prepared in an ether–water mixture at neutral pH by coupling the diazonium ion with formamide. The obtained triazenes were described as reasonably stable in contradiction to reports [2] of formylphenyltriazenes isolated from aqueous solutions.

In spite of the doubt which has been cast upon the applicability of the method of Oddo and Algerino [2] for the preparation of acetyltriazenes [5] we report that formyl-, acetyl- and benzoyltriazenes can be prepared by their method.

Triazene formation from diazonium ions and amides were interesting as an extension of our work with triazenes.[6,7] The presence of a carbonyl group attached to nitrogen could change the normal tautomer distribution and the reaction of diazonium ions with nitrogen atoms less nucleophilic than amine nitrogen has not been thoroughly investigated and could be used as a model for reaction with proteins.

$$\underset{1}{R-CO-NH_2} + \underset{2}{{}^{+}N_2-R'} \rightarrow \underset{3}{R-CO-NH-N=N-R'}$$

Scheme 1. Compound, R,R': *a*, CH_3, *p*-$CH_3C_6H_4$; *b*, C_6H_5, *p*-$CH_3C_6H_4$; *c*, H, *p*-$CH_3OC_6H_4$; *d*, H, *p*-$CH_3C_6H_4$; *e*, H, *p*-$NO_2C_6H_4$; *f*, H, *p*-ClC_6H_4; *g*, H, *p*-BrC_6H_4; *h*, CH_3, *p*-$NO_2C_6H_4$; *i*, CH_3, *p*-$C_2H_5OCOC_6H_4$; *j*, CH_3, *p*-$CH_3OC_6H_4$; *k*, C_6H_5, *p*-$CH_3OC_6H_4$.

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The attempts to prepare formyl-, acetyl- and benzoyltriazene by coupling diazonium ion with the respective amide in aqueous sodium hydroxide solution was carried out as depicted in Scheme 1.

The coupling reaction is fairly good for acetamide giving *3a*, but gave low yields for formamide and benzamide (*3c* and *3b*). For all other reactions no triazene was characterized.

Application of the procedure for preparation of *3d* and *3e* gave rather unstable compounds decomposing vigorously upon heating to 80 °C. In $CHCl_3$ solution they decompose rapidly at room temperature, in DMSO solution slowly, which is why they were not characterized further. Attempts to obtain NMR spectra of these compounds in order to establish their tautomeric structure failed. However, it turned out that formyl-*p*-methoxyphenyltriazene *3c* was stable at room temperature in solution. The method did not work for preparation of acetyl- (*3j*) and benzoyl-*p*-methoxyphenyltriazene *3k*. No triazene was isolated presumably owing to much faster decomposition of the diazonium ion compared with the coupling reaction. We also tried to prepare formyl-*p*-chlorophenyltriazene *3f* and formyl-*p*-bromophenyltriazene *3g* by coupling the relevant diazonium ion with formamide in sodium hydroxide solution. On account of the reported stability [3] we hoped to obtain a stable product but in both cases the explosive compounds which are believed to be bis-*p*-halogenophenyldiazo ether $((X-C_6H_4N_2)_2O)$ [6] were formed presumably owing to the electrophilic character of these diazonium ions. *3f* and *3g* could not be isolated even by reaction at slightly alkaline or at neutral pH. The attempts at making acetyl-*p*-nitrophenyl- (*3h*) and acetyl-*p*-(ethoxycarbonyl)phenyltriazene *3i* gave two compounds in high yields, but the compounds were unstable, giving off nitrogen immediately after they were filtered off. They were not characterized.

The actual tautomeric structure of the acylaryltriazenes A or B could be established by observing the ^{13}C chemical shift values for the quaternary carbon atoms in the *p*-substituted phenyl groups.

For the *p*-tolyl groups the chemical shift values for the two quaternary carbon atoms are normal-

$$\underset{A}{R-CO-NH-N=N-C_6H_4-CH_3-p}$$

$$\underset{B}{R-CO-N=N-NH-C_6H_4-CH_3-p}$$

Scheme 2.

ly seen [6,7] for *p*-tolyldiazo (A) at 146.3 and 138.8 ppm and for *p*-tolylamino (B) at 139.3 and 132.6 ppm. For compound *3a* we find 139.0 and 146.3 ppm and for compound *3b* 139.5 and 146.3 ppm, both in accordance with the tautomeric structure A. For compound *3c* the chemical shift values are 141.7 and 160.2 ppm for the quaternary carbon atoms. These values are also in accordance with structure A as the use of substitution constants [9] for 4-methyl and 4-methoxy groups gives calculated values of 146.5 and 137.7 ppm for the *p*-tolyl group.

All three acylaryltriazenes were weak acids which could be dissolved in diluted sodium hydroxide and reprecipitated by adding diluted hydrochloric acid.

Experimental. The experimental [10] equipment was reported earlier. Melting points are uncorrected.

General procedure for preparation of 3-acyl-1-aryltriazenes. A solution of the diazonium chloride was prepared from amine (0.1 mol), hydrochloric acid (0.3 mol) in water (100 ml) and sodium nitrite (0.1 mol) in water (30 ml). The diazonium chloride solution was added dropwise to a stirred mixture of amide (0.20 mol), sodium hydroxide (0.20 mol) and water (100 ml) at 0 °C. After addition the mixture was stirred for 15 min and the foaming brown precipitate filtered off. The filtrate was acidified with hydrochloric acid and the triazene filtered off, dried and recrystallized from toluene–light petroleum.

*3-Acetyl-1-*p*-methylphenyltriazene* 3a. Yield 23 %, m.p. 120–121 °C. Anal. $C_9H_{11}N_3O$: C,H,N. ^{1}H NMR ($CDCl_3$): δ 2.35 (3 H, s), 2.43 (3 H, s), 7.10–7.70 (4 H, m), 10.88 (1 H, s). IR ($CHCl_3$, cm^{-1}): 3300 (m), 1710 (s), 1500 (s), 1380 (s), 1210 (s), 1150 (s). ^{13}C NMR (DMSO-d_6): δ 170.4, 146.3, 139.0, 129.8, 121.5, 21.5, 20.8.

*3-Benzoyl-1-*p*-methylphenyltriazene* 3b. Yield 7 %, m.p. 90–92 °C. Anal. $C_{14}H_{13}N_3O$: C,H,N. ^{1}H NMR ($CDCl_3$): δ 2.37 (3 H, s), 7.05–8.05 (9 H, m), 10.53 (1 H, s) IR ($CHCl_3$, cm^{-1}): 3290 (m), 1690 (s), 1605 (m), 1475 (s). ^{13}C NMR (DMSO-d_6): δ 165.1, 146.3, 139.5, 132.5, 132.2, 129.9, 128.4, 121.7, 20.8.

*3-Formyl-1-*p*-methoxyphenyltriazene* 3c. Yield 6 %, m.p. 102 °C. Anal. $C_8H_9N_3O_2$: C,H,N. ^{1}H NMR ($CDCl_3$): δ 3.85 (3 H, s), 6.82–7.75 (4 H, m), 9.16 (1 H, d), 10.30 (1 H, d). IR ($CHCl_3$, cm^{-1}): 3260 (w), 1715 (s), 1605 (m), 1495 (m). ^{13}C NMR (DMSO-d_6): δ 166.2, 160.2, 141.7, 123.0, 114.5, 55.4.

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A Convenient Synthesis of Mutagenic 3*H*-Imidazo[4,5-*f*]-quinolin-2-amines and Their 2-^{14}C-Labelled Analogues

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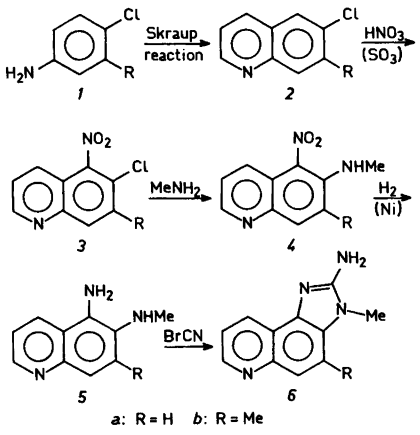
Several compounds, identified in meat or fish after heating, show high mutagenic activity. In Ames' test,[1] the title compounds *6a* ("IQ") and *6b* ("MeIQ") are among the most potent mutagens known for *Salmonella typhimurium* TA 98 after activation by liver S-9 fraction.[2] Both *6a*[3] and *6b*[4] have been synthesized. The last step in each synthesis is the introduction of the *N*-methyl group. The reaction yields a mixture of methyl derivatives, which is particularly regrettable when isotopically labelled *6a* or *6b* is needed (*e.g.*, in metabolic studies). This difficulty is avoided in the present syntheses of *6a* and *6b*, the last step being cyclization with cyanogen bromide, which is readily available in (^{13}C- or) ^{14}C-labelled form.[5]

The synthetic route is outlined in Scheme 1 and is based on work by Soviet scientists. This work includes the synthesis of *5a* essentially according to the scheme[6] and its cyclization to 3*H*-imidazo[4,5-*f*]quinolines other than *6*.[7] Among the apparently unknown intermediates *2b–5b*, *2b* was accompanied by its known[8] 5-methyl isomer and *3b* by a small amount of its 8-nitro isomer.



Scheme 1.

The unwanted isomers were readily removed by crystallization.

Experimental. All reactions and purifications were monitored by TLC on silica gel (Riedel-de Haën, SIF) or by GLC on a 25 m×0.24 mm i.d. capillary column coated with CP Sil 5 and heated from 100 to 250 °C at 10 °C/min. The TLC spots were visualized by UV irradiation. Column chromatography (CC) of unlabelled mixtures was performed by the "flash" technique.[9] Evaporations were performed at reduced pressure below 40 °C. Melting points are corrected. Mass spectra were recorded at 70 eV, using electron impact and direct insertion. ^{1}H NMR spectra were recorded at 90 MHz and *ca.* 30 °C.

*3-Methyl-3*H*-imidazo[4,5-*f*]quinolin-2-amine* (*6a*) was prepared from 6-chloroquinoline (*2a*)[10] exactly as described below for the sequence *2b–6b*, but the separation of isomers was, of course, superfluous.

Compound	*3a*	*4a*	*5a*	*6a*
Yield (%)	86	88	65	75
Cryst. from	CCl_4	Me_2CO	PhMe	C_5H_5N
M.p. (°C) obs.	129	194	168–169	>300
M.p. (°C) lit.	129[10]	190[6]	179–180[6]	>300[3]

Although the melting points of *4a* and *5a* differed from those reported,[6] the mass and ^{1}H NMR spectra completely confirmed their formulas. The product *6a* was identical (MS, ^{1}H NMR, UV) with a sample provided by T. Sugimura.[3]

6-Chloro-7-methylquinoline (2b). 4-Chloro-*m*-toluidine (*1b*)[11] was subjected to the Skraup reaction as described for aniline.[12] GLC analysis of the product (yield *ca.* 80 %) showed almost only *2b* and its 5-methyl isomer, eluted in that order and with the relative peak heights 2:1. About half of the *2b* was isolated by crystallization from diethyl ether. The isomers in the mother liquor were readily separated by crystallization of their picrates from *N*,*N*-dimethylformamide. From the less soluble picrate, 1 M sodium hydroxide liberated *2b*. After crystallization from light petroleum (b.p. 40–60 °C), the total yield of *2b*, m.p. 82.5–83 °C, was 45–50 %. Anal. $C_{10}H_8ClN$: C, H, N. MS, *m/e* (rel. int.): 177 (100, M), 142 (98), 179 (31), 141 (30), 63 (19), 71 (17), 115 (16), 140 (15), 114 (15), 178 (14). ^{1}H NMR ($CDCl_3$): δ 2.59 (Me, d), 7.36 (3-H, dd), 7.83 (5-H, s), 7.97 (8-H, m), 8.05 (4-H, dm), 8.87 (2-H, dd); $|J|$ 0.85 (7,8), 1.65 (2,4), 4.2 (2,3), 8.3 (3,4) Hz.

6-Chloro-5-methylquinoline, m.p. 79.5–80 °C (lit.[8] 81–82 °C), was obtained from its picrate as described for *2b*. The ^{1}H NMR spectral data agreed with those reported.[8]

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6-Chloro-7-methyl-5-nitroquinoline (3b). Conc. sulfuric acid (3 ml) was added dropwise to *2b* (8.9 g, 50 mmol), stirred with a glass rod. After cooling, the resulting salt was stirred with more acid (30 ml) until completely dissolved. To the stirred and cooled solution was added fuming sulfuric acid (65 % SO_3, 9 ml) in one portion, followed by ≥96 % nitric acid (9 ml) added over 30 min below 20 °C. After another 10 min without cooling, the solution was poured on to ice (500 g), neutralized with ammonia and extracted with chloroform. The extract was washed with water, analysed by GLC and evaporated. The analysis showed almost only *3b* and its 8-nitro isomer, eluted in that order and with the relative peak heights 19:4. Crystallization of the residue from methanol yielded *3b* (8.4 g, 75 %), m.p. 127–127.5 °C. Anal. $C_{10}H_7ClN_2O_2$: C, H, N. MS, *m/e* (rel. int.): 141 (100), 222 (69, M), 140 (39), 164 (37), 192 (34), 176 (29), 114 (26), 63 (24), 224 (22), 113 (22). ^{1}H NMR ($CDCl_3$): δ 2.66 (Me, d), 7.52 (3-H, dd), 7.98 (4-H, ddd), 8.15 (8-H, m), 8.98 (2-H, dd); $|J|$ 0.85 (4,8), 0.95 (7,8), 1.65 (2,4), 4.2 (2,3), 8.7 (3,4) Hz.

6-Chloro-7-methyl-8-nitroquinoline, m.p. 213–213.5 °C, was obtained from the mother liquor by evaporation, CC ($CHCl_3$–EtOAc, 1:1 v/v) and crystallization from methanol. Anal. $C_{10}H_7ClN_2O_2$: C, H, N. MS, *m/e* (rel. int.): 141 (100), 222 (65, M), 205 (41), 140 (40), 192 (34), 63 (32), 114 (31), 113 (28), 128 (26), 129 (25). ^{1}H NMR ($CDCl_3$): δ 2.55 (Me, s), 7.51 (3-H, dd), 7.99 (5-H, s), 8.14 (4-H, dd), 8.97 (2-H, dd); $|J|$ 1.7 (2,4), 4.3 (2,3), 8.4 (3,4) Hz.

N,*7-Dimethyl-5-nitro-6-quinolinamine* (4b). 40 % aq. methylamine (17 g, 220 mmol) was added over 1 h to a refluxing solution of *3b* (8.0 g, 36 mmol) in 95 % ethanol (100 ml). After refluxing for another 4 h, GLC or TLC ($CHCl_3$–EtOAc, 1:1 v/v) showed no *3b*. The solution was then poured on to ice-water and extracted with chloroform. The extract was washed with water and evaporated. Crystallization of the residue from methanol yielded *4b* (6.6 g, 85 %), m.p. 161–162 °C. Anal. $C_{11}H_{11}N_3O_2$: C, H, N. MS, *m/e* (rel. int.): 217 (100, M), 142 (52), 143 (46), 170 (40), 156 (31), 144 (29), 141 (28), 172 (28), 200 (27), 115 (27). ^{1}H NMR ($CDCl_3$): δ 2.46 (7-Me, d), 3.00 (NMe, d), 5.2 (NH, broad s), 7.40 (3-H, dd), 7.88 (8-H, m), 8.18 (4-H, ddd), 8.69 (2-H, dd); $|J|$ 0.8 (4,8), 0.85 (7,8), 1.5 (2,4), 4.3 (2,3), 5.6 (*N,N*), 8.7 (3,4) Hz.

N^6,*7-Dimethyl-5,6-quinolinediamine* (5b). A vigorously stirred mixture of *4b* (3.26 g, 15.0 mmol), Raney nickel (3 teaspoons) and abs. ethanol (185 ml) was hydrogenated under ambient conditions. After *ca.* 90 min, the calculated amount (1.1 l) of hydrogen had been absorbed. The catalyst was then filtered off quickly and the filtrate evaporated. CC ($CHCl_3$–MeOH, 19:1 v/v) of the dark residue yielded *5b* (1.94 g, 69 %). After crystallization from toluene, *5b* melted at 131–132.5 °C. Anal. $C_{11}H_{13}N_3$: C, H, N. MS, *m/e* (rel. int.): 172 (100), 187 (92, M), 145 (60), 173 (13), 188 (12), 144 (12), 117 (10), 142 (9), 159 (8), 51 (8). ^{1}H NMR ($CDCl_3$): δ 2.48 (7-Me, d), 2.74 (NMe, s), 4.5 (NH, broad s), 7.23 (3-H, dd), 7.40 (8-H, m), 8.09 (4-H, ddd), 8.76 (2-H, dd); $|J|$ 0.85 (4,8), 0.95 (7,8), 1.65 (2,4), 4.2 (2,3), 8.5 (3,4) Hz.

*3,4-Dimethyl-3*H*-imidazo[4,5-f]quinolin-2-amine* (6b). Two procedures are given: *A* for unlabelled and *B* for labelled *6b*.

A. Cyanogen bromide (1.06 g, 10.0 mmol) and *5b* (1.87 g, 10.0 mmol) were dissolved in methanol (40 ml). The hydrobromide of *6b* separated gradually. After 4 h, conc. ammonia (1.0 ml) was added and the mixture evaporated. CC ($CHCl_3$–MeOH, 5:1 v/v) of the residue yielded *6b* (1.65 g, 78 %). After crystallization from 95 % ethanol, *6b* melted at 296–298 °C (sealed tube) and was identical (m.p., MS, ^{1}H NMR, UV) with a sample provided by T. Sugimura.[4]

B. Potassium cyanide (65 mg, 1.00 mmol), labelled with ^{13}C or ^{14}C, was dissolved in methanol (2 ml) and added over 20 min to a stirred solution of bromine (160 mg, 1.00 mmol) in methanol (2 ml), cooled in ice-water, *cf.* Ref. 5. Potassium bromide separated gradually. *5b* (187 mg, 1.00 mmol) was immediately dissolved in the colourless mixture. After 4 h at 20 °C, *6b* (165 mg, 78 %) was isolated as in *A*, but CC was performed at atmospheric pressure.

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Added in proof. In the last step (*5*→*6*), the reaction mixture should be kept at 0 °C overnight. The hydrobromide of *6* may then be collected, washed with cold methanol and dissolved in warm water (40 ml/g). Pure *6* separates from the solution on adding aqueous ammonia and cooling. A partly similar synthesis of *6a* and *6b* was published recently.[13]

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Nickel-complex Catalysis in the Reaction between Grignard Reagents and Substituted Pyrimidines

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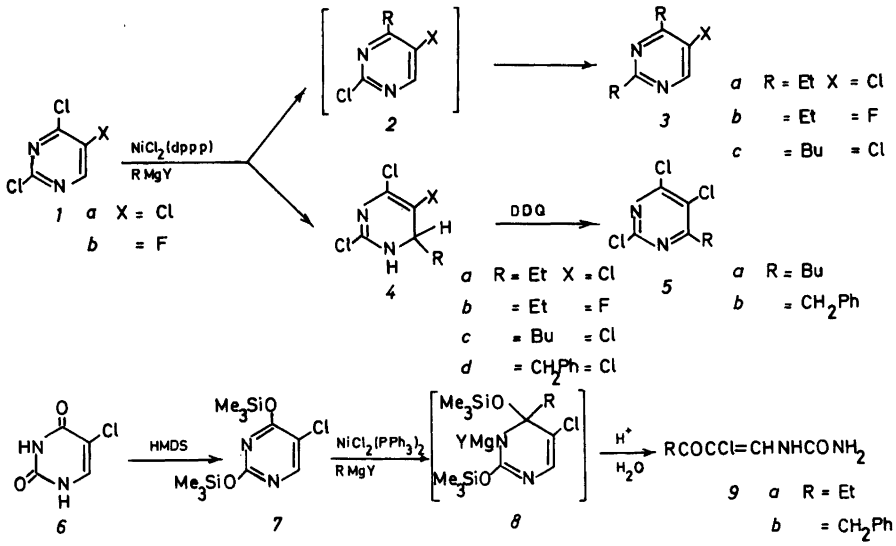
Recently we reported that phenylmagnesium bromide in reactions catalyzed by dichloro-[1,3-bis(diphenylphosphinopropane]nickel(II), $NiCl_2$(dppp), or dichloro-bis(triphenylphosphine)nickel(II), $NiCl_2(PPh_3)_2$, adds the carbon nucleophile selectively to the vacant position in 2,4-dichloro-5-chloro(fluoro)pyrimidine rather than undergoing substitution by cross-coupling.[1] We now report that the main reaction path for alkyl Grignard reagents is different; in this case the expected cross-coupling occurs with substitution of the halogen atoms in the 2- and the 4-position. Thus in the reaction of ethylmagnesium bromide in the presence of $NiCl_2$(dppp) the major new product is the diethylated pyrimidine *3a* even when only one molar equivalent of the Grignard reagent was used; attempts to effect monosubstitution were not successful. In the minor product, the adduct *4a*, the carbon nucleophile has entered the vacant pyrimidine position in *1a*; product ratio *3a*–*4a* is 7:1. The adduct *4a* is not formed in significant amounts if heating of the reaction mixture is avoided. The 5-fluoro analogue *1b* reacted in the same way. Butylmagnesium bromide in its reaction with *1a* gave an analogous product ratio of *3c* and *4c*. The latter, however, was very readily aromatized to *5a* by air oxidation.

As an example from the aralkyl group, the Grignard reagent from benzyl chloride was reacted with *1a*; the product was the adduct *4d*. It is thus notable that the benzyl reagent behaves differently from the alkyl reagents and, in fact, reacts in the same manner as the aryl reagent previously reported upon.[1] The structure *4d* assigned to the product, has been confirmed by a dehydrogenation reaction to furnish *5b* using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).

The silyl ether *7* was studied as part of a search for pyrimidines with less reactive 2,4-substituents than in *1* in order to attain regioselectivity in substitution reactions. The silyl ether *7* is readily available from 5-chlorouracil *6* by heating with hexamethyldisilazane. The reaction of *7* with both the ethyl and benzyl Grignard reagents using either $NiCl_2(PPh_3)_2$ or $NiCl_2$(dppp) catalysis appears to proceed initially as desired by the addition of the Grignard reagent to the 4-position. During the reaction, or more likely during the aqueous work-up, however, the adduct suffers opening of the ring to furnish the novel *N*-vinylurea derivatives *9*. The reaction sequence *6*→*9* therefore provides a convenient route to this class of compounds.

Experimental. The data from the mass spectra



Scheme 1.

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are reported as MS[70 eV; *m/z*(% rel. int.)].

General procedure for the Grignard coupling reactions. The Grignard reagent (12 mmol) in ether (25 ml) was added under nitrogen over 10 min at 0 °C to a stirred suspension from the halopyrimidine *1* (10 mmol) and $NiCl_2(dppp)$[2] or $NiCl_2(PPh_3)$[3] (0.2 mmol) in dry ether (100 ml). There was an immediate change of colour through yellow to brown. The mixture was allowed to reach room temperature, left stirring overnight and finally heated under reflux for 10 h. The cold mixture was hydrolyzed with 2 M HCl, the organic layer separated, the aqueous layer extracted with ether (3×25 ml), the combined ether solutions washed with water, with saturated sodium carbonate solution and water again, and the dried ($MgSO_4$) ether solution evaporated. The product was further purified by recrystallization or chromatography as described.

2,4-Diethyl-5-chloropyrimidine 3a *and 2,4,5-trichloro-6-ethyl-1,6-dihydropyrimidine* 4a were obtained from ethylmagnesium bromide and 2,4,5-trichloropyrimidine[4] using $NiCl_2(dppp)$ admixed with some starting material. The products were separated by preparative TLC (ether–light petroleum; 1:1).

3a. Colourless oil, yield 47 %. Anal. $C_8H_{11}ClN_2$: C,H. ^1H NMR (CCl_4): δ 1.30 and 2.83 (Et), 8.25 (H-6). MS: 172/170 (19/61, M), 171 (40), 169 (100), 157 (8), 155 (24), 149 (24), 147 (40), 120 (10).

4a. Colourless crystals, m.p. 105 °C (heptane), yield 7 %. Anal. $C_6H_7Cl_3N_2$: C, H, N. ^1H NMR (CCl_4): δ 0.9–2.0 (CH_3 and CH_2), 4.6 (t, H-6), 10.2 (NH; H–D exchange in D_2O). IR (KBr): 3120 cm^{-1} (NH). MS: 214 (8), 212 (13, M), 195 (97), 193 (100), 76 (6), 149 (27), 147 (45).

When the reaction was run at 0 °C and room temperature the product was a mixture of *3a* and *1a*.

When the reaction was run with 2 molar equivalents of ethylmagnesium bromide the yield of *3a* was 70 % and of *4a* 9 %.

2,4-Diethyl-5-fluoropyrimidine 3b was obtained from ethylmagnesium bromide, 2,4-dichloro-5-fluoropyrimidine[5] and $NiCl_2(dppp)$. Small amounts of the adduct *4b* was also formed but were not isolated. *3b* was isolated from the reaction mixture by preparative TLC (ether–pentane; 1:4); yield 53 % of colourless oil. Anal. $C_8H_{11}FN_2$: C, H. ^1H NMR (CCl_4): δ 1.3 and 2.8 (Et), 8.1 (H-6). MS: 154 (67, M), 153 (100), 126 (17), 100 (3), 98 (8).

2,4-Dibutyl-5-chloropyrimidine 3c *and 2,4,5-trichloro-6-butylpyrimidine* 5a were obtained from butylmagnesium bromide and 2,4,5-trichloropyrimidine using $NiCl_2(dppp)$. The products were separated by preparative TLC (ether–pentane; 1:5). During the isolation work the part of the product which was the adduct *4c* was oxidized by air to furnish *5a* which was the product isolated.

3c. Colourless oil, yield 40 %. Anal. $C_{12}H_{19}ClN_2$: C, H. ^1H NMR (CCl_4): δ 0.9, 1.5, 2.8 (Bu), 8.22 (H-6). MS: 228/226 (1/3, M), 213 (3), 210 (6), 197 (6), 149 (53), 113 (13), 57 (100).

5a. Colourless oil, yield 8 %. Anal. $C_8H_9Cl_3N_2$: ^1H NMR (CCl_4): δ 0.95, 1.65, 2.90 (Bu). MS: 238 (4, M), 202 (4), 200 (30), 198 (90), 164 (3), 162 (9).

When the reaction was run in the presence of 2.2 mol equivalents of the Grignard reagents, the yields of *3c* and *5a* were increased to 65 and 15 %, respectively.

6-Benzyl-2,4,5-trichloro-1,6-dihydropyrimidine 4d was formed almost exclusively from benzylmagnesium chloride and 2,4,5-trichloropyrimidine using $NiCl_2(dppp)$ as above; colourless crystalline material in 86 % yield, m.p. 168 °C ($CHCl_3$). Anal. $C_{11}H_9Cl_3N_2$: C, H. ^1H NMR (DMSO-d_6): δ 2.85 (CH_2), 4.7 (H-6), 4.95 (NH; H–D exchange in D_2O), 7.2 (Ph). IR (KBr): 3125 cm^{-1} (NH). MS: 239 (3, M–Cl), 187 (30), 185 (90), 183 (100), 149 (21), 147 (30), 91 (36).

When $NiCl_2(PPh_3)$ was used as the catalyst, the yield of *4d* dropped to 78 %.

6-Benzyl-2,4,5-trichloropyrimidine 4d. A solution of 6-benzyl-2,4,5-trichloro-1,6-dihydropyrimidine (0.55 g, 2 mmol) and DDQ (0.45 g) in dioxan (25 ml) was stirred at room temperature for 48 h. The precipitated material was then filtered off, the filtrate evaporated under reduced pressure and the product isolated from the residual material by preparative TLC (ether–pentane; 1:3); yield 39 %, m.p. 85 °C (hexane). Anal. $C_{11}H_7Cl_3N_2$: C, H. ^1H NMR ($CDCl_3$): δ 4.25 (CH_2), 7.2 (Ph). MS: 274/272 (66/100, M), 239 (35), 237 (56), 204 (4), 202 (15), 91 (67).

2,4-Bis(trimethylsilyloxy)-5-chloropyrimidine 7. A mixture of 5-chlorouracil[6] (14.5 g, 0.1 mol), hexamethyldisilazane (50 ml) and ammonium sulfate (0.08 g) was heated under reflux and stirring under anhydrous conditions for 5 h. Fractional distillation yielded the title compound in 95 % yield, b.p. 92 °C/1 mmHg. ^1H NMR ($CDCl_3$): δ 0.35 (SiMe), 8.10 (H-6).

2-Chloro-1-ureido-1-penten-3-one 9a. Ethylmagnesium bromide, prepared from Mg (0.28 g, 0.012 g.atom) and ethyl bromide (1.3 g, 0.012 mol) in anhydrous ether (25 ml), was added gradually to a solution of 2,4-bis(trimethylsilyloxy)-5-chloropyrimidine (2.9 g, 0.01 mol) and $NiCl_2(PPh_3)$ (130 mg, 0.2 mmol) in anhydrous ether (100 ml) at 0 °C. The mixture was stirred overnight, heated under reflux for 24 h, the cooled mixture hydrolyzed with 2 M HCl, the

organic layer separated, the aqueous layer extracted with ethyl acetate (4×25 ml), the combined organic solutions washed with water, with saturated aqueous sodium carbonate and again with water, and the dried ($MgSO_4$) solution evaporated; yield 56 %, m.p. 218 °C (EtOAc). Anal. $C_6H_9ClN_2O_2$: C, H. ^1H NMR (DMSO-d_6): 0.9, 1.5 (Et), 4.6 (=CH), 3.8, 7.75, 10.4 (NH, H–D exchange in D_2O). IR (KBr): 3320, 3180, 3050 (NH, NH_2), 1700 cm^{-1} (CO). MS: 178/176 (8/24, M), 149 (13), 147 (40), 106 (14), 104 (42), 93 (27), 58 (100).

2-Chloro-4-phenyl-1-ureido-1-buten-3-one 9b was prepared from benzylmagnesium chloride as above; yield 52 %, m.p. 207 °C (EtOAc; decomp.). Anal. $C_{11}H_{11}ClN_2O_2$: C, H. ^1H NMR (DMSO-d_6): 2.95 (CH_2), 4.15 (=CH), 7.3 (Ph), 3.5, 7.8, 11.1 (NH and NH_2, H–D exchange in D_2O). IR (KBr): 3900, 3200, 3100 (NH, NH_2), 1720 cm^{-1} (CO). MS: 203 (3, M–Cl), 149 (2), 147 (6), 91 (100).

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The Application of the "Reaction Order Approach" to a Complex Electrode Mechanism. Pitfalls in Drastic Media Changes

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Amatore, Pinson and Savéant [1] have recently attempted to discredit the so-called "reaction order approach" developed in this laboratory [2] for the analysis of electrode mechanisms. The approach was developed especially for complex reaction schemes for which theoretical data are not available and involves the determination of the apparent rate law for a process without carrying out any calculations. However, Amatore, Pinson and Savéant claim that the approach only works for simple reactions and fails when the rate is determined by more than a single step.[1] It is the purpose of this communication to demonstrate that this is not the case.

More recently, Amatore, Pinson and Savéant [3] have published another note dealing with the anion radical–proton donor complex mechanism for the dimerization of anion radicals. This mechanism was observed during the electrodimerization of diethyl fumarate anion radical in DMF and can be described by eqns. (1) and (2).[4]

$$A^{\cdot-} + H_2O \overset{K_1}{\rightleftharpoons} A^{\cdot-}/H_2O \qquad (1)$$

$$A^{\cdot-}/H_2O + A^{\cdot-} \xrightarrow{k_2} A_2^{2-}/H_2O \qquad (2)$$

Amatore, Pinson and Savéant apply this mechanism to the dimerization of 9-cyanoanthracene anion radical and attempt to direct the mechanism to reaction (3) as the rate determining step by going to very high (16 M) water concentrations.[3]

$$2\ A^{\cdot-}/H_2O \xrightarrow{k_3} A_2^{2-}/2\ H_2O \qquad (3)$$

A non-linear dependence of the apparent rate constant on $[H_2O]$ was observed and interpreted in terms of a significant contribution of (3). Although this is an expected consequence of going to very high water concentrations, the data are inconclusive because there could be a large effect of the change of the medium from essentially aprotic to 16 M water.

The rate laws for the two cases with different rate determining steps are (4) and (5).

$$\text{Rate} = k_2K_1[A^{\cdot-}]^2[H_2O] \qquad (4)$$

$$\text{Rate} = k_3K_1^2[A^{\cdot-}]^2[H_2O]^2 \qquad (5)$$

A deuterium equilibrium isotope effect was observed earlier for rate law (4).[4] The fact that the exponent of K_1 differs in the two rate laws suggests a possible way to verify the occurrence of reaction (3). At low $[H_2O]$, only rate law (4) is expected to apply when K_1 is small as it is when $A^{\cdot-}$ is $ANCN^{\cdot-}$. The apparent deuterium isotope effect for this case would be that for equilibrium (1). Under conditions where rate law (5) is significant, the apparent isotope effect should reflect the contribution from K_1^2 and should be either greater or less than that at low $[H_2O]$ depending upon whether $(K_1)_H/(K_1)_D$ is greater or less than unity.

In order to test for a dependence of the apparent kinetic isotope effect on $[H_2O]$, experiments were carried out in both dimethylsulfoxide (DMSO) and dimethylformamide (DMF) in which the water (or deuterium oxide) concentration was varied from 1 to 16 M. Over the entire range in DMSO and up to 14 M (H_2O) in DMF the values of $(k_H/k_D)_{app}$ were observed to be independent of $[H_2O]$ and equal to 1.07 ± 0.07 (DMSO) and 0.87 ± 0.03 (DMF). At the highest water concentration (16 M) $(k_H/k_D)_{app}$ in DMF changed from less than one to 1.1. Aside from the questionable change in the last case there did not appear to be any significant contribution from rate law (3).

In order to test for a possible substrate concentration effect on $(k_H/k_D)_{app}$ [ANCN] was varied from 0.125 to 1.00 mM in both solvents 16 M in H_2O (or D_2O). The apparent kinetic isotope effects were independent of substrate concentration (Table 1) within experimental error. However, the data are most revealing. The fourth column gives the "reaction order approach" criterion for a reaction second order in anion radical, *i.e.* v_c/[ANCN] is required to be constant to be consistent with either rate law (4) or (5).[2] The latter varies by a factor of almost 4 in both solvents. Thus, the "reaction order approach" rules out either rate law under the conditions of the experiments. Furthermore v_c is essentially constant in both solvents at [ANCN] of 0.5 to 1.00 mM. This means that $R_{A/B}$ which is the sum of the reaction orders in ANCN and $ANCN^{\cdot-}$ is equal to 1 in this concentration range.

But what was the basis for the proposal by Amatore, Pinson and Savéant [3] that rate laws (4)

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Table 1. Reaction order and deuterium kinetic isotope effect analysis of the reactions of 9-cyanoanthracene anion radical in aqueous DMF and DMSO.[a]

[ANCN]/mM	Solvent	v_c/V s^{-1} [b]	v_c/[ANCN]	k_H/k_D
0.125	DMF	125.8	1006	1.18
0.250	DMF	184.4	738	1.19
0.375	DMF	240.0	640	1.19
0.500	DMF	264.1	528	1.08
0.750	DMF	272.7	364	1.16
1.000	DMF	253.6	254	1.10
				1.15(0.05)
0.125	DMSO	64.6	517	0.89
0.250	DMSO	116.1	464	1.19
0.500	DMSO	166.1	322	1.01
0.750	DMSO	171.7	229	1.00
1.000	DMSO	166.0	166	1.03
				1.02(0.11)

[a] In solvent containing H_2O or D_2O (16 M) at 293.4 K. [b] The subscript c indicates that v was evaluated at a constant value of the derivative peak ratio; 0.500 in DMF and 0.400 in DMSO.

Table 2. Linear sweep voltammetry data for the reduction of 9-cyanoanthracene in aqueous dimethylsulfoxide.[a]

[H_2O]/M	Electrode	dE^p/d log v [b]	$-$dE^p/d log C_A [c]
1.39	Au	13.5 (0.5)	16.3 (200)
1.39	Au	17.0 (1.0)	13.3 (400)
1.39	Au	18.1 (2.0)	7.6 (1000)
2.78	Au	16.9 (0.5)	15.4 (200)
2.78	Au	17.2 (1.0)	14.3 (400)
2.78	Au	19.8 (2.0)	12.1 (1000)
8.33	Hg	19.6 (0.5)	11.0 (200)
8.33	Hg	20.4 (1.0)	11.8 (400)
8.33	Hg	19.7 (2.0)	11.0 (1000)
13.9	Hg	20.1 (0.5)	11.2 (200)
13.9	Hg	20.1 (1.0)	12.0 (400)
13.9	Hg	21.6 (2.0)	9.8 (1000)
13.9	Au	20.4 (0.5)	12.3 (200)
13.9	Au	20.7 (1.0)	12.3 (400)
13.9	Au	22.3 (2.0)	10.1 (1000)

[a] In solvent containing Bu_4NBF_4 (0.1 M) at 18.8 °C. [b] In mV/decade. The number in parentheses refers to the substrate concentration. [c] In mV/decade. The numbers in parentheses refer to v.

and (5) describe the reactions of $ANCN^{\cdot-}$ in aqueous DMSO? They apparently only studied the kinetics at a *single* concentration, *i.e.* 1.0 mM. They make the following statement: " Linear sweep voltammetric peak shift at low sweep rates (*i.e.*, in conditions where the reduction wave is chemically irreversible) with ANCN concentration and sweep rate (19 mV per log unit at 20 °C) indicate the occurrence of a radical–radical coupling dimerization process in the whole water concentration range (0–16 M)". This statement is difficult to understand in view of the fact that the reduction wave is chemically reversible at low sweep rates in dry solvents (see

Table 2 of Ref. 5) and it was not possible to get meaningful linear sweep voltammetry (LSV) results in DMF for this reason. Thus, it was necessary to re-investigate the LSV behaviour of ANCN in aqueous DMSO (Table 2). At the lowest [H_2O], 1.39 M, the data resemble that observed in dry DMF.[5] The value of $dE^p/d \log v$ was observed to be dependent upon [ANCN] and $dE^p/d \log C_A$ was dependent upon v. At [H_2O] greater than 2.78 M, $dE^p/d \log v$ was close to the value expected for second order dimerization, *i.e.* 19.3 mV/decade at 291.9 K, but in all cases $dE^p/d \log C_A$ was considerably lower than the theoretical value. Obviously, at high water concentrations the LSV data are not consistent with the dimerization mechanism and indicate either competing reactions or a complex rate law.

Both derivative cyclic voltammetry reaction order analysis and the LSV data indicate that there is a mechanism change in going from low to high water concentration in DMSO during the reduction of ANCN. In order to determine just what the mechanism is would require further experimental work. In the opinion of the author, the effort is not warranted. The complication of the change in mechanism was brought about by studying the reaction under very unusual conditions, in aprotic solvents containing up to 16 M H_2O. The reactions are much more well-defined in dry solvents.

This work shows that the "reaction order approach" can give valuable information when applied to complex reactions. In this case it reveals a complex kinetic pattern which is inconsistent with the previous mechanism proposal.[3] This work also points out the pitfalls in attempting to extend mechanisms from aprotic solvents to highly aqueous systems. It is apparent that it is not safe to assume that the reaction orders and the mechanism of a reaction remain the same when drastic changes are made in the reaction conditions.

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SCPT-INDO Calculations of NMR Carbon–Carbon Coupling Constants for Some Alcohols with Different Structures and Their Corresponding Hydrocarbons

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A large number of $^{13}C,^{13}C$-coupling constants have been measured in the last decade, and there is a widespread interest in their interpretation since these values provide information about the electronic structure of organic molecules.[1–3]

As known from the work of Ramsey,[4] spin–spin coupling constants [J(AB)] arise from three different coupling mechanisms: the orbital [$J(AB)^O$], dipolar [$J(AB)^D$] and Fermi contact [$J(AB)^{FC}$] interactions.

$$J(AB) = J(AB)^O + J(AB)^D + J(AB)^{FC}$$

If the Fermi contact mechanism is predominant, a linear relationship is valid between ^1J(CC) and the percentage s-character of the two bonding carbon hybrid orbitals.[5] This arises because the Fermi contact contribution depends upon the product of the s-orbital densities at the coupled nuclei, *i.e.* $S^2_C(O)\cdot S^2_C(O)$.

For some singly and multiply bound carbon atoms, such a relationship may be less reliable due to significant contributions to the spin–spin coupling interaction by the orbital and dipolar mechanisms.[6] Both of these non-contact interactions are proportional to the product of the one-centre integrals, $\langle r^{-3}\rangle_C$, for the coupled carbons, where $\langle r^{-3}\rangle_C$ is the expectation value of r^{-3} for the valence shell p-orbitals on the carbon atom concerned. The integral products, $S^2_C(O)$ and $\langle r^{-3}\rangle_C$, used in this study, were evaluated by Blizzard and Santry.[7]

Following our previous study [8] of $^{13}C,^{13}C$-couplings for methylcycloalkanes by the self-consistent perturbation approach, at the INDO level of approximation,[7] we now report the calculated contributions to the carbon–carbon coupling constants ^1J(C-1,C-2) of the compounds *1–6, 8–10, 12, 14* and *16–22*. The numbering of the atoms in the compounds is different from the rules given by IUPAC. This gives the same symbols for comparable coupling constants. The experimental coupling constants for compounds *1–22* have been published in previous papers.[2,3,9–12]

In the present paper we evaluate the influence of hydroxyl groups on the magnitude of the coupling constants by comparison of the calculated data for the alcohols with the values obtained for the corresponding hydrocarbons.

Results and discussion. The calculated contributions of the Fermi contact (FCT)-, orbital (OT)- and dipolar (DT)-terms to the ^1J(C-1, C-2) coupling constants for the compounds *1–22* are summarized in Tables 1 and 2, together with the experimental data.

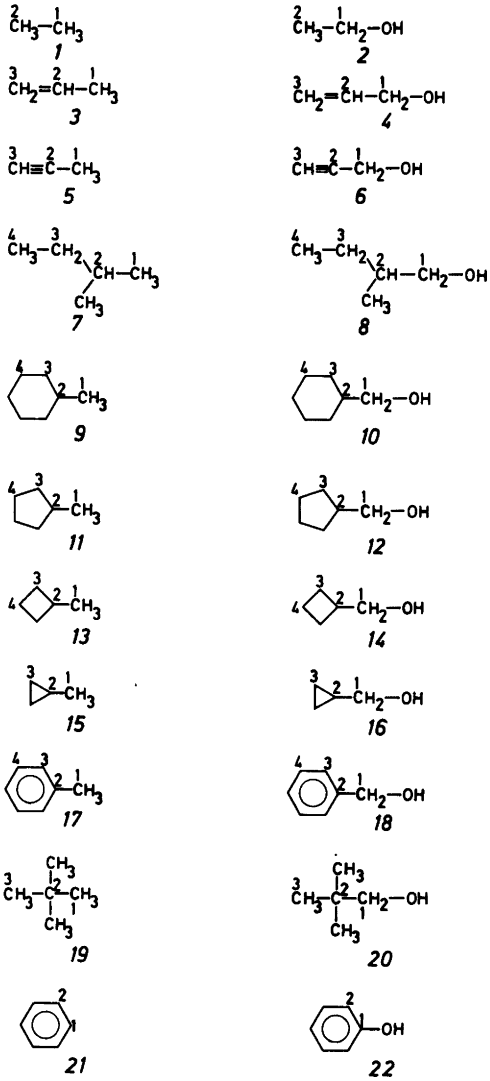

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Table 1. Calculated and experimental ^1J(C-1,C-2) coupling constants (in Hz) of the hydrocarbons.

Compound	Calculated				Exp.[a]
	FCT	OT	DT	Total	
1	35.64	−2.91	0.82	33.55	34.6
3	47.58	−3.12	0.71	45.17	41.9
5	66.54	−2.65	0.53	64.42	67.4
7[b]	37.50	−2.47	0.82	35.85	35.4
9	34.66	−2.51	0.84	32.99	35.7
11[b]	35.22	−2.49	0.83	33.56	36.2
13[b]	39.07	−2.58	0.76	37.25	36.1
15[b]	49.19	−2.59	0.68	47.28	43.4
17	42.97	−2.88	1.22	41.31	44.2
19	35.23	−2.29	0.82	33.76	33.7
21	65.44	−12.83	2.70	55.31	57.0

[a] Data taken from Ref. 12 (and references therein). [b] Calculated data taken from Ref. 8.

Table 2. Calculated and experimental ^1J(C-1,C-2) coupling constants (in Hz) of the alcohols.

Compound	Calculated				Exp.[a]
	FCT	OT	DT	Total	
2	42.61	−2.75	1.32	41.18	37.7
4	55.39	−3.06	1.17	53.50	45.4
6	78.08	−2.38	0.86	76.56	72.3
8	44.28	−2.51	1.28	43.05	37.7
10	40.79	−2.56	1.33	39.56	38.3
12	41.62	−2.56	1.33	40.39	38.8
14	45.76	−2.64	0.73	43.85	39.3
16	56.52	−2.61	1.09	55.00	47.8
18	50.31	−2.90	1.14	48.55	47.7
20	41.38	−2.42	1.27	40.23	37.5
22	73.76	−11.93	2.71	64.54	65.6

[a] Data taken from Ref. 12 (and references therein).

As shown in the tables the ^1J(C-1,C-2) coupling constants are dominated by the Fermi contact terms. The two non-contact terms add up to −1.8 Hz (±0.6 Hz), thus being almost negligible, except for compounds *21* and *22*. In these compounds (benzene and phenol) the carbon atoms are multiply bound. This is the reason why the orbital and dipolar terms are much larger than the non-contact terms for the compounds with common single carbon–carbon bonds [13] and, therefore, cannot be neglected.[7]

Changes in the size of the $^{13}C,^{13}C$-coupling constants caused by structural variations were observed both for the calculated and the experimental values. The calculated data agree mostly with the experimental findings. The largest deviations were found for the cyclopropane derivatives and the compounds with multiply bonded carbons.

Comparisons of the calculated coupling constants for the alcohols with the data obtained for the corresponding hydrocarbons show that an introduction of a hydroxyl group in an organic molecule leads to an average increase of the ^1J(C-1,C-2) values of about 7 Hz. Exceptions were observed for the alcohols *4*, *6* and *22*, with

Δ^1J values [$\Delta^1J = {}^1J$ (alcohol) $- {}^1J$ (hydrocarbon)] of 8.33 Hz, 12.14 Hz and 9.23 Hz, respectively. The value for the propyne system (*5* and *6*) is very large, presumably due to the fact that one of the carbons concerned is *sp*-hybridized.

Finally, it should be emphasized that the calculations confirm the experimental findings, although the calculated Δ^1J-values are larger than the experimental ones.

Experimental. The calculations were based on the self-consistent perturbation theory (SCPT) approach, within the INDO (intermediate neglect of differential overlap) framework, as developed by Blizzard and Santry.[7]

They were performed with the SCPT-INDO program of Blizzard and Santry [7] on the IBM 3032 computer system of the University of Münster, Germany. We used a modified version of the program with values of 3.7387 and 2.8793 a.u. for $S^2_C(O)$ and $\langle r^{-3}\rangle_C$, respectively. Bond distances and bond angles were based on the standard geometrical model.[14]

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The Mechanism of the Dimerization of Acetophenone Anion Radical in Acetonitrile. The Formation and the Dimerization of the Anion Radical–Water Complex

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Anion radicals form 1:1 complexes with water which in some cases enhance the rate of dimerization. So far, the only dimerization mechanism of this type that has been demonstrated involves equilibrium (1) followed by dimer forming reaction (2).[1-3] The corresponding dimerization of the complex (3) has been claimed to be important in the dimerization of 9-cyanoanthracene anion radical [4] but this has been shown to be erroneous.[5]

$$A^{\cdot-} + H_2O \overset{K_1}{\rightleftarrows} A^{\cdot-}/H_2O \quad (1)$$

$$A^{\cdot-}/H_2O + A^{\cdot-} \xrightarrow{k_2} A_2^{2-}(H_2O) \quad (2)$$

$$2\,A^{\cdot-}/H_2O \xrightarrow{k_3} A_2^{2-}(H_2O)_2 \quad (3)$$

The rate constant for reaction (2) has been estimated to be of the order of 10^6 times as great as that for the dimerization of uncomplexed diethyl fumarate anion radical.[2] This large rate difference is most likely due to less charge repulsion in the transition state for reaction (2) as compared to the dimerization of the uncomplexed anion radical. One might expect that the same factor could cause k_3 to be much larger than k_2.

The reason that reaction (3) has not been observed is that K_1 is in most cases so small that it cannot be measured. A large peak potential shift has been reported during voltammetry studies of the reduction of acetophenone in acetonitrile upon the addition of water.[6] This implies that K_1 may be large in this case. High substrate concentrations were employed in the voltammetric study [6] and since the anion radical undergoes a rapid second order reaction the potentials are not very reliable. The purpose of this work was to determine K_1 ($A^{\cdot-}$=acetophenone anion radical) and to study the kinetics of the reactions of the complex.

Reversible electrode potentials were determined for the reduction of acetophenone in acetonitrile containing water. Second harmonic *A.C.* voltammetry was used for the measurements at a substrate concentration of 0.1 mM. The data are gathered in Table 1. It was not possible to go to higher water concentrations because of a phase shift in the *A.C.* response most likely due to adsorption phenomena. The value of K_1 at 292.2 K was observed to be 16.6 M^{-1}.

Table 2. The influence of base and substrate concentration on the rate of dimerization of acetophenone anion radical in acetonitrile.[a]

C_A [b]/mM	$[Bu_4NOH]$/mM	$\tau_{0.4}$/ms	$(\tau_{0.4}C_A)^{-1}$
0.50	0	3.80	–
0.50	2.0	4.75	–
0.50	4.0	5.11	0.391
1.00	4.0	2.90	0.345
1.50	4.0	1.88	0.355
2.00	4.0	1.42	0.352

[a] In solvent containing Et_4NBF_4 (0.1 M) and H_2O (0.55 M) at 18.7 °C. [b] Substrate concentration.

Table 1. Determination of the equilibrium constant for the association of acetophenone anion radical with water in acetonitrile.[a]

$[H_2O]$/mM	$-E_{zc}$(300 Hz) [b]/mV	ΔE_{rev}/mV	(K_1/M^{-1}) [c]
0	342.8(0.1)	–	–
27.8	333.2(0.2)	9.6	16.7
55.6	326.5(0.2)	16.3	16.4

[a] In solvent containing Et_4NBF_4 (0.1 M) and acetophenone (0.1 mM) at 292.2 K. [b] The quadrature component zero current crossing potential of the second harmonic *A.C.* signal referred to a bias potential of −1.400 V. *vs.* Ag/Ag^+ in acetonitrile. The numbers in parentheses are the standard deviations in 5 replicate measurements. [c] Calculated from the expression, $K_1=(\exp(\Delta E_{rev}/(RT/F))-1)/[H_2O]$.

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Table 3. The reaction order in water during the dimerization of acetophenone anion radical in acetonitrile.[a]

$[H_2O]$/M	$[A^{\cdot-}/H_2O]/[A^{-}]$	$\tau_{0.4}$/ms	$(\tau_{0.4}[H_2O])^{-1}$
0.0556	0.923	52.2	0.345
0.111	1.85	21.9	0.411
0.166	2.76	10.5	0.574
0.221	3.67	6.46	0.700
0.276	4.58	4.39	0.825
0.331	5.49	3.55	0.851
0.386	6.41	2.62	0.989
0.441	7.32	2.06	1.10
0.498	8.27	1.72	1.17
0.550	9.13	1.48	1.23

[a] In solvent containing Et_4NBF_4 (0.1 M) and acetophenone (1.0 mM) at 19.1 °C.

The kinetics of the reactions of acetophenone anion radical in the same media were studied by double potential step chronoamperometry. Some deviation of the kinetics from second order were observed with the apparent rate constants decreasing with increasing substrate concentrations. This is indicative of the generation of an inhibiting species during reaction. The data in Table 2 show both the inhibition by hydroxide ion and a reasonably good fit to second order kinetics in a buffered solution. The quantity measured was $\tau_{0.4}$ which is the pulse width necessary to maintain the current ratio at a constant value equal to 0.400.[7]

The data in Table 3 show the effect of water on the rate of the dimerization. The second column gives the ratio, $[A^{\cdot-}/H_2O]/[A^{-}]$, calculated from the equilibrium expression. The column headed $(\tau_{0.4}[H_2O])^{-1}$ is the reaction order approach criterion for a reaction first order in water.[7] The data indicate that as $[A^{\cdot-}/H_2O]/[A^{-}]$ becomes larger the reaction order in water approaches 1. The apparent reaction order which can be derived from the data in the last column has to be corrected for the increase in the ratio of complexed to uncomplexed anion radical shown in the second column of Table 3. Thus, under conditions where equilibrium (1) is shifted to the right the reaction is still accelerated by water and appears to be first order in water.

Under conditions where (1) is far enough to the right that reaction (2) is insignificant, the kinetics are consistent with reversible reaction (3) followed by protonation reaction (4). The inhibition could be due to some reversibility of (4).

$$A_2^{2-}(H_2O)_x + H_2O \xrightarrow{k_4} HA_2^{-}(H_2O)_x + OH^{-} \qquad (4)$$

Since nothing is known about the association of the dianion with water the number of H_2O is designated as x.

This work shows that when K_1 is large as it is when A is acetophenone that the species undergoing dimerization is the anion radical–water complex. There is no evidence for the association of the anion radicals with more than one molecule of water.

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Acid Promoted Molecular Fragmentation of 6,7-Diazatricyclo-[3.2.2.$0^{2,4}$]nona-6,8-diene *N*-Oxide *

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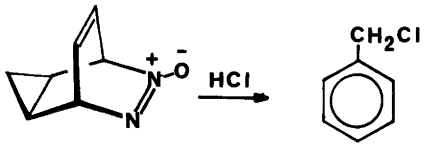
Previously we have reported several reactions in which *cis*-1,2-diazene *N*-oxides undergo thermally and photolytically induced rearrangements.[1] In this communication we turn our attention to an acid catalyzed transformation in which 6,7-diazatricyclo[3.2.2.$0^{2,4}$]nona-6,8-diene *N*-oxide *1* [2] in the presence of hydrogen chloride undergoes fragmentation to yield benzyl chloride, nitrogen and water.[3]



1 2

When *1* is mixed at room temperature with a solution of HCl in methanol (6 M), a vigorous reaction takes place with evolution of gas. The ^{1}H NMR spectrum of the crude reaction mixture (DCl, CD_3OD) was extremely simple, exhibiting only two singlets at δ 7.30 (5 H) and 4.60 (2 H), respectively, corresponding to benzyl chloride (80–85 % yield).***

The formation of benzyl chloride suggests that the reaction involves initial protonation at the *N*-oxide oxygen in *1*. Further transformation may proceed by heterolytic cleavage to give cation *3*. Collapse of *3 via* cyclopropane ring-opening produces diazotic acid *4*. Finally, aromatization by extrusion of nitrogen and water accounts for the observed product. The depicted heterolytic cleavage is reminiscent of the behaviour of azoxy-2-methylpropane under the influence of trifluoroacetic acid [4] and 3-chloro-3-phenyldiazirine in the presence of *m*-chloroperbenzoic acid.[5] In contrast to the behaviour of *1*, 2,3-diazabicyclo[2.2.2]octa-2,6-diene *N*-oxide [2] is unchanged after analogous treatment with HCl for three months. Clearly the attachment of a cyclopropane ring significantly alters the energetics of the fragmentation reaction. This is in accordance with the well-documented unique stability of the cyclopropylcarbinyl cation.[6] Since nitrous oxide was not detected in the reaction *1*→*2*, nitrogen is undoubtedly expelled during the aromatization depicted. The latter step is further supported by the work of Moss *et al.*[7] on the solvolysis of alkanediazotates.

* Rearrangement of 1,2-Diazene *N*-Oxides. 5. For Part 4, see Ref. 1.

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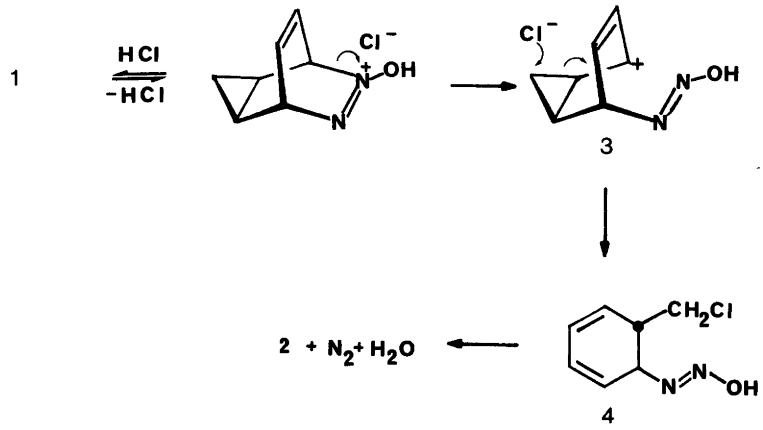
*** GLC–MS-analysis of the crude reaction mixture indicated the presence of minor amounts of methyl benzyl ether (2–5 %).



0302-4369/83 $2.50

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Stereochemistry of the Reactions of CuI Catalyzed Grignard Reagents with Ethyl (*E*)- and (*Z*)-β-Chlorocinnamates

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Ethyl (*E*)- and (*Z*)-3-chloro-3-phenyl-2-propenoate were prepared and separated. The (*Z*)-isomer gave β-alkylated ethyl cinnamates in good yield when reacted with alkyl Grignard reagents in the presence of 10 % CuI. The (*Z*)-isomer reacted with retention of configuration, whereas the (*E*)-isomer reacted with loss and retention of configuration.

Ever since the observation of Kharasch[1] that Grignard reagents add in the 1,4-manner to conjugated enones in the presence of catalytic amounts of cuprous salt, organocopper chemistry has been a standard synthetic technique. The different types of organocopper complexes useful in synthetic chemistry have been reviewed by Normant[2] and Posner.[3]

The stoichiometry and the kinetics of the copper catalyzed reactions between various Grignard reagents and alkyl halides have been examined by Kochi and Tamura.[4] Normant and co-workers[5] have reported that vinylic iodides undergo substitution with Grignard reagents in the presence of catalytic amounts of copper salts and that the reactions occur with retention of configuration. Alkyl-, aryl- and alkenylnickel-phosphine complex catalyzed cross-coupling of Grignard reagents with aryl and alkenyl halides have also been reported.[6] In our studies concerning the stereoselective synthesis of α-β-unsaturated esters it was of interest to examine the regio- and stereoselectivity of copper(I) iodide catalyzed Grignard substitution reactions with ethyl (*Z*)- and (*E*)-β-chlorocinnamates.

RESULTS AND DISCUSSION

The results from the reactions according to Scheme 1 are presented in Table 1. Primary, secondary and tertiary Grignard reagents reacted with ethyl (*Z*)-3-chloro-3-phenyl-2-propenoate (*1*) by replacement of the β-chloro substituent to produce β-alkyl substituted ethyl cinnamates (*3*)

Table 1. The reactions of CuI catalyzed RMgX with ethyl (*Z*)- and (*E*)-3-chloro-3-phenyl-2-propenoate according to Scheme 1.

Substrate	Reagent R	Product ratio[a] *E* (3) %	*Z* (4) %	Total yield[a] *E* + *Z* %
1	Me	100	0	82
1	Et	100	0	72
1	Pr	100	0	76
1	i-Pr	100	0	86
1	i-Bu	100	0	73
1	*sec*-Bu	100	0	80
1	*t*-Bu	100	0	70
1	2-MeBu	100	0	71
1	*C*-pentyl	100	0	86
1	*C*-hexyl	100	0	91
2	Me	85	15	72
2	Et	82	18	71
2	Pr	83	17	68
2	i-Pr	74	26	82
2	i-Bu	80	20	68
2	*sec*-Bu	72	28	72
2	*t*-Bu	62	38	63
2	2-MeBu	81	19	71
2	*C*-pentyl	72	28	82
2	*C*-hexyl	70	30	84

[a]As determined by ^{1}H NMR spectroscopy.

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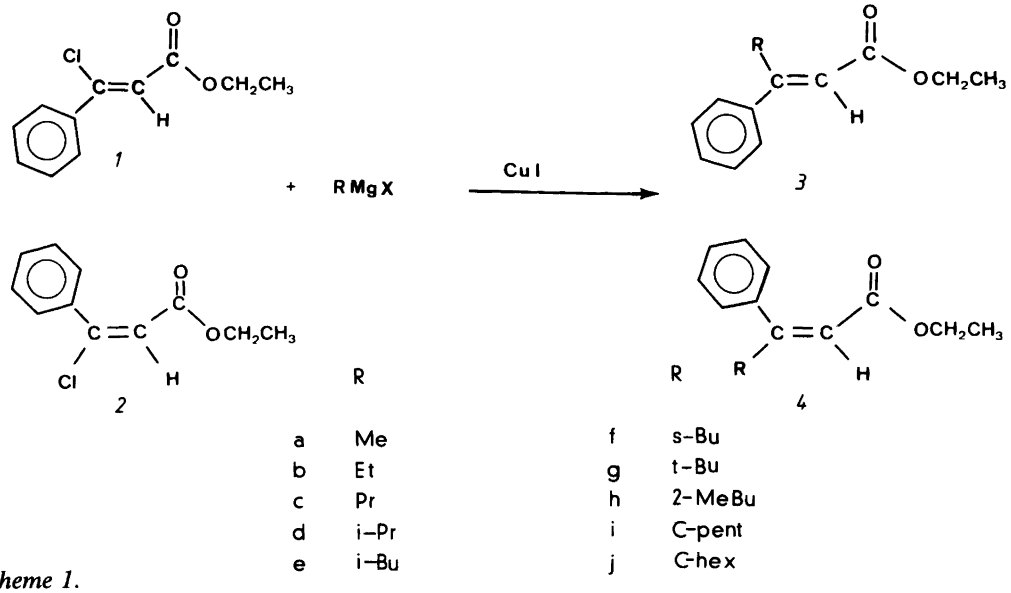

Scheme 1.

in good yield by a copper(I) iodide catalyzed reaction at a temperature above which the corresponding organocopper reagents rapidly decompose. The reactions proceeded by retention of configuration and were successful only if the Grignard reagents were added to the substrate suspended with copper(I) iodide in diethyl ether at 0 °C. The substitution products were not formed if the order of addition was reversed. If the reagent–substrate ratio was 3:1 β,β-dialkylated ketones were formed by conjugate addition and 1,2-addition. The CuI catalyzed reaction with *t*-butylmagnesium chloride was very fast at 0 °C and yielded small amounts of conjugate and 1,2-addition products besides substitution products although the reagent–substrate ratio was 1.2:1.

Ethyl (*E*)-3-chloro-3-phenyl-1-propenoate reacted slower than the corresponding *Z*-isomer and with loss and retention of configuration. The degree of isomerization was somewhat higher with primary than with secondary and tertiary Grignard reagents.

No isomerization of recovered starting material was observed when the reaction was quenched before complete conversion. In a previous study,[7] concerning the reaction of *t*-butylmagnesium chloride with ethyl (*Z*)-cinnamates, we found that the isomerization of the *Z*-form proceeded faster than conjugate addition since ethyl (*E*)-cinnamate was recovered. This isomerization was supposed to be the result of a rapid conversion of the less stable *Z*-isomer to the *E*-isomer *via* a radical anion intermediate. If a radical anion is formed in the present study, the alkylation step has to be much faster than the equilibration of the radical anion to the *Z*-isomer. Formation of ethyl cinnamate was not observed, when the reaction was quenched before complete conversion, which indicates that copper–halogen exchange, involving a copper–enoate intermediate, does not occur in these reactions, or that this intermediate is of very short duration. Moreover the concentration of this intermediate, if present, is of course low since the reactions proceed with catalytical amounts of copper. A metal–halogen exchange has, however, been observed in the copper iodide catalyzed reactions of Grignard reagents with vinyl iodides.[5]

The higher reactivity of the *Z*-isomer compared with that of the *E*-isomer can be due to different reaction mechanisms or to the formation of a complex (*9*) between copper and the *Z*-isomer, which would activate the olefinic double bond and weaken the carbon—chlorine bond.

For comparison it can be mentioned that the corresponding acid of the *Z*-isomer formed a solid complex with barium chloride while the

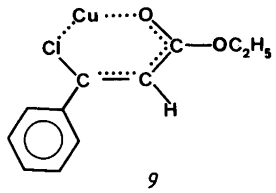

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Fig. 1.

E-isomer remained in solution. This difference was used for the separation of *Z*- and *E*-isomers.[8]

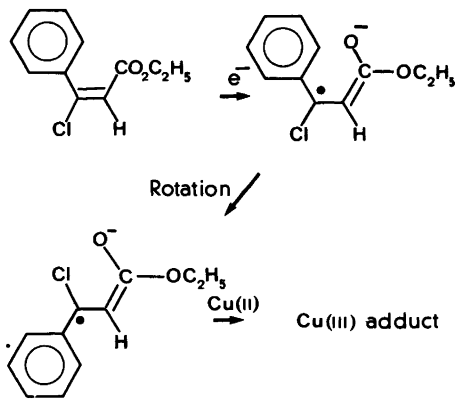
A qualitative order of reactivity of the Grignard reagents, in the reactions with the *E*-isomer (*2*), as estimated from the amount of starting material left after a reaction time of 10 min at 0 °C, showed the following sequence *t*-Bu>*sec*-Bu, C-hexyl, C-pentyl, i-Pr>Pr, Et, i-Bu>Me (see Experimental). This observation and the presence of isomerization products, indicate that a direct S_N2 mechanism is unlikely in the reaction with the *E*-isomer.

Chlorine was not substituted by a hydroxyl group when ethyl (*Z*)- and (*E*)-β-chlorocinnamates were reacted with an excess of sodium hydroxide in a mixture of ethanol and water (1:1). Instead, an elimination of hydrogen chloride took place and phenylethynoic acid was formed almost quantitatively.

The attempts to substitute chlorine in *1* and *2* by an uncatalyzed Grignard reaction were not successful although we found in a prior study[9] that the methoxy group in ethyl 1-methoxy-1-inden-2-carboxylate was almost quantitatively exchanged by phenyl and alkyl groups with pure Grignard reagents.

In most substitution reactions to RYC = CXR (Y = activating group, X = leaving group) the outcome has mainly been retention of configuration regardless of whether the precursor has an *E* or a *Z* configuration.[10] These reactions are supposed to proceed by a nucleophilic attack perpendicularly to the plane of the double bond and elimination of the nucleofuge either by a single-step or a multi-step process. The reactions of highly electrophilic olefins YY'C = CXR (Y, Y' = activating groups, X = leaving group) with nucleophiles resulted, however, in complete and partial stereoconversion.[11]

A single electron transfer to form an intermediary radical anion in the reaction of the *Z*-isomer (*1*) is hardly likely since the reaction

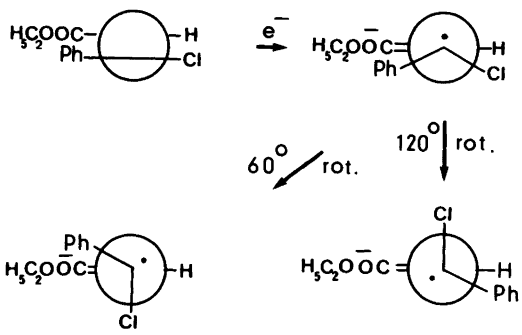


Scheme 2.

proceeded with complete retention of configuration. Retention of configuration suggests rather a nucleophilic substitution to form a Cu(III) adduct with subsequent elimination of chlorine so that the ethylenic bond remains mainly a double bond through the reaction. Even if a direct transfer of the alkyl group from copper, in a process not involving a Cu(III) adduct, cannot be ruled out.

If the barrier to rotation around the C_α–C_β bond is lowered, due to the formation of a carbonyl metal bond, the steric interactions between the substituents in the transition state should be dominating for the stereochemical outcome.

The reaction of the *E*-isomer (*2*) which proceeded mainly by isomerization of configuration can be consistent with a single electron transfer mechanism (Scheme 2). Since the *E*-isomer (*2*) in the present study reacted mainly with isomerization of configuration the assumed electron attack perpendicularly to the plane of the double bond has to be followed by a 120° counterclockwise rotation and elimination of chlorine from a position perpendicularly to the plane of the double bond simultaneously with the formation of the Cu(III) adduct. The latter step has to be fast since no isomerization of recovered starting material was observed. The steric interactions in the transition state of 120° counterclockwise rotation are consistent with the smaller steric interactions (Cl, H) compared with those of 60° rotation (Ph, COOEt) (Scheme 3). If the reaction proceeds by a nucleophilic addition mechanism to form a Cu(III) adduct before rotation the 120° counterclockwise rotation involves a greater steric interaction (Cl, H and Cu-complex,



Scheme 3.

COOEt) compared with the interactions on 60° rotation (Ph. COOEt), which indicates that rotation precedes the formation of a Cu(III) adduct since the dominating outcome is an isomerization product in the reaction of the *E*-isomer.

EXPERIMENTAL

All ^{1}H NMR spectra were recorded with a Jeol FX-60 FT spectrometer at 59.75 MHz and ^{13}C NMR spectra with the same instrument operating at 15.03 MHz. The spectra were taken in $CDCl_3$ with TMS as an internal standard. Mass spectra were recorded on an LKB 9000 combined GLC-MS instrument equipped with a (50 m×0.25 mm ID) capillary column coated with SE-30.

Grignard reagents. Grignard reagents were prepared in an inert atmosphere from the appropriate alkyl halides and synthetic grade magnesium turnings. The concentration in dry diethyl ether varied between 1,2 to 1,8 M as determined by standard titrations.

Ethyl (Z)- and (E)-3-chloro-3-phenylpropenoate.[8] The (*Z*)- and (*E*)-3-chloro-3-phenylpropenoic acids were prepared from methyl benzoylacetate. The (*Z*)- and (*E*)-isomers were formed in the proportion 60 to 40 %. They were separated as their barium-complexes on the basis of different solubilities in diluted ammonia. The acids were esterified with ethanol and the ethyl esters *1* and *2* distilled at 135–138 °C 2kPa.

Ethyl (Z)-3-chloro-3-phenylpropenoate (1). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.33 (3H, t, *J* 6.7 Hz), 4.27 (2H, q, *J* 6.7 Hz), 6.54 (1H, s), 7.2–7.7 (Ph). ^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.2 (CH_3), 60.6 (CH_2), 116.4 (C-2), 127.2, 128.6, 130.7, 137.2 (Ph), 146.1 (C-3), 164.1 (C-1).

Ethyl (E)-3-chloro-3-phenylpropenoate (2). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.11 (3H, t, *J* 6.7 Hz), 4.17 (2H, q, *J* 6.7 Hz), 6.35 (1H, s), 7.4 (Ph). ^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.8 (CH_3), 60.6 (CH_2), 119.9 (C-2), 127.9, 128.5, 129.9, 136.6 (Ph), 149.7 (C-3), 163.7 (C-1).

General procedure for the reaction of ethyl (Z)- and (E)-3-chloro-3-phenylpropenoates with CuI catalyzed Grignard reagents. The Grignard reagent (3.0 mmol) was added dropwise using a burette (Metrohm E 485 equipped with a 50 cm^3 cylinder) to a suspension of ethyl (*Z*)-β-chlorocinnamate (20 mmol) and CuI (2 mmol) in 50 cm^3 anhydrous diethyl ether at 0 °C. After addition the reaction mixture was stirred at 20 °C for 2 h. The suspension was then stirred with a mixture of ice and dilute hydrochloric acid for 30 min and extracted three times with diethyl ether. The organic phase was treated with Na_2CO_3 solution then dried with Na_2SO_4 and the diethyl ether evaporated. The residue was subjected to qualitative and quantitative analyses (Table 1). The reactions of ethyl (*E*)-β-chlorocinnamate were carried out in the same manner as those of the *E*-isomer. The qualitative order of reactivity of the Grignard reagents with the *E*-isomer (*2*) was estimated by withdrawing samples from the reaction mixture held at 0 °C for 10 min. Integration of the ^{1}H NMR signals of the phenyl protons and the ethylenic protons allowed the determination of ethyl (*E*)-3-chloro-3-phenyl-2-propenoate consumed and the yield of reaction products. The amounts of ethyl (*E*)-3-chloro-3-phenyl-2-propenoate left in the reaction with the Grignard reagents studied were as follows (reagent, %) Me, 32; i-Bu, 28; Et, 27; Pr, 25; i-Pr, 18; C-pentyl, C-hexyl, 16; *sec*-Bu 14; *t*-Bu. 11. The order of reactivity of the Grignard reagents with the *Z*-isomer (*1*) was not determined since the reactions were completed during the course of addition of the Grignard reagents. The ^{1}H, ^{13}C NMR and MS data of the identified reaction products are presented below.

Ethyl (E)-3-phenyl-2-butenoate (3a). MS [IP 70 eV; *m/e* (% rel. int.)]: 190 (59, M), 175 (4), 161 (38), 145 (100, M-OC_2H_5), 144 (45), 118 (19), 117 (40), 116 (27), 115 (47), 105 (4), 103 (4), 102 (4), 91 (16).

^{1}H NMR (59.75 MHz, $CDCl_3$): 1.1 (3H, t, *J* 6.2 Hz), 2.5 (3H, d, *J* 1.3 Hz), 4.1 (2H, q, 6.2 Hz), 6.17 (1H, q, *J* 1.3 Hz), 7.2–7.5 (5H).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.4 (CH_3), 17.8 (C-4), 59.7 (CH_2), 117.2 (C-2), 126.3, 128.6, 129.0, 142.4 (Ph), 155.4 (C-3), 166.6 (C-1); *J* (C-2, H-4) 4.6 Hz, *J* (C-4, H-2) 7.8 Hz.

Ethyl (E)-3-phenyl-2-pentenoate (3b). MS [IP 70 eV; *m/e* (% rel. int.)]: 204 (100, M), 176 (11), 175 (13), 159 (70), 158 (99), 131 (22), 129 (35),

115 (23), 91 (31).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.0 (3H, t, J 7.0 Hz), 1.3 (3H, t, J 6.5 Hz), 3.1 (2H, q, d, J 7.0 and 0.6), 4.2 (2H, q, J 6.5 Hz), 6.0 (1H, t, J 0.6 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.4 (C-5), 14.3 (CH_3), 24.4 (C-4), 59.7 (CH_2), 116.9 (C-2), 126.7, 128.5, 128.8, 141.3 (Ph), 161.9 (C-3), 166.4 (C-1); J (C-2, H-4) 4.4 Hz.

Ethyl (E)-3-phenyl-2-hexenoate (3c). MS [IP 70 eV; *m/e* (% rel.int.)]: 218 (100, M), 203 (13), 190 (13), 189 (16), 173 (64), 157 (38), 131 (35), 129 (40), 115 (31), 91 (31).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 0.9 (3H, t, J 7.0 Hz), 1.1 (3H, t, J 6.5 Hz), 1.4 (2H, m), 3.1 (2H, t, d, J 7.0 and 0.6 Hz), 4.2 (2H, q, J 6.5 Hz), 6.1 (1H, t, J 0.6 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.0 (C-6), 14.2 (CH_3), 22.3 (C-5), 32.9 (C-4), 59.6 (CH_2), 116.9 (C-2), 126.7, 128.6, 129.2, 141.3 (Ph), 163.5 (C-3), 166.4 (C-1), J (C-2, H-4) 4.4 Hz.

Ethyl (E)-4-methyl-3-phenyl-2-pentenoate (3d). MS [IP 70 eV; *m/e* (% rel. int.)]: 218 (100, M), 190 (8), 189 (11), 175 (5), 173 (43), 172 (53), 145 (73), 143 (37), 131 (20), 129 (34), 128 (22), 91 (25), 77 (20), 43 (15).

^{1}H NMR (59.75 MHz, $CDCl_3$): 1.09 (6H, d, J 7.1 Hz), 1.29 (3H, t, J 7.1 Hz), 4.15 (1H, sept, J 7.1 Hz), 4.19 (2H, q, J 7.1 Hz), 5.7 (1H, d, J 0.6 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.3 (CH_3), 21.4 (C-5), 29.6 (C-4), 59.6 (CH_2), 118.6 (C-2), 127.3, 127.5, 127.7, 141.0 (Ph), 166.1 (C-3), 166.4 (C-1); J (C-2, H-4) 3.9 Hz.

Ethyl (E)-5-methyl-3-phenyl-2-hexenoate (3e). MS [IP 70 eV; *m/e* (% rel. int.)]: 232 (62, M), 217 (30), 203 (8), 190 (33), 189 (43), 187 (63), 171 (64), 161 (33), 145 (100), 144 (62), 143 (62), 131 (33), 129 (39), 128 (28), 118 (59), 117 (43), 116 (37), 115 (79), 91 (40).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 0.87 (6H, d, J 6.5 Hz), 1.3 (3H, t, J 7.1 Hz), 1.67 (1H, sept), 3.1 (2H, dd, J 7.1 and 0.5 Hz), 4.2 (2H, q, J 6.5 Hz), 6.1 (1H, t, J 0.5 Hz), 6.9–7.2 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.4 (CH_3), 22.4 (C-6), 27.9 (C-5), 38.9 (C-4), 59.8 (CH_2), 118.6 (C-2), 126.8, 128.5, 128.7, 141.8 (Ph), 160.1 (C-3), 166.6 (C-1), J (C-2, H-4) 4.4 Hz.

Ethyl (E)-4-methyl-3-phenyl-2-hexenoate (3f). MS [IP 70 eV; *m/e* (% rel. int.)]: 232 (85, M), 217 (18), 187 (50), 171 (58), 159 (51), 157 (50), 145 (42), 144 (51), 143 (58), 131 (53), 129 (100), 117 (64), 115 (57), 105 (51), 91 (85).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.0 (3H, t, J 7.1 Hz), 1.2 (3H, d, J 7.2 Hz), 1.3 (3H, t, J 6.5 Hz), 1.5 (2H, m), 3.8 (1H, m) 4.2 (2H, q, J 6.5 Hz), 5.75 (1H, d, J 0.5 Hz), 7.3 (5H), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 12.3 (C-6), 14.3 (CH_3), 19.2 (CH_3), 28.2 (C-5), 36.6 (C-4), 59.8 (CH_2), 119.7 (C-2), 127.7, 141.0 (Ph), 166.0 (C-3), 166.3 (C-1), J (C-2, H-4) 3.9 Hz.

Ethyl (E)-4,4-dimethyl-3-phenyl-2-pentenoate (3g). MS [IP 70 eV; *m/e* (% rel. int.)]: 232 (55, M), 217 (85), 203 (5), 187 (40), 175 (6), 159 (100), 102 (28).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.2 (9H,s), 1.3 (3H, t, J 7.1 Hz), 4.2 (2H, q, J 7.1 Hz), 5.65 (1H, s), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.0 (CH_3), 30.2 (C-5), 36.4 (C-4), 60.1 (CH_2), 120.4 (C-2), 126.7, 127.4, 127.5, 144.0 (Ph), 163.3 (C-3), 167.1 (C-1).

Ethyl (E)-5-methyl-3-phenyl-2-heptenoate (3h). MS [IP 70 eV; *m/e* (% rel. int.)]: 246 (14), 231 (4), 217 (25), 201 (20), 190 (100), 171 (25), 161 (20), 145 (29), 144 (30), 143 (23), 118 (84), 115 (32), 91 (23).

^{1}H NMR (59.75 MHz, $CDCl_3$); δ 1.3 (3H, t J 7.1 Hz), 0.76–1.5 (9H, m), 3.2 (2H, d, J 7.4 disturbed), 4.2 (2H, q, J 7.1 Hz), 6.05 (1H, d, J 0.6 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 11.4 (C-7), 14.4 (CH_3), 18.7 (CH_3), 29.6 (C-6), 34.0 (C-5), 36.9 (C-4), 59.8 (CH_2), 118.8 (C-2), 126.8, 128.5, 141.9 (Ph), 160.2 (C-3), 166.7 (C-1), J (C-2, H-4) 4.4 Hz.

Ethyl (E)-3-cyclopentyl-3-phenyl-2-propenoate (3i). MS [IP 70 eV; *m/e* (% rel. int.)]: 244 (100, M), 227 (4), 216 (9), 215 (9), 199 (35), 198 (21), 197 (23), 171 (35), 170 (39), 157 (18), 141 (20), 129 (39), 115 (18), 91 (52).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.3 (3H, t, J 7.0 Hz), 1.5–1.9 (9H, broad), 4.2 (2H, q, J 7.0 Hz), 5.7 (1H, d, J 0.7 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.35 (CH_3), 25.3, 31.5 (C-pent.), 41.8 (C-4), 59.8 (CH_2), 119.4 (C-2), 127.5, 127.7, 141.2 (Ph), 164.96 (C-3), 166.4 (C-1), J (C-2, H-4) 3.9 Hz.

Ethyl (E)-3-cyclohexyl-3-phenyl-2-propenoate (3j). MS [IP 70 eV; *m/e* (% rel. int.)]: 258 (100, M), 213 (13), 211 (16), 185 (29), 183 (25), 170 (25), 167 (15), 141 (21), 131 (23), 129 (25), 117 (25), 115 (16), 91 (46).

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.3 (3H, t, J 7.0 Hz), 1.2–1.4 (11H, broad), 4.2 (2H, q, J 7.0 Hz), 5.7 (1H, d, J 0.6 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): 14.2 (CH_3), 25.9, 26.4, 31.6 (CH_2 C-hexyl), 40.5 (C-4), 59.6 (CH_2), 118.6 (C-2), 127.2, 127.5, 127.6, 141.4 (Ph), 166.1 (C-3), 166.8 (C-2), J (C-2, H-4) 4.4 Hz.

The corresponding *Z*-isomers were not isolated. Their ^{1}H and ^{13}C NMR spectra were deduced from the spectra of both isomers by comparison with the spectra of the pure *E*-

isomers. The mass spectra of the *Z*-isomers are not presented here because they did not differ to any greater extent from those of the *E*-isomers.

Ethyl (Z)-3-phenyl-2-butenoate (4a). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.1 (3H, t, *J* 6.2 Hz), 2.1 (3H, d, *J* 1.34 Hz), 3.8 (2H, d, *J* 6.2 Hz), 5.8 (1H, q, *J* 1.34 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.9 (CH_3), 27.0 (C-4), 59.7 (CH_2), 117.8 (C-2), 126.2, 128.5, 128.9, 140.9 (Ph). 155.2 (C-3), 165.7 (C-1), *J* (C-2, H-4) 6.3 Hz, *J* (C-4, H-2) 6.8 Hz.

Ethyl (Z)-3-phenyl-2-pentenoate (4b). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.0 (3H, t, *J* 7.1 Hz), 1.3 (3H, t, *J* 7.0 Hz), 2.4 (2H, q, d, *J* 7.0 and 1.3 Hz), 4.2 (2H, q *J* 7.0 Hz), 5.85 (1H, t, *J* 1.3 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 12.1 (CH_3), 13.9 (C-5), 24.7 (C-4), 59.8 (CH_2), 116.5 (C-2), 127.1, 127.5, 128.8, 140.5 (Ph), 162.0 (C-3), 166.4 (C-1).

Ethyl (Z)-4-methyl-3-phenyl-2-pentenoate (4d). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.0 (3H, t, *J* 7.1 Hz), 1.1 (6H, d, *J* 7.1 Hz), 2.76 (1H, sep, *J* 7.1 Hz), 3.95 (2H, q, *J* 7.1 Hz), 5.85 (1H, d, *J* 1.1 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.9 (CH_3), 21.1 (C-5), 37.3 (C-4), 59.6 (CH_2), 115.9 (C-2), 127.3, 127.5, 127.7, 140.3 (Ph), 164.9 (C-3), 167.1 (C-1), *J* (C-2, H-4) 3.4 Hz.

Ethyl (Z)-5-methyl-3-phenyl-2-hexenoate (4e). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 0.87 (6H, d, *J* 7.0 Hz), 1.3 (3H, t, *J* 6.5 Hz), 1.7 (1H, m), 2.3 (2H, d, *J* 6.5 Hz), 4.0 (2H, q, *J* 6.5 Hz), 5.85 (1H, t, *J* 1.1 Hz), 7.2–7.5 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.4 (CH_3), 22.4 (C-6), 27.8 (C-5), 50.0 (C-4), 59.8 (CH_2), 118.4 (C-2), 127.2, 127.6, 127.8, 140.0 (Ph), 158.6 (C-3), 166.0 (C-1), *J* (C-2, H-4) 5.8 Hz.

Ethyl (Z)-4-methyl-3-phenyl-2-hexenoate (4f). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 0.9-1.6 (8H, m), 2.8 (1H, sex, *J* 7.1 Hz), 4.2 (2H, q, *J* 7.0 Hz), 5.85 (1H, d, *J* 1.1), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 11.6 (C-6), 13.9 (CH_3) 18.6 (CH_3), 27.5 (C-5), 44.5 (C-4), 59.7 (CH_2), 117.0 (C-2), 127.3, 127.6, 127.7, 140.1 (Ph), 163.9 (C-3), 166.4 (C-1), *J* (C-1, H-4) 5.4 Hz.

Ethyl (Z)-4,4-dimethyl-3-phenyl-2-pentenoate (4g). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 0.99 (3H, t, *J* 7.1 Hz), 1.13 (9H, s), 3.9 (2H, q, *J* 7.1 Hz), 6.01 (1H, s), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.8 (CH_3), 29.0 (C-5), 37.2 (C-4), 59.4 (CH_2), 116.3 (C-2), 127.1, 127.4, 128.3, 139.3 (Ph), 163.7 (C-3), 166.1 (C-1).

Ethyl (Z)-5-methyl-3-phenyl-2-heptenoate (4h). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 0.85–1.4 (12H, broad), 2.2–2.4 (2H, m), 4.1 (2H, q, *J* 7.0 Hz), 5.85 (1H, t, *J* 1.0 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 11.4 (C-7), 14.0 (CH_3), 18.9 (CH_3), 29.7 (C-6), 34.8 (C-5), 47.5 (C-4), 59.8 (CH_2), 118.5 (C-2), 126.8, 128.5, 140.1 (Ph), 158.2 (C-3), 166.5 (C-1).

Ethyl (Z)-3-cyclopentyl-3-phenyl-2-propenoate (4i). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.1 (3H, t, *J* 7.0 Hz), 1.4–1.9 (9H, broad), 4.1 (2H, q, *J* 7.0 Hz), 5.9 (1H, d, *J* 1.3 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 14.0 (CH_3), 24.6, 31.8 (CH_2 C-pentyl), 46.3 (C-4), 59.8 (CH_2), 115.9 (C-2), 127.1, 127.5, 127.7, 140.7 (Ph), 162.8 (C-3), 166.4 (C-2), *J* (C-2, H-4) 5.4 Hz.

Ethyl (Z)-3-cyclohexyl-3-phenyl-2-propenoate (4j). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.1 (3H, t, *J* 7.0 Hz), 1.2–1.4 (11H, broad), 3.9 (2H, q, *J* 7.0 Hz), 5.83 (1H, d, *J* 1.1 Hz), 7.2–7.4 (5H, broad).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.9 (CH_3), 26.0, 26.9, 30.24, 47.4 (C-hexyl), 59.6 (CH_2), 116.1 (C-2), 127.5, 127.7, 128.3, 140.6 (Ph), 164.4 (C-3), 166.8 (C-1), *J* (C-2, H-4) 4.4 Hz.

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Structural Studies of Curcuminoids. II. Crystal Structure of 1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione–Methanol Complex

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The crystal and molecular structure of the curcumin derivative 1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione-methanol complex (B4HPHDD) has been determined at 121 K by X-ray crystallographic methods using 2090 reflections observed by counter methods. The crystals are monoclinic, space group $P2_1/n$ with unit cell dimensions a=6.781(2) Å, b=7.606(1) Å, c=33.024(5) Å, β=93.73(2)°. The structure was refined to a conventional R-factor of 0.033. Estimated standard deviations are 0.003 Å and 0.2° in interatomic distances and angles when hydrogen atoms are not involved. The enol-ring is found to be asymmetric.

Three diarylheptanoides have been isolated from the plant *Curcuma longa* L. (Fig. 1). These three compounds constitute the main coloured substances isolated from the plant material. Whereas the chromatographic analysis (HPLC) of the natural occurring diarylheptanoides will be published elsewhere,[1] we report here the X-ray crystallographic study of a synthetic compound found by HPLC/TLC to be equivalent to one of the compounds found in plant material, namely the one marked as III in Fig. 1. The crystal structure of the compound indicated as I has been reported previously.[2] These structure investigations are carried out in order to study the effect of the conjugation in curcuminoid molecules on the structure and stability of the 3,5-dione group as well as on the conformation of such molecules. By studying similar molecules in different crystalline environments it may also be possible to estimate the influence of molecular packing on the structure and conformation.

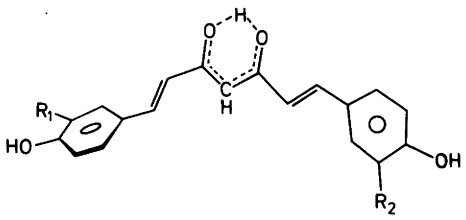

Fig. 1. Diaryl heptanoides isolated from *Curcuma longa* L. I, R_1=R_2=OCH_3: curcumin. II, R_1=OCH_3, R_2=H: demethoxycurcumin. III, R_1=R_2=H: bisdemethoxycurcumin.

EXPERIMENTAL

The title compound is a natural product[3] and was prepared from 2,4-pentanedione and 4-hydroxybenzaldehyde.[4] The compound was recrystallized from a mixture of ethyl-acetate and methanol and deep orange coloured plate-formed crystals separated. A crystal of dimensions 0.6×0.5×0.1 mm was used for the collection of X-ray data through the procedure described under Experimental Conditions. Cell parameters were determined by a least squares fit to the diffractometer settings for 15 general reflections. The standard deviations in the measured intensities were calculated as $\delta(I)=[C_T+(0.02\ C_N)^2]^{1/2}$, where C_T is the total number of counts and C_N is the scan count minus the background count. The intensity data were

0302-4369/83 $2.50

Table 1. Fractional atomic coordinates and thermal parameters multiplied by 10^4. The anisotropic temperature factor is given by $-2\pi^2(U_{11}a^{*2}h^2+\ldots+2U_{12}a^*b^*hk+\ldots)$. Estimated standard deviations in parentheses.

Atom	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O1	–4644(2)	4706(2)	6512(0)	318(9)	357(9)	228(8)	–1(8)	–80(7)	17(7)
O2	3083(2)	3189(2)	8549(0)	256(9)	313(9)	205(8)	–35(8)	6(7)	34(7)
O3	6180(2)	3615(2)	8999(0)	269(8)	280(8)	206(8)	–20(8)	5(7)	35(7)
O4	16870(2)	8605(2)	9738(0)	286(9)	424(10)	298(9)	11(9)	–64(7)	36(8)
O5	8611(2)	896(2)	9221(0)	275(9)	288(9)	254(8)	–25(8)	–70(7)	3(7)
C1	–3191(3)	4457(3)	6819(1)	314(13)	190(12)	185(11)	50(11)	39(10)	–55(9)
C2	–1261(3)	5057(3)	6783(1)	280(12)	197(12)	173(11)	–10(11)	20(10)	–9(9)
C3	122(3)	4815(3)	7104(1)	252(13)	192(12)	221(11)	–8(11)	20(10)	–18(9)
C4	–369(3)	3965(3)	7460(1)	245(12)	187(12)	180(10)	10(11)	17(9)	–19(9)
C5	–2306(3)	3321(3)	7472(1)	308(13)	214(12)	184(11)	–14(11)	39(10)	–2(9)
C6	–3694(3)	3560(3)	7154(1)	254(13)	252(12)	217(11)	–4(11)	13(10)	–29(9)
C7	961(3)	3767(3)	7823(1)	292(14)	202(12)	196(11)	7(12)	39(10)	–7(9)
C8	2717(3)	4532(3)	7903(1)	265(13)	219(12)	195(11)	–11(11)	14(10)	5(10)
C9	3882(3)	4246(3)	8281(1)	251(13)	199(11)	197(11)	15(11)	49(10)	–12(9)
C10	5707(3)	4990(3)	8364(1)	252(13)	229(12)	192(11)	–16(11)	38(10)	14(9)
C11	6833(3)	4661(3)	8734(1)	237(12)	208(11)	209(11)	24(11)	33(10)	–32(9)
C12	8742(3)	5519(3)	8810(1)	250(13)	232(12)	217(12)	6(11)	18(10)	11(10)
C13	9667(3)	5556(3)	9180(1)	256(13)	234(12)	224(12)	10(11)	39(10)	–4(10)
C14	11554(3)	6356(3)	9308(1)	228(13)	224(12)	231(11)	4(11)	14(10)	–21(9)
C15	12251(3)	6226(3)	9713(1)	315(14)	320(13)	194(11)	–51(13)	11(10)	13(10)
C16	14011(3)	6985(3)	9855(1)	350(15)	334(14)	181(12)	–26(12)	–31(11)	3(10)
C17	15137(3)	7891(3)	9589(1)	234(13)	251(12)	272(12)	–20(11)	–10(10)	–23(10)
C18	14490(3)	8020(3)	9181(1)	238(13)	271(13)	230(12)	15(12)	7(10)	26(10)
C19	12722(3)	7271(3)	9044(1)	258(13)	255(12)	187(11)	40(11)	–7(10)	–6(9)
C20	10446(4)	1248(4)	9457(1)	272(13)	332(15)	301(13)	–3(14)	–82(11)	–25(11)

Table 2. Fractional atomic coordinates and isotropic thermal parameters for the hydrogen atoms. Estimated standard deviations in parentheses.

Atom	*X*	*Y*	*Z*	*B*	Atom	*X*	*Y*	*Z*	*B*
HO1	-0.419(4)	0.515(4)	0.629(1)	6.2(8)	HO4)	1.741(4)	.928(4)	0.954(1)	6.8(9)
HO5	0.789(4)	0.194(4)	0.917(1)	6.8(8)	HX	0.406(4)	0.317(3)	0.877(1)	4.5(7)
H2	-0.091(3)	0.569(3)	0.654(1)	1.8(4)	H3	0.150(3)	0.527(3)	0.708(1)	2.3(5)
H5	-0.266(3)	0.271(3)	0.771(1)	1.5(4)	H6	-0.503(3)	0.310(3)	0.717(1)	2.4(5)
H7	0.049(3)	0.299(3)	0.803(1)	1.9(4)	H8	0.331(3)	0.531(3)	0.772(1)	2.0(5)
H10	0.625(3)	0.573(3)	0.817(1)	1.7(4)	H12	0.929(3)	0.610(3)	0.858(1)	2.0(4)
H13	0.897(3)	0.497(3)	0.940(1)	1.8(4)	H15	1.152(3)	0.555(3)	0.991(1)	3.4(5)
H16	1.447(3)	0.689(3)	1.013(1)	2.1(4)	H18	1.533(3)	0.868(3)	0.900(1)	2.5(5)
H19	1.225(3)	0.741(3)	.875(1)	1.8(4)	H2O1	1.130(4)	0.201(3)	0.930(1)	3.8(6)
H2O2	1.110(4)	0.010(4)	0.951(1)	4.2(6)	H2O3	1.016(3)	0.181(3)	0.972(1)	3.6(5)

corrected for Lorentz and polarization effects. The variation in the intensities of the test reflections were less than 1.5 % and no corrections were made on this basis. Scattering factors used were those of Doyle and Turner[5] for O and C, and of Stewart, Davidson and Simpson[6] for H.

CRYSTAL DATA

1,7-Bis(4-hydroxyphenyl)-1,6-heptadien-3,5-dione-methanol. $C_{19}O_4H_{16}\cdot CH_3OH$, monoclinic, a=6.781(2) Å, b=7.606(1) Å, c=33.024(5) Å, β=93.73(2)°, V=1699.7 Å^3, FW=340.2, Z=4, F_{000}=720, space group $P2_1/n$.

EXPERIMENTAL CONDITIONS

Instrument	SYNTEX $P\bar{1}$
Radiation	Graphite crystal monochromated Mo$K\alpha$ λ=0.71069 Å
Crystal dimensions/mm	0.6×0.5×0.1
Scanning mode	ω
Scan speed/° min^{-1}	2
Scan range/°	1.5
Background counts	For 0.35 of scan time at scan limits
Temperature/K	121
2θ range/°	2.5–45.0
Number of reflections meas.	2634
Number of reflections $I>2.5\sigma(I)$	2090
Number of standard reflections	3
Number of reflections between standard reflections	57

STRUCTURE DETERMINATION

The structure was solved by direct methods using the program assembly MULTAN.[7] Successive Fourier syntheses yielded the positions of all the non-hydrogen atoms, and revealed the existence of one molecule of methanol per asymmetric unit. After a least squares refinement with isotropic temperature factors, all the hydrogen atomic positions were found from difference syntheses. All positional parameters, anisotropic

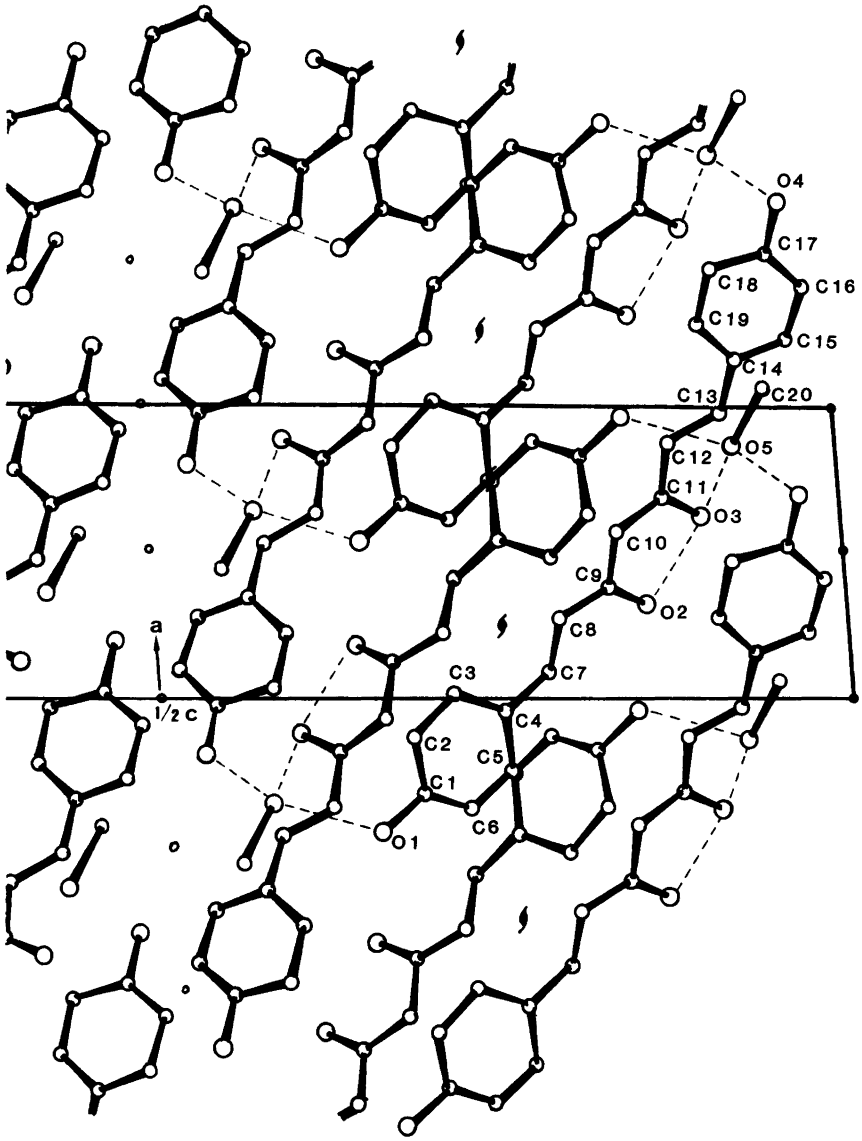

Fig. 2. Numbering of the atoms and the molecular packing in crystals of B4HPHDD·M as seen along the *b*-axis.

temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms were refined in the final least squares calculations giving an *R*-factor of 0.033 and a goodness of fit $S=[\Sigma w\Delta^2/(m-n)]^{1/2}=2.9$. The final parameters are given i Tables 1 and 2. Tables of observed and calculated structure factors are available from the authors.

DESCRIPTION AND DISCUSSION

The labelling of the atoms is given in Fig. 2, which also illustrates the molecular packing and the hydrogen bond system. Bond lengths and angles are given in Table 3 and some of the torsion angles in Table 4. The packing of the molecules in the present structure is different

Table 3. Bond lengths and angles. Estimated standard deviations are $(2–3)\times10^{-3}$ Å in bond lengths and 2×10^{-1} in angles.

Bond lengths (Å)		Bond angles (°)	
C1–O1	1.359	O1–C1–C2	123.2
C1–C2	1.394	O1–C1–C6	117.1
C2–C3	1.382	C1–C2–C3	120.1
C3–C4	1.401	C2–C3–C4	121.3
C4–C5	1.404	C3–C4–C5	117.3
C5–C6	1.377	C4–C5–C6	121.8
C6–C1	1.388	C5–C6–C1	119.9
C4–C7	1.459	C3–C4–C7	124.6
C7–C8	1.336	C5–C4–C7	118.0
C8–C9	1.448	C4–C7–C8	127.9
C9–O2	1.337	C7–C8–C9	122.4
C9–C10	1.372	C8–C9–C10	123.1
C10–C11	1.421	C8–C9–C2	116.0
C11–O3	1.283	O2–C9–C10	120.9
C11–C12	1.457	C9–C10–C11	121.8
C12–C13	1.337	C10–C11–C12	119.3
C13–C14	1.455	C10–C11–O3	120.4
C14–C15	1.395	C3–C11–C12	120.2
C15–C16	1.380	C11–C12–C13	121.6
C16–C17	1.386	C12–C13–C14	128.8
C17–O4	1.357	C13–C14–C15	119.1
C17–C18	1.394	C14–C15–C16	121.9
C18–C19	1.377	C15–C16–C17	119.5
C19–C14	1.400	C16–C17–C18	119.8
C20–O5	1.447	C16–C17–C4	118.0
		O4–C17–C18	122.2
		C17–C18–C19	120.1
Mean value of the X–H distances		C18–C19–C14	121.1
C–H	0.98(2)	C19–C14–C15	117.6
O–H	0.91(4)	C19–C14–C13	123.3

Table 4. Torsion angles in B4HPHDD.

Angle	
C3–C4–C7–C8	9.9
C4–C7–C8–C9	178.9
C7–C8–C9–C10	178.5
C7–C8–C9–O2	–1.1
C8–C9–C10–C11	–178.9
O2–C9–C10–C11	0.7
C9–C10–C11–O3	1.5
C9–C10–C11–C12	–178.7
C10–C11–C12–C13	165.8
O3–C11–C12–C13	–14.4
C11–C12–C13–C14	–179.6
C12–C13–C14–C15	–179.7

from that found for curcumin molecules.[2] The presence of the methanol molecules results in a hydrogen bond system where there is no direct hydrogen bond between B4HPHDD molecules. The alcoholic oxygen atom is involved in three hydrogen bonds to three different B4HPHDD molecules, as donor to the O3 oxygen and to O1 and O4 as acceptor. In this way molecules related by screw axes are bonded together in layers parallel to, and separated by, (002) planes. Between these layers there appears to be only weak van der Waals forces, the closest contact being C16–C16′ related by a center of symmetry (3.414 Å). (H16–C16′:3.12 Å). Within the hydrogen bonded layers there also appears to be interactions between the aromatic parts of the molecules in a way often encountered in crystal structures of such molecules.[6] These contacts are illustrated in Fig. 3.

Only one of the oxygen atoms of the enol-ring

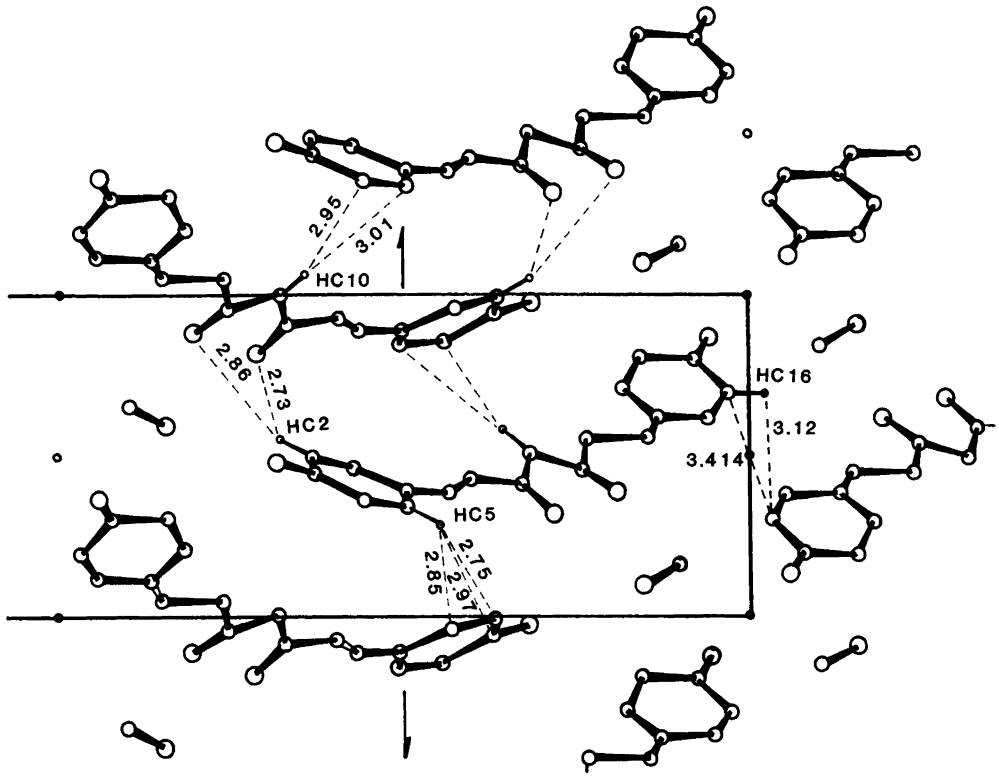

Fig. 3. Molecular packing of B4HPHDD·M units as seen along the *a*-axis, showing the distances between the aromatic moieties.

is engaged in an intermolecular hydrogen bond and in comparison with curcumin this introduces an element of asymmetry, which may explain the asymmetry of the entire enol-ring geometry in the present structure. Thus the two C–O bonds are significantly different as are the two bonds C9–C10 and C10–C11 signaling a localization of double bonding between C9–C10 and C11–O3. The intramolecular hydrogen bond between O2 and O3 is found to be established and the

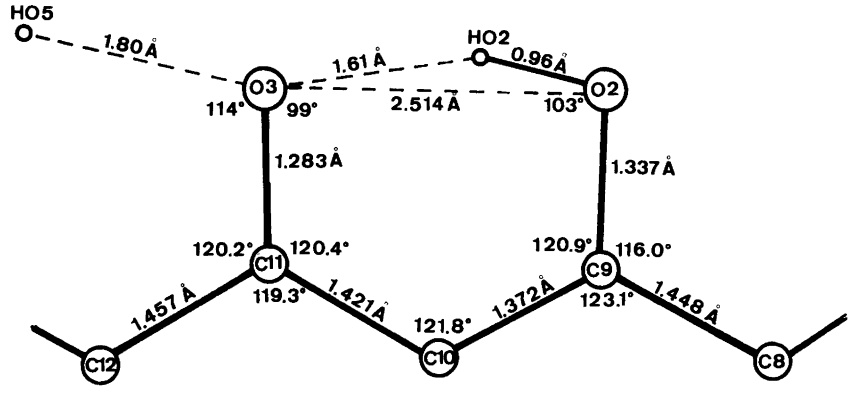

Fig. 4. The geometry of the enol ring and relevant hydrogen bonds.

geometry of the enol-ring and the relevant hydrogen atoms is shown in Fig. 4. The five atoms of the enol-ring are within 0.015 Å from a least squares plane through these atoms, whereas HO2 is not significantly (0.036 Å) out of that plane.

The O2–O3 distance of 2.514 Å is significantly longer than the corresponding distance found in the crystal structure of curcumin.

The overall conformation of the B4HPHDD molecules in the crystal of the methanol complex is also different from that found in the curcumin crystals. The torsion-angles about the C11–C12 and C13–C14 bonds are in the present study found to be –14.4 and –179.7°, respectively, as compared to –163.3 and 25.2° in curcumin. Thus the devition from coplanarity between the three groups connected through the C7–C8 and the C12–C13 bonds is less pronounced in the present crystals than in those of curcumin. The angles between the terminal phenyl groups are found to be 16.2° as compared to 47° in the curcumin crystals. The shortening of the C13–C14 bond which might be expected with a decrease of 25° in the torsion angle about C13–C14 appears also to be present, the C13–C14 bond being 1.455 Å as compared to 1.471 in curcumin and the C4–C7 and C13–C14 bonds are of equal lengths in the present study in contrast to the findings in the curcumin crystals. The C14–C19 ring is planar within the accuracy of the study, however, the C1–C6 ring appears to display a slight deviation from strict planarity as C1 and C4 is situated 0.015 Å above a least squares plane through the six ring atoms, whereas the other ring atoms lie about 0.008 Å beneath that plane. The angle between the planes C1–C2–C3–C4 and C4–C5–C6–C1 is found to be 2.2°.

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The Reaction between Diazoalkanes and Allylic Halides Carrying Electronegative γ-Substituents. 1. Solvent Effects in the Decomposition of 4-(1-Bromo-1-methylethyl)-4,5-dihydro-3*H*-3,3-pyrazoledicarbonitrile

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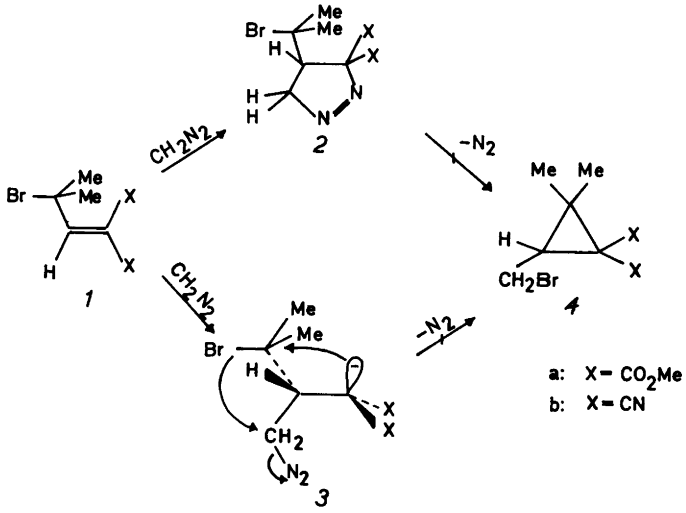
The formation of the title compound and its decomposition in several solvents is described. In nonpolar solvents both a cyclopropane and an olefinic product are formed; in polar solvents, both protic and aprotic, the cyclopropane is the only product observed. Rate of formation of the cyclopropane is very solvent dependent, while the olefin is formed at nearly the same rate in all solvents. The presence of the bromine atom in the side chain has a very great effect on the product distribution and on the rate of cyclopropane formation. Bonding in the proposed transition state is discussed.

Based on our studies on the reaction of nucleophiles with activated allylic halides to produce cyclopropanes,[1–5] further investigations involving attack on the double bond of such halides were initiated. In particular, we were interested in whether diazomethane in its reaction with the allylic bromides *1* yields the Δ^1-pyrazolines *2* and, if formed, how these compounds decompose under given conditions (Scheme 1).



Scheme 1.

0302-4369/83 $2.50

If, as generally accepted,[6] formation of pyrazolines results from a concerted 1,3-dipolar cycloaddition to the double bond of the dipolarophile, the reaction of diazomethane with the bromides *1* should give *2*. On the other hand, if a step-wise mechanism operates, as invoked in a few cases,[7,8] the carbanion being developed in the γ-position might competitively expel the bromide to form cyclopropane *4* (Scheme 1). The dipolar species *3* is conceptually similar to the one proposed in the reported cyclopropane-forming reactions.[1]

The reaction between diazomethane and bromides *1*, however, proceeded without detectable amounts of nitrogen being evolved. While *1a* reacted very slowly,[9] *1b* immediately consumed diazomethane at −15 °C with concurrent precipitation of a white product. Based on spectroscopic evidence, the products were given the Δ^1-pyrazoline structures *2a* and *2b*, respectively. The exclusive formation of pyrazolines strongly supports the simultaneous formation of the new σ-bonds in this type of cycloaddition reactions as both *1a* and *1b* react extremely fast with nucleophiles to give cyclopropanes with the assumed intermittency of carbanions similar to *3*.[1–3]

Thermolyses of Δ^1-pyrazolines analogous to *2* (*i.e.* with two electronegative 3-substituents) usually lead to mixtures of cyclopropanes and olefins.[10–11] The stereochemical outcome of such decompositions has received quite some attention,[10] and we will deal with these problems in forthcoming papers. With symmetrical substitution at the 3- and 5-positions, respectively, this problem is eliminated in our Δ^1-pyrazolines.

The thermal decomposition of *2a* to a Δ^2-pyrazolinium bromide is described earlier.[9]

In contrast to *2a*, the cyano analogue, *2b*, is very unstable. In dry condition it decomposes rapidly after a few minutes even at −35 °C with considerable smoke formation leaving only traces of unidentified materials behind. In solution, however, it decomposes controllably with nitrogen evolution to mixtures of cyclopropane *5* and alkene *6*; the structure of the latter compound easily verified spectroscopically. The assignment of structure *5* (and not the theoretically possible structure *4*) to the decomposition product was based on ^{13}C NMR where the carbon atom carrying the bromide atom was quarternary and on the C–H stretching vibrations in the IR-spectrum (see Experimental).

As seen from Table 1, the product composition shows a strong solvent dependency. In nonpolar aprotic solvents alkene *6* is dominating while in polar solvents, both protic and aprotic, cyclopropane *5* is formed almost exclusively.

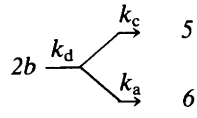
Table 1. Product distribution in the decomposition of Δ^1-pyrazoline *2b*.[a]

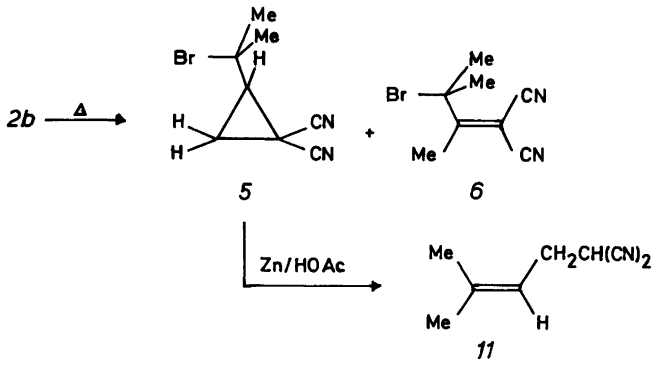
Solvent	*t* (°C)	*5*	*6*
C_5H_{12}	25.0	31	69
C_6H_6	27.3	13	87
	17.6	15	85
Et_2O	25.0	9	91
$CHCl_3$	25.0	20	80
	17.6	22	78
	6.7	25	75
	−5.5	30	70
	−16.2	40	60
CH_2Cl_2	25.0	28	72
$(CH_2Cl)_2$	27.2	31	69
	22.3	32	68
	10.8	40	60
	−10.4	57	43
Bu^iOH	−19.3	100	0
	−37.7	100	0
C_5H_5N	−9.5	100	0
	−40.1	100	0
Pr^iOH	−16.0	100	0
	−38.0	100	0
$(CH_3)_2CO$	25.0	85	15
	−19.3	100	0
	−43.4	100	0
EtOH	−25.0	100	0
	−55.2	100	0
MeOH	25.0	100	0
	−29.0	100	0

[a] Yields in percent.

The decomposition reaction was followed kinetically in several solvents at different temperatures by measuring the evolution of nitrogen. The decomposition of pyrazoline *2b* was strictly first order in all solvents and at all temperatures with correlation coefficients ranging from 0.989 to 1.000 (Table 2).

It thus seems reasonable to assume that the decomposition of *2b* consists of two competitive reactions each of first order in the pyrazoline:





Scheme 2.

Thus, $k_d=k_c+k_a$, where k_d is the rate constant for decomposition of *2b* and k_c and k_a are the rate constants for formation of cyclopropane *5* and alkene *6*, respectively. It can be shown that

$$k_c=k_d\cdot(5)_\infty/[(5)_\infty + (6)_\infty]$$

and

$$k_a=k_d\cdot(6)_\infty/[(5)_\infty + (6)_\infty]$$

Table 2. Experimental kinetic data for the decomposition of *2b* and calculated rate constants for formation of *5* and *6*.[a]

Solvent	t (°C)	$k_d\cdot10^5$	$k_a\cdot10^5$	$k_c\cdot10^5$	r^b
C_6H_6	27.3	553.0	482.0	71.0	1.000
	17.6	176.0	149.0	27.0	0.999
$CHCl_3$	17.6	251.0	196.0	55.0	0.999
	6.7	69.5	52.4	17.1	0.999
	−5.5	16.0	11.2	4.8	0.997
	−16.2	3.6	2.2	1.4	0.999
$(CH_2Cl)_2$	27.2	498.0	333.0	165.0	0.999
	22.3	330.0	223.0	107.0	0.998
	10.8	104.0	62.5	41.2	1.000
	−10.4	10.7	4.5	6.2	0.998
Bu^iOH	−19.3	225.0	0	225.0	0.999
	−29.7	64.4	0	64.4	1.000
	−37.7	14.3	0	14.3	0.999
C_5H_5N	−9.5	275.0	0	275.0	1.000
	−18.6	159.0	0	159.0	0.998
	−40.1	44.0	0	44.0	1.000
Pr^iOH	−16.0	680.0	0	680.0	0.988
	−29.0	112.0	0	112.0	0.989
	−38.0	28.0	0	28.0	0.998
$(CH_3)_2CO$	−19.3	324.0	0	324.0	0.992
	−28.3	169.0	0	169.0	0.997
	−43.3	51.2	0	51.2	0.996
EtOH	−25.0	815.0	0	815.0	0.990
	−29.0	405.0	0	405.0	0.999
	−49.1	55.0	0	55.0	0.995
	−55.2	11.0	0	11.0	0.995

[a] Unit for rate constants: sec^{-1}. [b] Correlation coefficients for first order plot.

Table 3. Activation parameters for the decomposition of Δ^1-pyrazoline *2b* at 244.2 K.[a]

Solvent	C_6H_6	$CHCl_3$	$(CH_2Cl)_2$	Bu^{t}OH	C_5H_5N	Pr^{i}OH	$(CH_3)_2C{=}O$	EtOH
$E_T(30)$[b]	144.3	163.5	175.2	204.9	168.1	203.2	176.5	217.0
Cyclopropane (*5*) formation								
$k_c \cdot 10^6$	0.86	2.6	5.0	589	882	1120	1620	4810
$\Delta G^{\neq}$	87.8±0.8	86.1±0.8	83.6±0.8	74.9±0.8	74.0±0.8	73.6±0.8	72.8±0.8	70.3±0.8
$\Delta H^{\neq}$	70.7±7.5	65.7±1.7	55.6±1.7	72.8±1.7	28.9±0.8	71.1±1.3	35.5±1.3	56.9±0.8
$\Delta S^{\neq}$	−70.6±26.3	−83.2±7.5	−114.2±7.1	−8.8±7.1	−185.3±4.6	−9.6±5.9	−152.2±5.4	−55.6±4.2
r[c]	– [d]	1.000	1.000	0.997	1.000	1.000	1.000	0.987
Alkene (*6*) formation								
$k_a \cdot 10^6$	1.4	2.9	3.1	(6.5)	(2.3)	(6.5)	(2.9)	(9.9)
$\Delta G^{\neq}$	86.9±0.8	85.7±0.8	85.3±0.8	(83.6)	(85.7)	(83.6)	(85.3)	(82.8)
$\Delta H^{\neq}$	86.1±7.5	80.7±1.7	74.4±1.7					
$\Delta S^{\neq}$	−3.3±5.4	−20.1±7.5	−44.7±7.1					
r[c]	– [d]	1.000	1.000					

[a] Units are kJ mol^{-1} for $\Delta G^{\neq}$ and $\Delta H^{\neq}$, J mol^{-1}K^{-1} for $\Delta S^{\neq}$. Extrapolated values in brackets. Limit of errors, see Experimental. [b] Solvent polarity parameters (kJ mol^{-1}) from Ref. 13. [c] Correlation coefficients for Arrhenius plots. [d] Only two temperatures.

where $(5)_\infty$ and $(6)_\infty$ represent the final concentrations of *5* and *6*.[12] Thus, by measuring the decomposition rate of *2b* and establishing the final product composition the individual rate constants k_a and k_c can be calculated.*

Table 2 gives the measured rates of decomposition of *2d* and the calculated rates of formation of *5* and *6*. Table 3 gives the relative rates of formation of *5* and *6* and the activation parameters as obtained from the Arrhenius-plots and the Gibbs-Helmholtz equation.

A striking feature from Table 3 is the very strong rate acceleration in the cyclopropane formation with increasing solvent polarity, an observation pointing to a transition state more polar than the ground state.

Several empirical parameters have from time to time been used to relate reaction rates or equilibria positions to solvent polarity.[13] The list of $E_T(30)$-values, obtained from the solvent-dependent transition-energies in the absorption spectra of pyridinum-N-phenoxide betaines, is probably the most comprehensive solvent scale to date and exhibits good linear correlation with kinetic data.[14] Fig. 1 relates the free activation energies for cyclopropane formation to $E_T(30)$. Except for the solvents pyridine and acetone a fairly good linear relationship is obtained (correlation coefficient 0.980). The 10^2-fold rate enhancement in pyridine and acetone (as calculated from the $\Delta G^{\neq}$ *vs.* $E_T(30)$ plot) is also reflected in the extremely low activation enthalpies (Table 3). These low values and the large negative activation entropies indicate a highly solvated transition state. It has been proposed that the decomposition of 1-pyrazolines with electronegative 3-substituents starts with heterolytic cleavage of the N2—C3 bond to form a zwitterion.[10] The carbanionic part is internally stabilized by the 3-substituents. This is demonstrated by a reduction in $\Delta G^{\neq}$ of 25–35 kJ/mol in the decomposition of the nonbrominated analogues of *2* when the two methoxycarbonyl groups at C3 were replaced with cyano groups.[10] In this connection it is interesting to note that while pK_A is two units lower for malononitrile than for dimethyl malonate (thermodynamic acidity), the rate of ionization (kinetic acidity) is

* Cyclopropane *5* decomposes in the gas chromatograph and therefore the product composition was estimated by ^{1}H NMR (see Experimental).

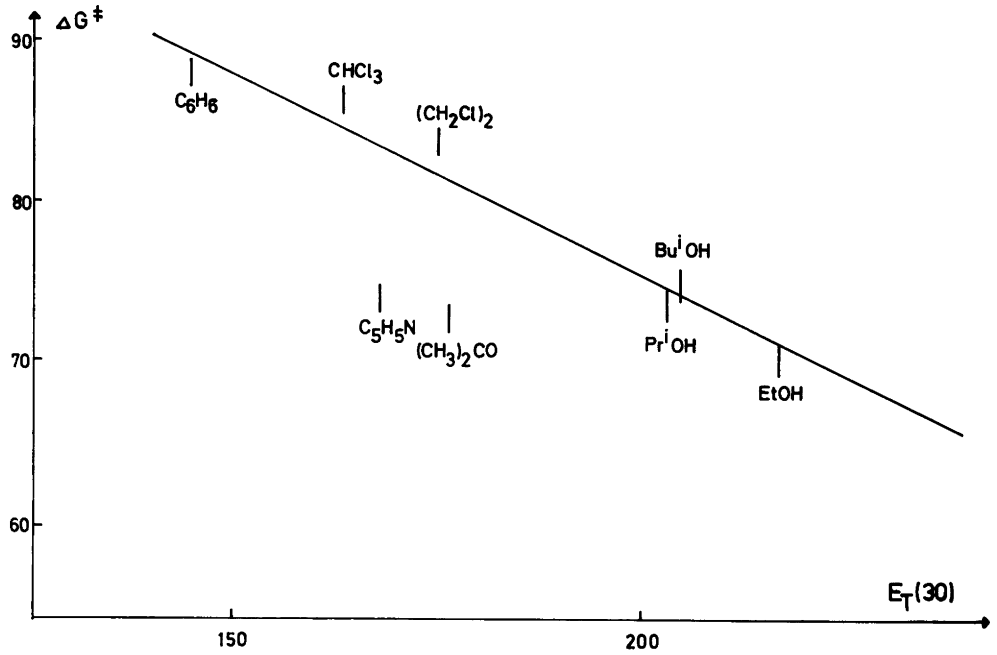


Fig. 1. Plot of the free activation energy *vs.* the solvent polarity parameter $E_T(30)$. Units kJ mol^{-1}. Least square line $\Delta G^{\neq} = -3.76\ E_T(30) + 482.9$ (r=0.980).

about 6×10^2 higher for the former compound.[15] The effect of changing the 3-substituents in our studies is a complete change in product formation. While *2b* undergoes ring cleavage, *2a* forms quantitatively a 2-pyrazoline as demonstrated in an earlier paper.[9]

The electron pair donation capacity (EPD) of pyridine as measured by the donation number (DN) * is higher than for ethanol, *i.e.* pyridine is a very good cation solvator. Acetone is also a good cation solvator, being an n-donor[17] or a coordinating solvent.[18]

A second feature from Table 3 is the relatively high activation enthalpies coupled with small entropy changes in the protic solvents. In contrast to the situation in aprotic solvents it looks as if solvation of the transition state (causing lowering of the activation entropy) is less important, obviously an erroneous conclusion. The reason for this observation must be sought in the structure of protic solvent where the molecules are held together by hydrogen bonds. By introducing charged particles (read: the charged transition state) into the solvent, energy is required to break these bonds (increasing $\Delta H^{\neq}$-term) and more degrees of freedom given to the solvent molecules (increasing $\Delta S^{\neq}$-term). As pointed out earlier, the negative part of the zwitterion from *2b* is internally stabilized, thus the unique capacity of protic solvents to solvate anions through hydrogen bondings is less demanded. The electron pair donation capacity of ethanol as measured by its donation number (*vide supra*) is lower than for pyridine making it an average cation solvator.[16] To conclude, the macroscopically measured activation enthalpies and entropies must both be higher in protic solvents since rupture of the solvent structure is involved.

In many reactions $\Delta H^{\neq}$ is linearly related to $\Delta S^{\neq}$.[19] Fig 2 represents such a plot where the line for the protic solvents (correlation factor 0.997) is almost parallel and lower than for the aprotic solvents (correlation factor 0.992). A similar "double line" plot is also found for the decomposition of triethylsulfonium bromide,[19,20] where the line for the protic solvent is higher than for the aprotic solvents. This is certainly expected

* Defined as the negative ΔH values for 1:1 adduct formation between antimon pentachloride and electron pair donor solvents.[16]

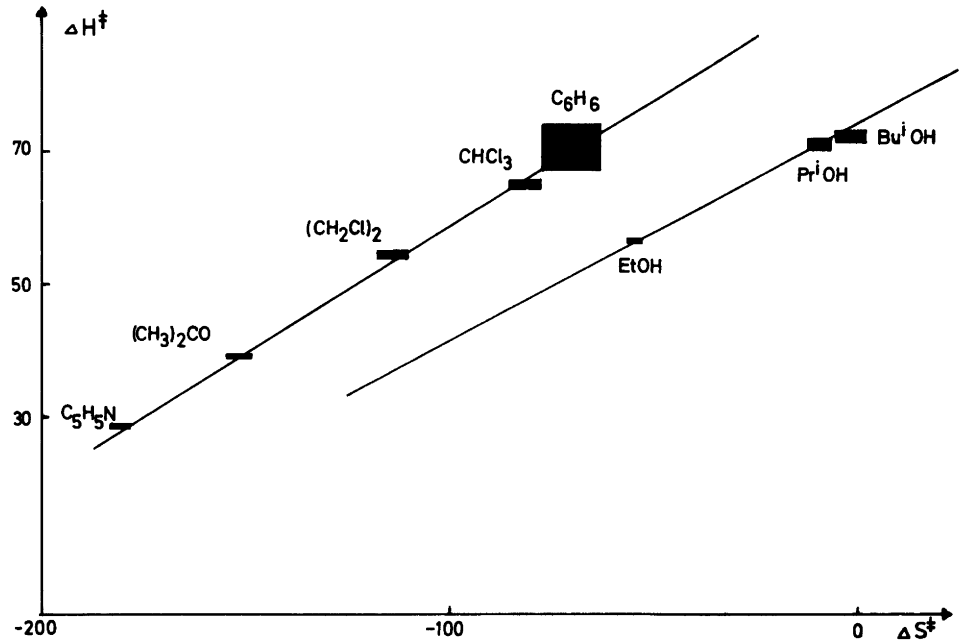
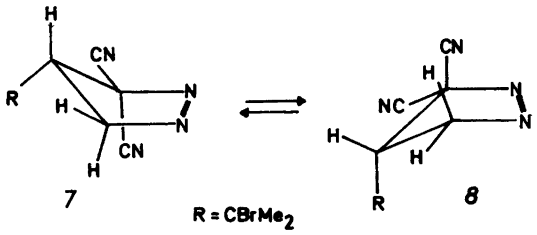


Fig. 2. Plot of activation enthalpy *vs.* activation entropy. Units for enthalpy kJ mol^{-1}, entropy J mol^{-1} K^{-1}. The black rectangles represent the maximum error along both axes. Least square lines:
Upper: $\Delta H^{\ddagger}=0.382\Delta S^{\ddagger}+97.5$ ($r=0.992$).
Lower: $\Delta H^{\ddagger}=0.324\Delta S^{\ddagger}+74.9$ ($r=0.997$).

since charge is dispersed in the transition state as reflected in the rate decrease with increasing polarity of the solvents. Fully aware of the danger in drawing physical conclusions based on such interrelationship of thermodynamic parameters,[19,21] it is nevertheless interesting to note that the difference in the intercept for these lines is 16–25 kJ/mol in the enthalpies which is of same order of magnitude as the strength of the hydrogen bond in alcohols (ethanol: 26 kJ/mol[22]).

Alkene formation is not observed, neither in the protic solvents nor in the polar aprotic solvents (pyridine, acetone) at low temperature (Table 1). Based on a linearity of the free activation energy *vs.* the $E_T(30)$ values for benzene, chloroform and 1,2-dichloroethane (correlation factor 0.990), extrapolated values for $\Delta G^{\ddagger}$ (and k_a) are given in brackets in Table 3 for the polar aprotic and protic solvents. These calculated $\Delta G^{\ddagger}$-values seem to be of the right order of magnitude, *i.e.* 8–12 kJ/mol higher than the $\Delta G^{\ddagger}$-values for cyclopropane formation (corresponding to 10^2–10^3 difference in the rate constants) as no alkenes were observed in these solvents. The relatively low solvent dependence in the alkene formation thus observed indicates a transition state having a comparatively small charge separation.

The effect of having a bromine atom in the side chain in the 4-position of *2b* is rather dramatic. The rate of decomposition is about 50-fold higher than that of the nonbrominated analogue,[10] and the product ratio (alkene–cyclopropane) is changed from 84:16 to 0:100 at 244.2 °K (Table 4). This accelerated decomposition rate is reflected in a 325-fold rate *increase* in cyclopropane formation and an 8-fold rate *decrease* in alkene formation. The latter decrease is probably caused by steric compression in the transition state. It is established by X-ray crystallography that Δ^1-pyrazolines exhibit C_s-symmetry in the solid phase,[23] with C4 at the flip of the envelope, the plane through C3–C4–C5 forming angles of 20–30° to the plane through C3–N2–N1–C5. In solutions conformational equilibrium between *7* and *8* is suggested (Scheme 3).[24]

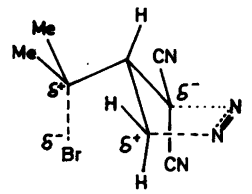


Scheme 3.

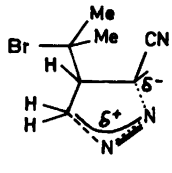
Analogous to cyclohexane systems, large groups in five-membered rings with C_s-symmetry will tend to prefer pseudoequatorial positions, thus it is highly likely that for *2b* the equilibrium *7*⇌*8* is more displaced to left than for the non-brominated analogue. For stereoelectronic reasons, alkene *6* most likely is formed from conformer *8* where overlap between the σ-electrons of the C–H bond at C4 and the back-lobe of the C5–N1 sp^3-orbital can take place.

The large increase ($\Delta\Delta G^{\neq}$~12 kJ mol^{-1}) in the rate of cyclopropane formation for *2b* as compared to its non-brominated analogue is more difficult to explain. As demonstrated above, the transition state for cyclopropane formation is highly polarized, hence any polarizable element in the molecule will tend to lower the free activation energy. A closer look at models of conformer *7* discloses that the bromine atom in *2b* may take positions close to C5.* In the transition state for cyclopropane formation some positive charge is built up at C5, as will be demonstrated in a forthcoming paper.[25] Thus dipole–dipole interactive stabilization of the transition state as indicated in *9* may facilitate cyclopropane formation from *2b* as compared to its nonbrominated analogue.

As mentioned above, the large solvent effects observed for cyclopropane formation point to a polarized transition state. In accordance with the fact that the stereochemistry is retained in the cyclopropane formation,[23c] it is assumed that the N2–C3 bond is not completely broken before bonding between C3 and C5 has started, and a transition state like *10* is proposed.



9



10

It may be argued that there is a possibility that the N2–C3 bond may be completely broken and that rotation around the C4–C5 bond is slower than C3–C5 bond formation and thus explaining the retained stereochemistry. Against this argument stands the very low rotation barrier in C–C single bonds with low substitution on the carbon atoms involved. The C5–N1 bond is probably only partially broken in the transition state. This is indicated by the low reaction constant found for 5-aryl substituted 1-pyrazolines.[25]

Table 4. Comparison of rate constants and activation parameters for the decomposition of *2b* and its nonbrominated analogue [a] in ethanol at 244.2 K.[b].

	2b	Non-brominated *2b*
Cyclopropane formation		
$k_c \cdot 10^6$	4810	14.8
$\Delta G^{\neq}$	70.3±0.8	82.0±0.4
$\Delta H^{\neq}$	56.9±0.8	74.0±0.8
$\Delta S^{\neq}$	−55.6±4.2	−32.2±4.2
r^c	0.987	1.000
Alkene formation		
$k_c \cdot 10^6$	(9.9)	77.3
$\Delta G^{\neq}$	(82.8)	78.2±0.4

[a] Calculated from the kinetic data in Ref. 10. [b] Units are kJ mol^{-1} for $\Delta G^{\neq}$ and $\Delta H^{\neq}$, J mol^{-1}K^{-1} for $\Delta S^{\neq}$. Extrapolation data in parentheses. Limit of error, see Experimental. [c] Correlation coefficients for Arrhenius plot.

EXPERIMENTAL

General. Melting points (uncorrected) were determined on a micro hot-stage. IR spectra were recorded on a Perkin-Elmer 457 Grating Infrared spectrophotometer, ^{1}H NMR spectra on a Varian HA 100-15D spectrometer operating at 98 MHz, ^{13}C NMR spectra on a JEOL FX-60 FT NMR

* In fact, in the crystal phase of the 5-phenyl substituted analogue of *2b*, the distance between C5 and the bromide atom is somewhat lower than the sum of their van der Vaal's radii.[23c]

spectrometer and mass spectra on an AEI MS 902 instrument. Elemental analyses were performed by I. Beetz, West Germany.

4-(1-Bromo-1-methylethyl)-4,5-dihydro-3H-3,3-pyrazoldicarbonitrile (2b). To a solution of 2-bromo-2-methylpropylidenemalononitrile (10 mmol) in diethyl ether (30 ml) was added diazomethane (11 mmol) in diethyl ether (25 ml) at −15 °C. During the addition of diazomethane *2b* precipitated as colourless needles. Decomposes rapidly in dry condition at or well below room temperature. ^{1}H NMR (acetone-d_6 at −70 °C): δ 1.99 (1H,s), 2.11 (3H,s), ABX-system: υ_A 5.71, υ_B 5.37, υ_X 3.62, J_{AX}=8.0 Hz, J_{BX}=9.0 Hz, J_{AB}=19.0 Hz.

2-(1-Bromo-1-methylethyl)-1,1-cyclopropanedicarbonitrile (5). Freshly made *2b* was decomposed in methanol at 25 °C to yield *5* as the sole product. M.P. 69–70 °C (ether–pentane). Anal. $C_8H_9N_2Br$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.93 (3H,s), 1.96 (3H,s), A_2B-system: υ_A 2.08, υ_B 2.43, J_{AB}=9.2 Hz. ^{13}C NMR ($CDCl_3$): δ 4.3 (C1), 23.2 (C3), 32.3+32.7 (2×Me), 42.6 (C2), 57.9 (*C*-Br), 112.9+115.0 (2×C=N). IR (KBr) 3090 (m), 3010 (m) and 2980 (m) cm^{-1}.

2-Bromo-1,2-dimethylpropylidenemalononitrile (6). Freshly made *2b* was decomposed in diethyl ether at 25 °C to give an oil consisting of 9 % *5* and 91 % *6*. Upon distillation *6* was obtained in pure state. B.p. 81–83 °C/0.07 mmHg. ^{1}H NMR ($CDCl_3$): δ 2.17 (6H,s), 2.50 (3H,s).

4-Methyl-3-pentene-1,1-dicarbonitrile (11). Cyclopropane (*5*) (13 mmol) was dissolved in acetic acid (25 ml) containing a few drops of water. Zn (2 g) was added and the mixture stirred overnight at room temperature. After dilution with water, extraction with pentane gave *11* (63 %) B.p. 43–44 °C/0.05 mm Hg. Anal. $C_8H_{10}N_2$: C, H. ^{1}H NMR (CCl_4-double resonance expts): δ 1.75 (3H,d, J=1.0 Hz), 1.84 (3H,d, J=0.9 Hz), 2.68 (2H,2d, J=6.9 Hz and J=7.4 Hz), 3.64 (1H,t, J=6.9 Hz), 5.20 (1H, triplet (J=7.4 Hz) of multiplets (J=1.0 Hz)).

Decomposition of 2b. Immediately after its preparation, samples (0.5 g) of *2b* were dissolved in different solvents (50 ml) and stirred at room temperature (25.0 °C) until nitrogen evolution had ceased. After an additional hour, solvents were evaporated and the residue subjected to NMR-analyses (Table 1).

Kinetic measurements. Solvents (250 ml) were temperature equilibrated and saturated with highly purified nitrogen before samples of *2b* (0.5–1.0 g) were added. Evolved nitrogen was measured with a gas burette. Rate constants were obtained from the slope of the plot of log (v_∞–v) against time (s^{-1}). Least square treatment of the parameters gave correlation coefficients ranging from 0.989–1.000 (Table 2). Measurements were carried out for three to four halflives (*i.e.* 85–90 % completion) and the solution left at ambient temperatures for seven halflives (>97 % reaction). Solvents were evaporated and the residue subjected to ^{1}H NMR analysis to determine the ratio *5*:*6* using the integrals of the *gem.* dimethyl group as measures. Five integral traces were run for each experiment and the average values (stand. dev. <1 %) used in the calculation of rate constant for alkene (k_a) and cyclopropane (k_c) formation (Table 2). The error limits for the activation parameters entered in Table 3 are the *maximum* calculated errors [27] based on estimated error in the rate constant of 5 % whenever the rate constant k_a and k_c had to be calculated (*i.e.* when both *5* and *6* were formed) and 3 % when only cyclopropane was formed.

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The Reaction between Diazoalkanes and Allylic Halides Carrying Electronegative γ-Substituents. 2. Formation and Decomposition of Dimethyl 4-(1-Bromo-1-methylethyl)-5-aryl-4,5-dihydro-3*H*-3,3-pyrazoledicarboxylates

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The title compounds were synthesized by the reaction of the proper *para*-substituted phenyldiazomethanes and dimethyl 2-bromo-2-methylpropylidene-malonates. *p*-Cyano- and *p*-nitrophenyldiazomethane did not give 1-pyrazolines, but decomposed at the reaction conditions. Upon thermolyses in various solvents only cyclopropanes were obtained. Only small solvent effects were observed. The kinetic data gave good linear correlation with σ^+-data, with a reaction constant, $\rho = -1.00$.

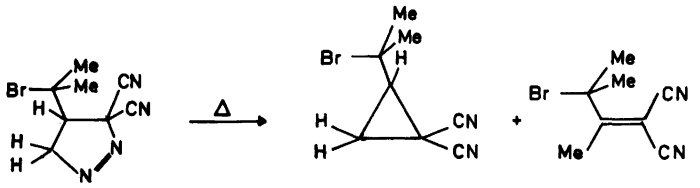
In the preceding paper of this series the decomposition of 4-(1-bromo-1-methylethyl)-4,5-dihydro-3*H*-3,3-pyrazoledicarbonitrile (*1*) was found to give cyclopropane *2* and alkene *3* (Scheme 1).[1] Formation of the former compound was shown to be strongly solvent dependent.

The transition state leading to the cyclopropane was suggested to have a considerable dipolar character with the positive end near C5. A well-known probe of charge build-up or destroying is the introduction of *p*- or *m*-substituted phenyl groups at such centers and to study rate influences exerted by these substituents, *i.e.* to test if a Hammett-type linear free energy relationship exists.[2]

5-Arylsubstituted 1-pyrazolines *6* were synthesized by reactions of the properly substituted diazomethanes (*4*) with the activated olefins *5* (Scheme 2). Unfortunately, 4-cyano- and 4-nitrophenyldiazomethanes did not react at all; after five days the diazo compounds had decomposed, leaving the olefin unreacted.

Only one of the possible stereoisomers of pyrazolines *6* was observed and the structure of *6b* was firmly established by X-ray crystallography to have *trans* configuration at the C4–C5 bond.[3] ^{1}H NMR spectra indicated analogous structures for *6a* and *6c-h*.

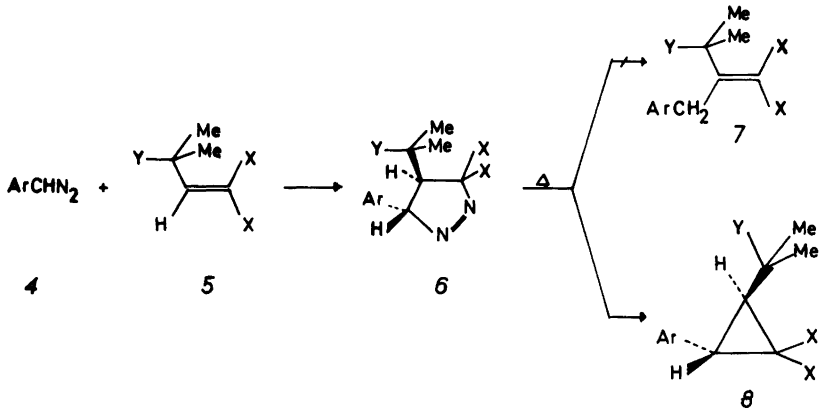
The introduction of 5-aryl substituents in the 1-pyrazolines had a great influence on their



1 2 3

Scheme 1.

0302-4369/83 $2.50



4 5 6

Scheme 2.

5a Y=Br, X=CN
5b Y=Br, X=CO_2Me
5c Y=Cl, X=CO_2Me
5d Y=H, X=CO_2Me

6a Ar=Ph, Y=Br, X=CN
6b Ar=Ph, Y=Br, X=CO_2Me
6c Ar=4-MeO–Ph, Y=Br, X=CO_2Me
6d Ar=4-Me–Ph, Y=Br, X=CO_2Me
6e Ar=4-Cl–Ph, Y=Br, X=CO_2Me
6f Ar=4-Br–Ph, Y=Br, X=CO_2Me
6g Ar=Ph, Y=Cl, X=CO_2Me
6h Ar=Ph, Y=H, X=CO_2Me

Table 1. Kinetic data for the decomposition of pyrazolines *6*.[a].

Compound	Solvent	*t* (°C)	10^4k	*r*[b]
6b	Toluene	43.0	1.5±0.1	0.991–0.997
		57.0	8.0±0.1	0.999
		72.0	54.0±3.0	0.997–0.999
	Chlorobenzene	43.0	1.2±0.1	0.990–0.999
		57.0	8.4±0.2	0.996–0.999
		72.0	53.0±2.0	0.998–0.999
	Cyclohexanone	43.0	0.9±0.1	0.991–0.997
		57.0	5.1±0.1	0.992–0.995
		72.0	31.0±2.0	0.995–0.998
	Butanol	43.0	2.0±0.1	0.998–0.999
		57.0	12.9±0.9	0.996–0.997
		72.0	84.0±4.0	0.987–0.998
6c	Toluene	31.0	2.9±0.1	0.993–0.999
		57.0	57.0±5.0	0.994–0.996
6d	Toluene	46.0	5.4±0.3	0.999
		57.0	21.5±0.1	0.999
6e	Toluene	57.0	7.1±0.4	0.998–0.999
		70.0	32.0±1.0	0.995–0.999
	Butanol	72.0	98.0±5.0	0.994–0.996
6f	Toluene	57.0	5.9±0.6	0.995–0.999
6g	Toluene	57.0	6.5±0.1	0.993–0.995

[a] Units for rate constants: s^{-1}. [b] Correlation coefficients span for first order plot; single entry means that the parallel runs had same *r*.

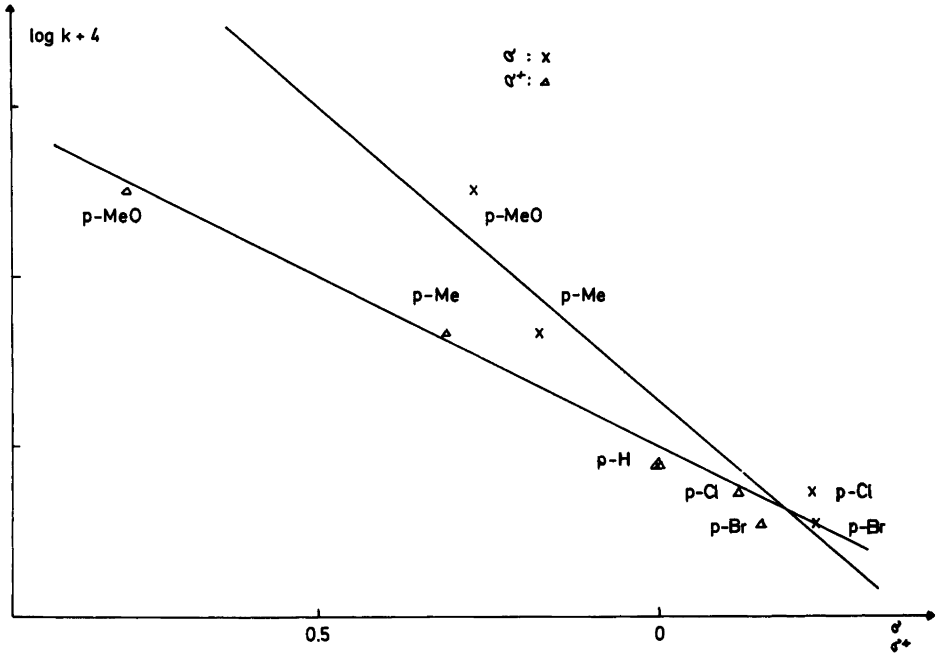


Fig. 1. Correlation of log k with σ and σ^+. Least square lines:
σ: log k= $-1.68\sigma-2.86$ (r=0.934)
σ^+: log k= $-1.00\sigma^+-3.02$ (r=0.998).

stability. Thus any attempt to isolate *6a* was in vain, it decomposes immediately to cyclopropane *8a*. However, at –70 °C it was possible to obtain its ^{1}H NMR spectrum.[4] Therefore we had to concentrate our decomposition studies on compounds with ester groups at C3 (*6b–h*).

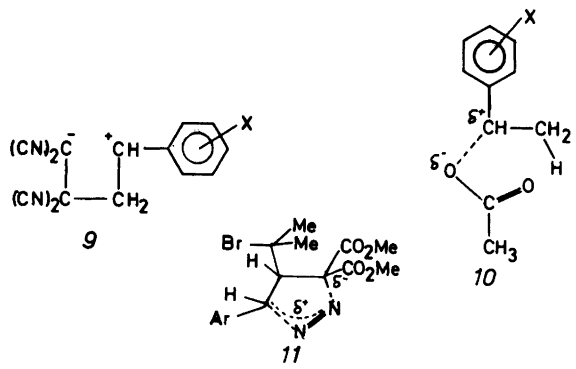
Decomposition of *6b-f* in various solvents at different temperatures gave only cyclopropane *8*, while *6g* gave small amounts of some unidentified material in addition to *8g*. *6h* gave a mixture consisting of 45 % *8h* and 55 % of an olefinic arising from isopropyl group migration from C4 to C5 during decomposition.

The decomposition reaction was followed kinetically in four solvents of different polarities, with $E_T(30)$ ranging from 141.8 to 210.0 kJ mol^{-1}.[5] The kinetics were found to be strictly first order with correlation coefficients ranging from 0.987 to 0.999 (Table 1).

In contrast to the decomposition of *1*,[1] the observed solvent dependence of the decomposition rate of *6b* was very small. Thus, while the decomposition rate of *1* increased by a factor of 1300 when going from benzene to isopropylalcohol ($\Delta E_T(30)$~60 kJ mol^{-1}), the decomposition rate for *6b* increased only by a factor of 2.5 from toluene to butanol ($\Delta E_T(30)$~70 kJ mol^{-1}).

The effect of changing the substituents in *para* position of the phenyl group is larger than the solvent effect. Thus the rate of decomposition is about 10-fold higher for *p*-MeO than for *p*-Br. With the limited number of substituents available, Fig. 1 seems to indicate that the correlation with σ^+ is better than with σ.

The correlation with σ^+ together with the negative reaction constant supports the earlier assumption that some positive charge is developing at C5 in the transition state. The rather low value of the reaction constant (ρ= –1.00) indicates that the C5–N1 bond is not completely broken. In comparison, in the S_N1 hydrolyses of substituted cumyl chlorides in 90 % aqueous acetone the reaction constant was estimated to –4.54,[6] and in the reaction of substituted styrenes with tetracyanoethylene a reaction constant of –7.1 was found, indicating a zwitterionic transi-



Scheme 3.

tion state, *9*,[7]

On the other hand, in the thermolysis of substituted 1-methylbenzylacetates a ρ-value of –0.66 was found, indicating a transition state with smaller charge separation, like *10*.[8] The reaction constant observed by us thus indicates a transition state with charge separation as shown in *11*.

As mentioned above, the introduction of a phenyl group in 5-position had great influence on the reaction rate. It changes the decomposition route too, as the 1-pyrazolinediester without 5-substituents rearranges to the corresponding 2-pyrazoline on heating.[9]

The effect of having a bromine atom in the side chain is reflected in a higher yield of cyclopropanes compared to alkenes (Table 2), which also was observed in the dicyanopyrazolines.[1,10]

The alkene formed in the decomposition of *6h* is a result of isopropyl group migration from C4 to C5. This is expected if the conformer with the migrating group pseudoequatorial is the more stable one.[11] We have found (*vide supra*) that in *6b* the phenyl and the bromoisopropyl groups take pseudoequatorial positions in the crystal phase.[3] Further, our studies on the conformations of 4,5-*trans*-disubstituted 1-pyrazolines have shown that an *increase* of the size of the 4- *and* 5-substituents tends to make the conformers with these substituents pseudo-*axial* more stable.[4] Thus, by *reducing* the size of the 4-substituent (bromoisopropyl→isopropyl) one would expect it to prefer a pseudoequatorial position, capable of migrating to the 5-position.

Contrary to the dicyanopyrazolines,[1,10] the rate of decomposition of the aryl-substituted diesterpyrazolines is much faster (approx. 12 times) *without* bromine in the side chain. Dissecting this rate into rates of formation of cyclopro-

Table 2. Rates and product distribution in the decomposition of pyrazolines *6b* and *6h* at 330.2 K.

	6b	*6h*
Rate of decomposition	$8.8.\times10^{-4}$	108.0×10^{-4}
Cyclopropane		
Yield (%)	100	45
Rate of formation	8.8×10^{-4}	50×10^{-4}
Alkene		
Yield (%)	0	55
Rate of formation	$<8.8\times10^{-6}$ [a]	58×10^{-4}

[a] Max. value as no alkene was observed.

pane and alkene, respectively, as shown before,[1] one finds that cyclopropane formation is approximately 6 times faster from the nonbrominated compound while alkene is formed more than 60 times faster from the same 1-pyrazoline (Table 2). The latter result is probably best explained by an assumed lower migratory aptitude of the bromoisopropyl group.

In the dicyanopyrazolines cyclopropane formation was approx. 325 times faster for the side-chain brominated 1-pyrazolines.[1,10] This was mainly attributed to the polarization of the C–Br bond thereby stabilizing the partly positive charge at C5 in the transition state. Such stabilizing effects from the C–Br dipole is less important in the present 1-pyrazolines since the aryl group at C5 acts as an "electron reservoir".

Likewise, the observed lack of solvent effect (*vide supra*) may be explained by intramolecular stabilization of the partly positive charge at C5 exerted by the aryl group.

EXPERIMENTAL

General. Melting points (uncorrected) were determined on a micro hot-stage. IR spectra were recorded on a JASCO IRA-1 spectrophotometer, ^{1}H NMR spectra on a Varian HA 100-15D spectrometer operating at 98 MHz, ^{13}C NMR spectra on a JEOL FX60 FT spectrometer and mass spectra on an AEI MS 902 instrument. Elemental analyses were performed by Ilse Beetz, West Germany.

Materials. The following substituted diazomethanes were synthesized according to standard literature procedures: Phenyl-,[11] 4-methoxyphenyl-,[12] 4-methylphenyl-,[11] 4-chloro- and 4-bromophenyl-,[11] 4-cyanophenyl- and 4-nitrophenyl-.[13]

*Reaction between 2-bromo-2-methylpropylidenemalononitrile (*5a*) and phenyldiazomethane (*4a*). Synthesis of 2-(1-bromo-1-methylethyl)-3-phenyl-1,1-cyclopropanedicarbonitrile (*8a*).* To *5a* (0.5 g, 2.5 mmol) in ether (10 ml) was added the red-colored solution *4a* (0.3 g, 2.5 mmol) in pentane (20 ml). Rapid decoloration with gas evolution took place within seconds and after cooling to –20 °C colorless crystals of *8a* appeared. Yield 78 %. M.p. 58–59 °C (ether–pentane). Found: C 58.6, H 4.8, N 9.8. Calc. for $C_{14}H_{13}BrN_2$: C 58.1, H 4.5, N 9.8. ^{1}H NMR (CCl_4): δ 7.37 (5H,s), 3.42 and 2.82 (2H, 2d. AB-system, J=9.0 Hz), 2.05 (3H,s), 1.95 (3H,s). IR(KBr): 2240 (m), 1380 and 1400 (s) cm^{-1}. MS: m/e 287–289 (M^+), 209 (M–Br). When the reaction was carried out at –78 °C, ^{1}H NMR signals were obtained indicating the intermediacy of *4-(1-bromo-1-methylethyl)-5-phenyl-4,5-dihydro-3H-3,3-pyrazoledicarbonitrile* (6a).[14]

*Syntheses of dimethyl 4-(1-bromo-1-methylethyl)-5-aryl-4,5-dihydro-3*H*-3,3-pyrazoledicarboxylate (*6b–h*).* The syntheses of *6b* and *6e* are described before,[9] and *6c, 6d* and *6f* were prepared analogously (reaction temperature, time and yield given in brackets): *5-(4-Methoxyphenyl), (6c)* (–20 °C, 18 d, 52 %): Dec. at room temperature. Purified by dissolving in chloroform below –10 °C and quickly cooling to –78 °C. ^{1}H NMR (acetone-d_6 –40 °C): δ 7.3–7.1 (4H, AA'XX'-quart.) 5.70 (1H,d, J=10.0 Hz), 4.00 (3H,s), 3.85 (3H,s), 3.82 (3H,s), 3.02 (1H,d, J=10.0 Hz), 1.94 (3H, s), 1.51 (3H,s). *5-(4-Methylphenyl),(6d)* (–20 °C, 14 d, 61 %): Dec. at about 50 °C. Recryst. from dichloromethane–pentane. ^{1}H NMR($CDCl_3$): δ 7.18 (4H,s), 5.75 (1H,d, J=10.0 Hz), 4.01 (3H, s), 3.83 (3H,s), 3.10 (1H,d, J=10.0 Hz), 2.38 (3H,s), 1.94 (3H,s), 1.49 (3H,s). *5-(4-Bromophenyl), (6f)* (–20 °C, 18 d, 71 %): M.p. 98–99 °C (dichloromethane–pentane). Anal. $C_{16}H_{18}Br_2N_2O_4$: C,H. ^{1}H NMR($CDCl_3$): δ 7.5–7.2 (4H, AA'XX'-quart.), 5.75 (1H,d, J=10.0 Hz), 4.00 (3H,s), 3.83 (3H,s), 3.07 (1H,d, J=10.0 Hz), 1.93 (3H,s), 1.50 (3H,s). IR(KBr); 1740 (s), 1540 (m) cm^{-1}.

*Dimethyl 4-(1-chloro-1-methylethyl)-5-phenyl-4,5-dihydro-3*H*-3,3-pyrazoledicarboxylate (*6g*).* Prepared analogously to *6b* from *5c*.[9] Yield 62 %, m.p. 109–110 °C (chloroform–pentane) Anal. $C_{16}H_{19}ClN_2O_4$: C,H,N. ^{1}H NMR($CDCl_3$): δ 7.6–7.3 (5H,m), 5.88 (1H,d, J=10.0 Hz), 4.13 (3H,s), 3.92 (3H,s), 3.44 (1H,d,J=10.0 Hz), 1.90 (3H,s), 1.42 (3H,s). IR (KBr): 1745 (s), 1550 (m) cm^{-1}.

*Dimethyl 4-isopropyl-5-phenyl-4,5-dihydro-3*H*-3,3-pyrazoledicarboxylate (*6h*).* Prepared analogously to *6b* from *5d*.[9] Yield 55 %. ^{1}H NMR($CDCl_3$, –60 °C): δ 7.6–7.1 (5H,m),5.39 (1H,d, J=10.0 Hz), 4.06 (3H,s), 2.76 (1H, 2d, both with J=10.0 Hz), 1.9–1.4 (1H,m), 0.99 (3H,d, J=6.0 Hz), 0.79 (3H,d, J=6.0 Hz).

Decomposition of pyrazolines 6b–h. The decomposition of *6b* and *6e* to give *8b* and *8e*, respectively, is described elsewhere.[9] Following the same procedure *6c, 6d* and *6f* were decomposed. Thus *6c* gave *dimethyl 2-(1-bromo-1-methylethyl)-3-(4-methoxyphenyl)-1,1-cyclopropanedicarboxylate* (8c): Yield 90 %. M.p. 50–52°C (chloroform–pentane). Anal. $C_{17}H_{21}BrO_5$: C,H. ^{1}H NMR($CDCl_3$): δ 7.3–7.1 (4H, AA'XX'-quart.), 3.62 (3H,s), 3.58 (3H,s), 3.43 (3H,s), 3.43 and 3.00 (2H, AB-quart. J=9.0 Hz), 1.97

(3H,s), 1.80 (3H,s). The decomposition products from *6d*, *6f* and *6g* were identified by their ^{1}H NMR spectra to be *dimethyl 2-(1-bromo-1-methylethyl)-3-(4-methylphenyl)-1,1-cyclopropanedicarboxylate* (8d): ^{1}H NMR ($CDCl_3$): δ 7.3–7.1 (4H, AA'XX'-quart.), 3.72 (3H,s), 3.37 (3H,s), 3.44 and 3.04 (2H, AB-quart, J=9.0 Hz), 2.25 (3H,s), 1.92 (3H,s), 1.77 (3H,s): *dimethyl 2-(1-bromo-1-methylethyl)-3-(4-bromophenyl)-1,1-cyclopropanedicarboxylate* (8f): ^{1}H NMR ($CDCl_3$): 7.4–7.1 (4H, AA'XX'-quart), 3.77 (3H,s), 3.45 (3H,s), 3.38 and 2.99 (2H, AB-quart. J=9.0 Hz), 1.95 (3H,s), 1.77 (3H,s); and *dimethyl 2-(1-chloro-1-methylethyl)-3-phenyl-1,1-cycloprropanedi carboxylate* (8g): ^{1}H NMR ($CDCl_3$): δ 7.28 (5H,s), 3.78 (3H,s), 3.43 (3H,s), 3.47 and 2.83 (2H, AB-quart. J=9.0 Hz), 1.78 (3H, s), 1.72 (3H,s), respectively. Similar decomposition of *6h* led to a mixture of cyclopropane *8h* (45 %) and an alkene, *dimethyl 3-methyl-2-phenylbutylidenemalonate* (55 %). Column chromatography gave *8h* in a state pure enough for ^{1}H NMR, while the alkene only was obtained as a mixture with *8h*. *Dimethyl 2-isopropyl-3-phenyl-1,1-cyclopropanedicarboxylate* (*8h*): ^{1}H NMR($CDCl_3$): δ 7.20 (5H,s), 3.77 (3H,s), 3.35 (3H,s), 3.10 (1H,d, J=8.0 Hz), 2.25 (1H, 2d, J=8.0 and 8.8 Hz), 1.8–1.3 (1H,m), 1.10 (3H,d, J=7.0 Hz), 0.95 (3H,d, J=7.0 Hz).

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The Reaction between Diazoalkanes and Allylic Halides Carrying Electronegative γ-Substituents. 3. The Crystal Structures of Dimethyl 4-(1-Bromo-1-methylethyl)-5-phenyl-4,5-dihydro-3*H*-pyrazole-3,3-dicarboxylate and Dimethyl 2-(1-Bromo-1-methylethyl)-3-phenyl-1,1-cyclopropane-dicarboxylate

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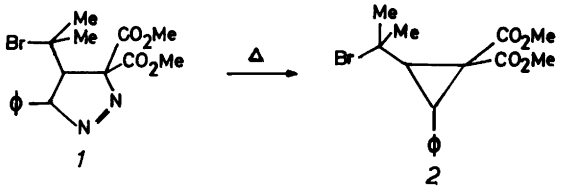
The structures of the title compounds have been determined by X-ray methods. Full-matrix least-squares refinements led to *R*-values of 0.061 (3289) and 0.099 (1989), respectively. (The numbers of observed reflections are in parentheses.)

The compounds are monoclinic with the following unit cell dimensions (at –150 ° C): The pyrazoline compound: a=7.171(2) Å; b=9.790(2) Å; c=25.121(4) Å; β=106.62(2)°; space group $P2_1$, Z=2. The cyclopropane compound: a=10.055(2) Å; b=12.135(2) Å; c=13.278(2) Å; β=103.57(2)°; space group $P2_1/c$, Z=4.

In both compounds the bromoisopropyl group and the phenyl group are situated *trans* with respect to the central ring. Bond lengths and angles are normal. In the cyclopropane compound a rotational disorder in the bromoisopropyl group was observed.

Synthesis of the title compound *1* and its thermal decomposition into *2* is reported earlier (Scheme 1).[1]

The decomposition of 1-pyrazolines to give cyclopropanes could in principle follow two different pathways: One involving a dipolar transition state and one involving diradical intermediates. The former is usually expected when the ring contains charge-stabilizing substituents and the latter pathway is preferred with unsubstituted or alkyl-substituted 1-pyrazolines[2] or when photolytically decomposed.[3] The stereochemical outcome of the decomposition has been at variance. Retention,[4] predominate inversion or lack of stereospecificity[4b] has been observed. In connection with our studies on the decomposition of 1-pyrazolines to give cyclopropanes it was of prime importance to know the



1 *2*

Scheme 1.

0302-4369/83 $2.50

stereochemistry of both the starting material and the product.[5-7]

Stereochemical relations between substituents in 1-pyrazolines are normally determined on the basis of (i) the concertedness of 1,3-dipolar cycloadditions giving retention of the stereochemistry of the starting alkene, along with the stereoselectivity observed when large substituents present in the 1,3-dipole and the dipolarophile interact in the transition state,[8] (ii) ^{1}H NMR coupling constants if vicinal hydrogens are present in the ring,[9] and (iii) the assumption that pseudoaxial hydrogens are located in the deshielding zone of the azo group and thus shifted to higher field relative to pseudoequatorial hydrogens.[9]

During the formation of *1* the bromoisopropyl group in the dipolarophile and the phenyl group in the 1,3-dipole are expected to interact in such a way that a *trans* adduct will be preferred. Furthermore, an observed coupling constant $J_{H_2H_3}$=10.0 Hz is probably due to coupling between two pseudoaxial hydrogens.[9,10] However, since the Karplus equation predicts large coupling constants both for large and small torsion angles, a *cis* relation between the 2- and 3-substituents (torsion angle about 30°) could just as well be justified by the observed coupling constant. The situation is further complicated by the proposed conformational equilibrium for 1-pyrazolines, where pseudoaxial and -equatorial positions are rapidly interchanged.[7-10]

The stereochemistry of the cyclopropane *2* was expected to be easily determined by the vicinal coupling constant, which normally takes its largest value between *cis* protons (torsion angle approx. 0°). Vicinal coupling constants in cyclopropane derivatives are reported to be 8.0–11.2 Hz for *cis* and 5.2–8.0 Hz for *trans* located hydrogens.[11] In dimethyl *trans*-2-methyl-3-phenyl-1,1-cyclopropanedicarboxylate J_{23}=8.2 Hz while in the *cis* analogue J_{23}=10.8 Hz.[12]

The observed coupling constant of 9.0 Hz for *2* indicates a *cis* relation between the phenyl group and the bromoisopropyl group, implying an inversion of the stereochemistry during the thermolytic decomposition which in turn indicates the intermediacy of a diradical.[3] On the other hand, compound *1* carries highly polar substituents and the rate of decomposition increases with enhanced charge-stabilizing capacities of the substituents.[6] Faced with the above-mentioned ambiguities with regard to the precise stereochemistry of *1* and *2*, a study of these compounds by X-ray diffraction was initiated.

EXPERIMENTAL

Data for the measurements of cell dimensions and intensity data were collected on a SYNTEX $P\bar{1}$ diffractometer with –150 ° C at the crystal site using graphite crystal monochromated Mo$K\alpha$ radiation (λ=0.71069 Å). Cell parameters were determined by a least-squares fit to the diffractometer settings for 15 general reflections. Intensity data were collected with the $\theta/2\theta$ scan technique, scan speed 3–6° min^{-1} (2θ) depending on the intensity; scan width 1.6° (2θ). All intensities in a quadrant of reciprocal space within sin θ/λ=0.63 Å^{-1} for *1* and sin θ=0.54 Å^{-1} for *2* were measured. Background counts were taken at each of the scan limits for 0.35 times the scan time. Three standard reflections were measured after every 100 reflections and the intensities of the data sets were adjusted according to the drift in the standard reflections. The numbers of reflections recorded for the two compounds were 3841 for *1* and 2332 for *2*. Of these 3289 (*1*) and 1989 (*2*) with $I>2.5\sigma(I)$ were retained for the structure determinations. The estimate of the standard deviation of the intensity was based on counting statistics with an additional term of 5 % of the net intensity. The data were corrected for Lorentz and polarization effects, but not for absorption or extinction.

A description of the computer programs applied for the structure analyses is given in Ref. 13. The quantity minimized in the full-matrix least-squares program was $\Sigma w\Delta F^2$, where w is the inverse of the variance of the observed structure factor.

Atomic form factors were those of Doyle and Turner[14] for Br, O, N and C, and of Stewart, Davidson and Simpson[15] for H.

CRYSTAL DATA

1. Dimethyl 4-(1-bromo-1-methylethyl)-5-phenyl-4,5-dihydro-3*H*-pyrazole-3,3-dicarboxylate, $C_{16}H_{19}BrN_2O_4$, m.p. 104–105 °C (ether–pentane). Monoclinic, a=7.171(2) Å; b=9.790(2) Å; c=25.121(4) Å; β=106.62(2)°; V=1689.9 Å^3, (t= –150 °C). M=383.25; Z=2; $F(000)$=784; μ(Mo$K\alpha$)=26.0 cm^{-1}; D_x=1.506 g cm^{-3}. Space group $P2_1$ (No. 4).

2. Dimethyl 2-(1-bromo-1-methylethyl)-3-phenyl-1,1-cyclopropanedicarboxylate,

Table 1. Fractional atomic parameters and standard deviations.

Atom	*x*	*y*	*z* (Cpd. *1*)	*x*	*y*	*z* (Cpd. *2*)
BR	0.8838(1)	0.8755(0)	0.6234(3)	0.0771(1)	0.0466(0	0.1285(0)
C10	0.6207(12)	0.9493(19)	0.5813(13)	0.2051(18)	0.0851(16)	0.2589(6)
C11	0.5745(12)	0.8885(11)	0.5227(3)	0.3334(8)	0.1347(6)	0.2338(6)
C12	0.6426(13)	1.1047(10)	0.5783(4)	0.2394(3)	–0.0423(2)	0.3259(2)
C1	0.4087(11)	0.7626(3)	0.6171(0)	0.1751(8)	0.2042(6)	0.4226(6)
C2	0.4673(10)	0.9138(8)	0.6112(3)	0.1294(8)	0.1668(6)	0.3087(6)
C3	0.5099(11)	0.9676(8)	0.6710(3)	0.0403(8)	0.1386(6)	0.3828(6)
C4	0.4443(11)	1.1129(9)	0.6781(3)	–0.0977(8)	0.1894(6)	0.3754(6)
C5	0.2574(12)	1.1564(9)	0.6501(4)	–0.1451(8)	0.1984(6)	0.4634(6)
C6	0.1999(12)	1.2894(10)	0.6583(4)	–0.2766(9)	0.2440(7)	0.4580(7)
C7	0.3263(13)	1.3776(10)	0.6949(4)	–0.3548(9)	0.2795(7)	0.3655(7)
C8	0.5134(12)	1.3332(10)	0.7235(4)	–0.3061(9)	0.2690(7)	0.2768(7)
C9	0.5715(12)	1.1997(9)	0.7157(3)	–0.1761(8)	0.2255(6)	0.2817(6)
C13	0.5681(11)	1.6554(8)	0.6245(3)	0.2944(9)	0.1520(6)	0.5007(6)
C14	0.8429(13)	0.5515(10)	0.6868(4)	0.3663(9)	0.0259(7)	0.6341(7)
C15	0.2337(11)	1.7180(9)	0.5704(3)	0.1536(8)	0.3249(6)	0.4427(6)
C16	–0.0036(13)	0.5477(10)	0.5399(4)	0.1883(10)	0.4637(6)	0.5734(7)
O1	0.6747(8)	1.6436(6)	0.6774(2)	0.2548(5)	0.0813(4)	0.5626(4)
O2	0.1651(8)	0.5994(7)	.5817(2)	0.2070(5)	0.3491(4)	0.5415(4)
O3	0.5923(9)	0.5898(6)	0.5864(6)	0.4111(6)	0.1741(4)	0.5000(4)
O4	0.1676(8)	0.7819(7)	0.5275(2)	0.0930(6)	0.3887(4)	0.3781(4)
N1	0.4013(9)	0.8724(8)	0.6978(3)			
N2	0.3461(9)	0.7651(7)	0.6702(3)			
H111	0.431	0.926	0.498	0.382	0.077	.202
H112	0.572	0.777	0.525	0.398	0.162	.300
H113	0.686	0.922	0.503	0.308	0.198	.185
H121	0.754	1.127	0.557			
H2	0.354	0.964	0.581	0.111	0.205	.241
H122	0.691	1.147	0.621			
H123	0.504	1.149	0.555			
H3	0.665	0.971	0.698	0.005	0.069	.407
H5	0.158	1.087	0.622	–0.142	0.222	.220
H6	0.055	1.324	0.636	–0.311	0.249	.517
H7	0.283	1.482	0.701	–0.443	0.309	.365
H8	0.611	1.398	0.753	–0.361	0.296	.212
H9	0.717	1.164	0.738	–0.142	0.222	.220
H141	0.921	0.550	0.732	0.330	–0.026	.678
H142	0.942	0.591	0.664	0.429	0.080	.678
H143	0.795	0.449	0.673	0.421	–0.017	.592
H161	–0.052	0.450	0.553	0.233	0.471	.649
H162	0.033	0.534	0.500	0.089	0.479	.561
H163	–0.124	0.623	0.533	0.233	0.513	.532

$C_{16}H_{19}BrO_4$, m.p. 61–62 °C (heptane). Monoclinic, a=10.055(2) Å; b=12.135(2) Å; c=13.278(2) Å; β=103.57(2)°; V=1574.9 Å^3, (t= –150 °C). M=355.23; Z=4; F(000)=728; μ(Mo$K\alpha$)=27.8 cm^{-1}; D_x=1.498 g cm^{-3}. Space group $P2_1/c$ (No. 14).

STRUCTURE DETERMINATIONS

The structures were determined by Patterson methods followed by successive Fourier syntheses. Positions were calculated for hydrogen atoms and these were included as fixed contributors in the least-squares calculations. During the

Table 2. Structural data.

Bond	Bond lengths (A) Cpd. *1*	Cpd. *2*
BR–C10	2.015(8)	
C10–C11	1.534(11)	1.530(12)
C10–C12	1.533(13)	
C2–C10	1.539(11)	1.496(12)
C1–C2	1.566(11)	1.542(11)
C1–C3		1.544(11)
C2–C3	1.538(10)	1.516(12)
C1–N2	1.524(9)	
N1–N2	1.259(10)	
C3–N1	1.493(10)	
C3–C4	1.524(11)	1.501(12)
C–C(Ph)*	1.396(11)	1.385(10)
C1–C13	1.524(10)	1.527(11)
C1–C15	1.516(11)	1.513(12)
C13–O1	1.336(9)	1.313(10)
C13–O3	1.206(9)	1.206(11)
O1–C14	1.471(10)	1.455(11)
C15–O2	1.324(10)	1.328(10)
C15–O4	1.219(9)	1.209(10)
O2–C16	1.449(10)	1.476(10)

Torsion angles (°)	Cpd. *1*	Cpd. *2*
C10–C2–C3–C4	85.7(8)	–135.5(8)
C10–C2–C1–C13	34.5(10)	–7.2(12)
H2–C2–C3–H3	145	135
C10–C2–C1–C15	–92.8(8)	141.7(8)

Angles between planes	Cpd. *1*
C1–N2–N1–C3 and C1–C2–C3	22.9(10)
C1–N2–N1–C3 and C4–C3–C1–C15	91.4(8)
C1–N2–N1–C3 and phenyl	85.5(8)

Bond angles (°)	Cpd. *1*	Cpd. *2*
BR–C10–C11	106.5(6)	
BR–C10–C12	106.8(6)	
BR–C10–C2	110.8(5)	
C2–C10–C11	113.1(7)	112.2(7)
C2–C10–C12	110.3(7)	
C1–C2–C10	120.7(6)	124.3(7)
C1–C2–C3	102.6(6)	61.1(5)
C3–C2–C10	115.4(6)	125.2(7)
C1–C3–C4		122.1(7)
C2–C3–C4	116.9(7)	122.9(7)
C2–C3–N1	104.1(6))	
C4–C3–N1	108.1(6)	
C3–N1–N2	113.2(6)	
N1–N2–C1	118.1(6)	
N2–C1–C13	107.6(6)	
N2–C1–C15	107.2(6)	
C2–C1–C13	116.9(6)	123.3(7)
C2–C1–C15	112.8(6)	115.9(7)
C3–C1–C13		120.1(7)
C3–C1–C15		114.0(7)
C–C–C(Ph)	119.9(5)	120.0(4)
C3–C4–C5	120.7(7)	118.7(8)
C3–C4–C9	118.9(7)	120.9(8)
C1–C13–O3	122.9(7)	120.9(8)
C1–C13–O1		113.0(7)
C13–O1–C14	115.0(6)	114.2(7)
C1–C15–O4	124.2(8)	124.3(7)
C1–C15–O2		110.3(7)
C15–O2–C16	115.7(7)	116.2(7)
O3–C13–O1	125.0(7)	126.0(8)
O4–C15–O2	124.5(7)	125.4(8)
C11–C10–C12	109.5(7)	112.1(6)
C2–C1–C3		58.7(5)
C13–C1–C15	108.8(7)	113.8(7)
C2–C1–N2	102.9(6)	

refinements a disorder in the structure of *2* was revealed. The bromine atom of the bromoisopropyl group turned out to be partially interchanged with one of the methyl groups (C12) corresponding to a rotation of 120° about the C2–C10 bond. The positions of the two pairs of atoms could not be resolved; the model was thus refined with pseudo atoms situated at the Br and C12 sites where the population factors also were parameters. The results showed that about 1/4 of the bromine is replaced by methyl and *vice versa* at C12. Obviously no determination of the C10–C12 and C10–Br bond lengths could be made; the disorder probably also is the reason for the rather poor agreement factors arrived at for this compound.

The refinements converged to conventional R factors of 0.061 for *1* and 0.099 for *2*. The R_w were 0.068 and 0.14; the $S=[\Sigma w\Delta F^2/(m-n)]^{1/2}$ were 2.0 and 5.0, respectively.

Final atomic coordinates are listed in Table 1. Tables of the thermal parameters and the structure factor listings are available from the authors.

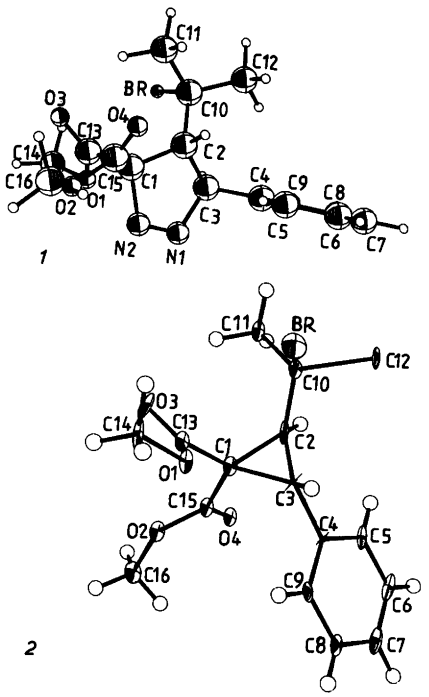


Fig. 1. ORTEP drawings of compounds *1* and *2*.

DISCUSSION

ORTEP drawings of the molecules are shown in Fig. 1 where the numbering scheme of the atoms is also indicated. In Table 2 the structural data are listed. Estimated standard deviations are calculated from the variance–covariance matrices.

As demonstrated earlier,[16] the 1-pyrazoline ring possesses envelope conformation with C3, N1, N2 and C1 situated in a plane forming an angle of 22.9° with the plane through C1, C2 and C3. The ring plane of the phenyl group forms an angle of 85.5° with the plane through C3, N1, N2 and C1. As may be seen from Fig. 1, the phenyl group and the bromoisopropyl group are situated *trans* to each other in both compounds.

For *1* the large groups positioned at C2 and C3 are placed pseudoequatorially with the torsion angle C10–C2–C3–C4 being 85.7°. The torsion angles C10–C2–C1–C13 and C10–C2–C1–C15 are 34.5 and 92.8°, respectively. The distance between the bromine atom and C3 is short (3.35 Å) compared to the sum of the van der Waals' radii (3.45 Å). If this is also the case in solution, the electrons of the bromine atom may contribute significantly to the stabilizing of a developing positive charge on C3 during thermolysis, and may explain the differences in thermolysis rates between brominated and non-brominated analogues of *1*.[5,17]

For the cyclopropane derivative *2* the torsion angle C10–C2–C3–C4 is 135.5°. Thus, the observed coupling constant in NMR between the adjacent hydrogens is larger than expected from the Karplus equation and from analogues found in the literature.[9,18]

Bond lengths and bond angles have the expected values in both compounds. The packing arrangements in the crystals are also quite normal with intermolecular separations to be expected from the van der Waals' radius sums.

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Oxidative Transformations of the Side-chains in the (12*E*)- and (12*Z*)-Abienols

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The reaction of (12*E*)-abienol (*3*) with *m*-chloroperbenzoic acid in chloroform yields, in addition to compounds *4*–*7*, two unexpected products which have now been formulated as the (12*R*,13*R*,14*R*)- and (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanols (*1*, *2*) on the basis of chemical results and X-ray analysis.

A probable route to *1* and *2* involves attack of the peracid on the 14,15-double bond in *3* and rearrangement to the (12*R*,13*E*)- and (12*S*,13*E*)-8,12-epoxy-13-labden-15-ols (*18*, *19*), which are then epoxidized. Its existence is borne out by the fact that *18* and *19* can be trapped from an epoxidation reaction carried out in a two-phase buffered system, and by the observation that both *18* and *19* undergo rapid and stereoselective epoxidations. Such a reaction sequence is of less importance for (12*Z*)-abienol (*22*), which furnishes compounds *29*–*32* by preferential attack of the peracid on the 12,13-double bond.

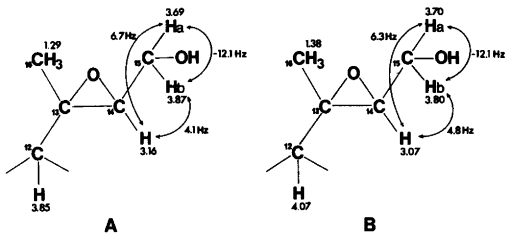
Alcohols *18* and *19* have been synthesized from (12*Z*)-abienol (*22*) *via* a stereospecific lead tetraacetate oxidation, which affords as the major products the (12*R*,13*S*)- and (12*S*,13*R*)-8,12-epoxy-14-labden-13-oyl acetates (*23*,*24*). The latter compounds were subjected to palladium(II)-catalyzed allylic rearrangements giving *25* and *26*, respectively, which were hydrolyzed.

A comparison of spectral data indicates that carterochaetol, previously formulated as (12*R*,13*Z*)-8,12-epoxy-13-labden-15-ol (*33*), is identical with (12*S*,13*E*)-8,12-epoxy-13-labden-15-ol (*19*). A revision of the stereochemistry at C-12 is also suggested for 16-hydroxycarterochaetol (*35*).

In a previous communication [1] we described the preparation of four tobacco diterpenoids:[2–4] The (12*R*,13*S*)- and (12*S*,13*R*)-8,12-epoxy-14-labden-13-ols (*4*, *5*) and the (12*R*,13*S*)- and (12*S*,13*R*)-8,13-epoxy-14-labden-12-ols (*6*, *7*) by treatment of (12*E*)-abienol (*3*) with *m*-chloroperbenzoic acid in chloroform. Two products of higher polarity, constituting some 20 % of the reaction mixture, were also obtained. We now report the stereostructures of these and discuss the mechanism of their formation in the light of results obtained by chemical transformations.

RESULTS

The two polar oxidation products (*1*, *2*) were identified as isomers of 8,12-13,14-diepoxy-15-labdanol on the basis of the following evidence. Both compounds (*1*, *2*) had the composition $C_{20}H_{34}O_3$ and gave rise to monoacetates (*8*, *9*) on acetylation. The allocation of the hydroxyl group in *1* and *2* to partial structures A and B, respectively, was determined by proton spin decoupling and spin simulation experiments. Additional support for these assignments was provided by the ^{13}C NMR spectra, which, besides



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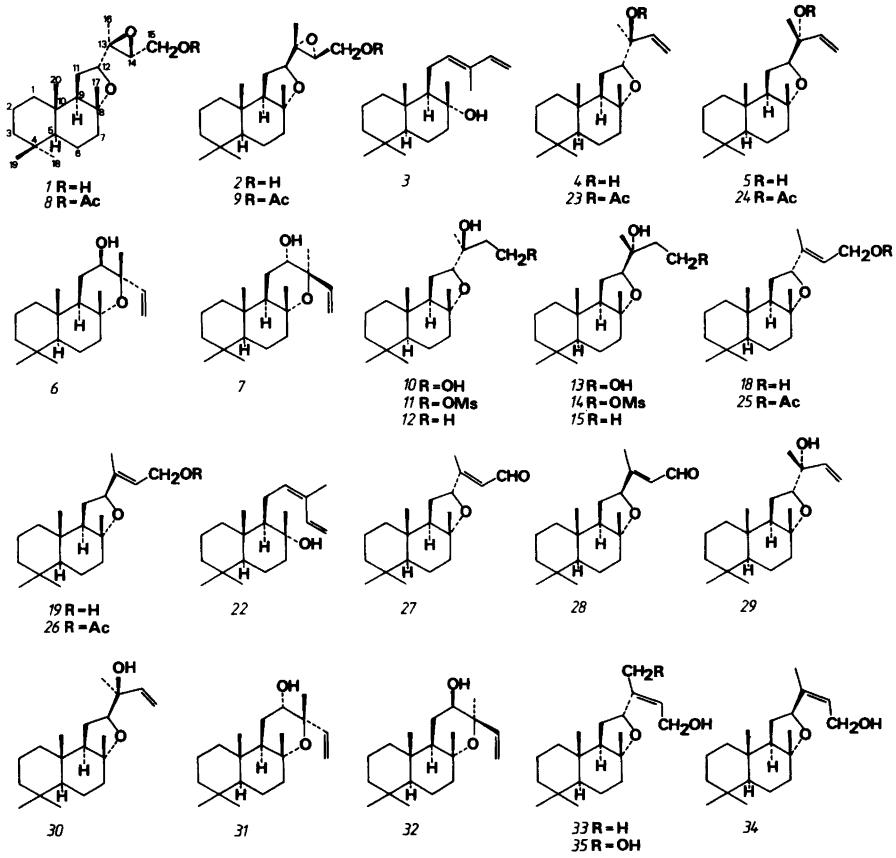


the C–15 resonance, present at δ 61.0 for *1* and at δ 60.9 for *2*, contained signals attributed to C-13 and C-14 at δ 61.6 (s) and 62.1 (d) for *1* and at δ 61.8 (s) and 60.1 (d) for *2* (*cf.* Table 1).

The presence of the 8,12-epoxy group followed from the chemical shift values of the signals due to the remaining oxygen-carrying carbon atoms, δ 81.8 (s) and 79.2 (d) for *1* and δ 81.1 (s) and 80.4 (d) for *2*.

The stereochemistry at C-12 and C-13 in alcohol *1* was deduced to be *R,R* by chemical correlation. Reduction using LAH converted alcohol *1* to a diol (*10*), which was reacted with methanesulfonyl chloride in pyridine. The monomesylate formed (*11*) was reduced using LAH to give a product, which was identical to (12*R*,13*S*)-8,12-epoxy-13-labdanol (*12*).

In a similar manner, alcohol *2* was converted *via* diol *13* and monomesylate *14* to (12*S*,13*R*)-8,12-epoxy-13-labdanol (*15*), hence demonstrating that its stereochemistry at C-12 and C-13 is *S,S*. While the assignment of an *R*-chirality to C-14 in alcohol *1* rests on chemical correlations detailed below, X-ray analysis was used to complete the determination of the stereochemistry of alcohol *2*.

Alcohol *2* formed needle-shaped crystals of space group $P2_1$. The crystal data, obtained on a Philips PW 1100 diffractometer, are: a=12.693, b=7.376 and c=11.116 Å, β=112.02°, Z=2. The present *R*-value including anisotropic thermal parameters for all non-hydrogen atoms is 0.098; location of the hydrogen atoms and further refinement being under way.[5] A stereoscopic view, which summarizes the X-ray results and demonstrates that alcohol *2* is (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanol, is shown in Fig. 1.

Three routes, A, B and C in Scheme 1, may be invoked to account for the generation of alcohols *1* and *2*. In route A, (12*E*)-abienol (*3*) is converted *via* the intermediacy of epoxides *16*

Table 1. Carbon-13 chemical shifts and assignments for compounds *1, 2, 8–10, 12, 13, 15, 18, 19* and *23–26.*[a].

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
1	40.0	18.4	42.4	33.1	57.4	20.6	39.7	81.8	59.9	36.4	25.3	79.2	61.6	62.1	61.0	12.9	21.4	33.5	21.1	14.8
2	40.0	18.4	42.4	33.1	57.0	21.0	40.3	81.1	60.6	36.3	23.7	80.4	61.8	60.1	60.9	14.7	24.1	33.5	21.0	15.6
8 [b]	40.0	18.4	42.5	33.1	57.4	20.6	39.7	81.7	59.9	36.4	25.4	78.5	61.4	58.6	63.2	13.4	21.4	33.6	21.1	14.8
9 [c]	40.1	18.5	42.5	33.1	57.1	21.0	40.4	81.0	60.7	36.4	23.7	80.0	61.4	56.8	63.1	15.0	24.0	33.5	21.0	15.6
10	39.8	18.3	42.4	33.0	57.3	20.5	39.6	81.2	60.0	36.3	23.9	81.6	74.5	38.9	58.9	22.4	21.3	33.5	21.1	14.7
12	39.9	18.4	42.5	33.1	57.4	20.6	39.8	80.9	60.4	36.4	23.8	81.9	73.9	29.9	7.7	22.9	21.3	33.6	21.1	14.8
13	40.2	18.5	42.5	33.1	57.1	21.4	40.9	81.1	60.9	36.4	23.8	85.8	73.6	38.8	58.9	23.9	25.4	33.5	21.0	16.0
15	40.3	18.5	42.6	33.2	57.2	21.5	41.0	80.8	61.1	36.5	24.3	85.8	72.5	30.1	7.9	23.7	25.4	33.6	21.0	16.0
18	39.7	18.4	42.5	33.1	57.3	20.6	39.9	81.3	59.2	36.3	28.2	79.6	140.0	123.7	59.2	12.9	21.6	33.5	21.1	15.0
19	40.1	18.4	42.5	33.1	57.2	21.0	40.6	81.3	61.0	36.4	28.8	81.9	140.2	122.0	59.1	13.4	24.8	33.5	21.0	15.8
23 [d]	40.0	18.3	42.4	33.0	57.4	20.5	39.6	81.2	59.5	36.2	24.4	80.3	84.0	139.4	114.9	18.9	21.3	33.5	21.1	14.8
24 [e]	40.2	18.5	42.5	33.1	57.2	21.3	40.9	81.5	60.6	36.4	24.1	85.3	83.4	139.6	114.6	20.0	25.1	33.5	21.0	15.9
25 [f]	39.9	18.4	42.5	33.1	57.3	20.6	39.7	81.2	59.1	36.2	28.2	79.3	142.3	118.4	61.0	13.1	21.5	33.5	21.1	15.0
26 [g]	40.6	18.4	42.5	33.1	57.1	21.0	40.1	81.2	61.0	36.3	28.8	81.6	142.7	116.6	61.0	13.6	24.6	33.5	21.0	15.8

[a] δ-Values in $CDCl_3$ relative to TMS. [b] $OCOCH_3$ 20.8. [c] $OCOCH_3$ 170.8; $OCOCH_3$ 20.8. [d] $OCOCH_3$ 169.7; $OCOCH_3$ 22.3. [e] $OCOCH_3$ 169.9; $OCOCH_3$ 22.1. [f] $OCOCH_3$ 170.9; $OCOCH_3$ 21.0. [g] $OCOCH_3$ 170.9; $OCOCH_3$ 21.0.

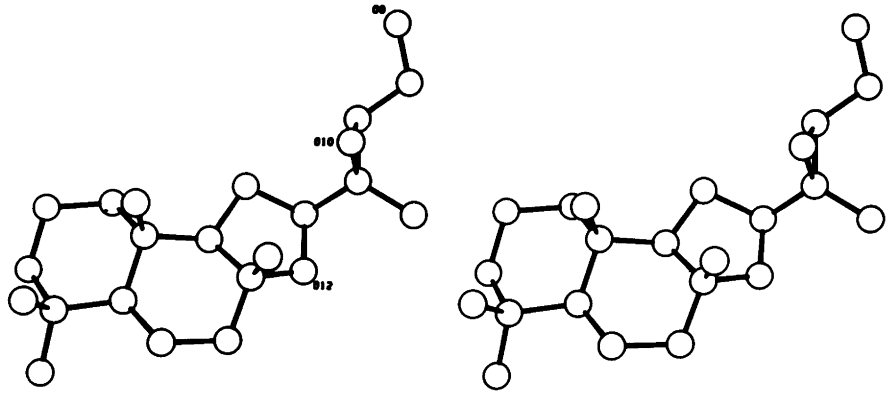


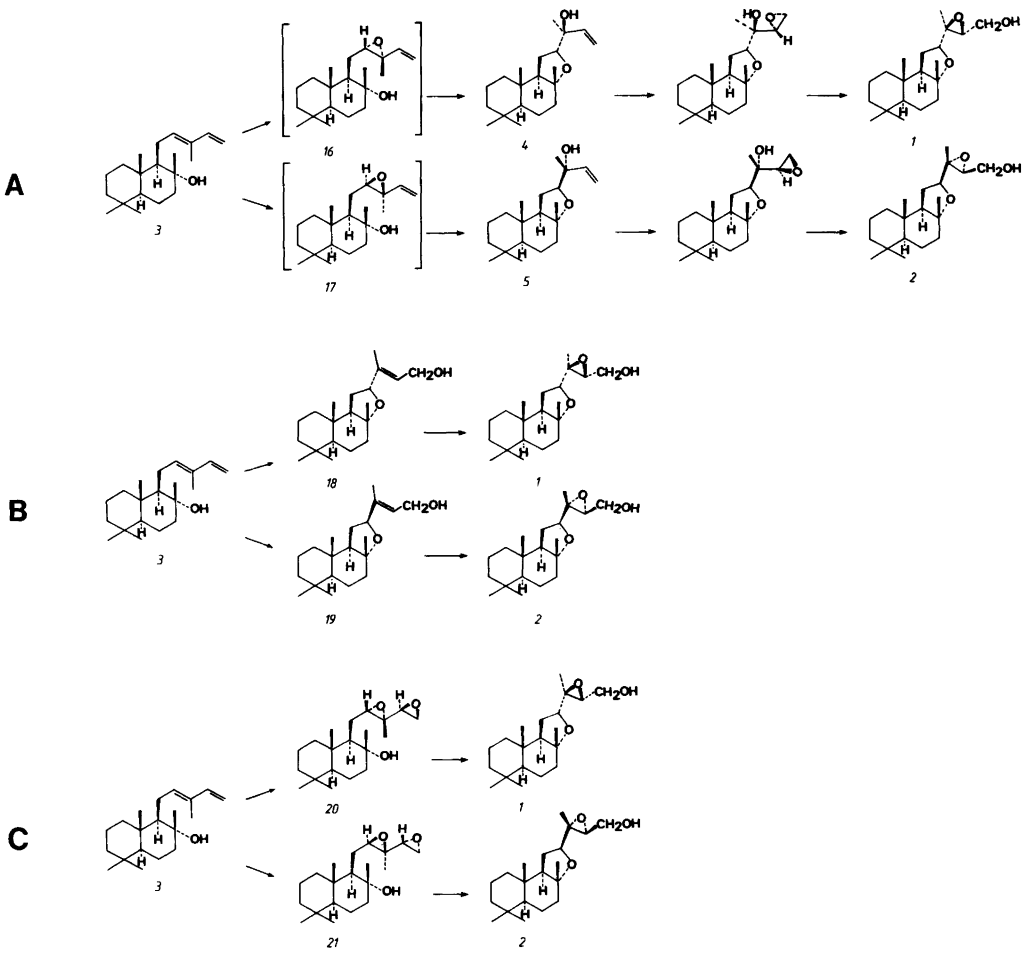
Fig. 1. A stereoscopic view of (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanol (*2*).

and *17*, or equivalent peracid complexes, to the 8,12-epoxides *4* and *5*, which undergo epoxidation of the 14,15-double bond and subsequent rearrangement. This possibility was ruled out, however, since *4* was recovered unchanged after treatment with *m*-chloroperbenzoic acid in chloroform for 2 h, *i.e.* the reaction time normally needed to convert (12*E*)-abienol (*3*) to alcohols *1* and *2*. Route B would involve an initial attack of peracid on the 14,15-double bond in (12*E*)-abienol (*3*), accompanied by acid-induced rearrangements to give alcohols *18* and *19*, which are rapidly and stereospecifically epoxidized. The formation of alcohols *1* and *2* *via* route C would require the intermediate 12,13-14,15-diepoxides *20* and *21* to have (12*S*,13*R*,14*S*)- and (12*R*,13*S*,14*R*)-stereochemistry, respectively. These two diastereoisomers are actually those expected to arise from (12*E*)-abienol (*3*), since the initially generated monoepoxides would exist mainly in s-*trans* conformations and attack of the peracid would occur *anti* to the epoxide oxygen.[6]

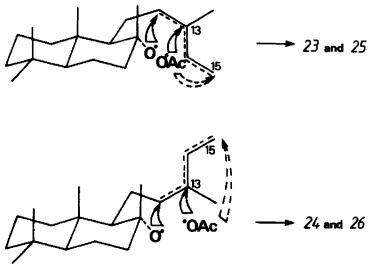
In order to evaluate the importance of routes B and C we decided to prepare the (12*R*,13*E*)- and (12*S*,13*E*)-8,12-epoxy-13-labden-15-ols (*18*, *19*) and to study the products of their epoxidation reactions. To this end (12*Z*)-abienol (*22*) was initially treated with lead tetraacetate (LTA) in benzene. Four products were obtained. The major two, which gave rise to alcohols *4* and *5* on alkaline hydrolysis, were identified as the (12*R*,13*S*)- and (12*S*,13*R*)-8,12-epoxy-14-labden-13-oyl acetates *23* and *24*, respectively. The minor two products (*25*, *26*) proved to be identical to the rearrangement products obtained by reacting acetates *23* and *24* with bis(acetonitrile)palladium(II) chloride in tetrahydrofuran. Their ^{1}H NMR spectra included signals due to a vinylic methyl group, a CH_2–OAc group and an olefinic proton. They were hence formulated as the (12*R*,13*E*)- and (12*S*,13*E*)-8,12-epoxy-13-labden-15-oyl acetates (*25*, *26*), the *E*-geometries assigned to their 13,14-double bonds being consistent with the proposed mechanism of the allylic rearrangement reaction [7] (for conclusive evidence, see below).

The LTA reaction deserves additional comments. Firstly, it follows that (12*Z*)-abienol (*22*) on LTA treatment in pure benzene gives rise to 8,12-epoxides (*23*–*26*) only. Addition of pyridine does not affect the outcome of this reaction. These results contrast with findings for other γ,δ-unsaturated alcohols, *e.g.* 4-penten-1-ol [8] and β-ionol,[9] which yield mixtures of tetrahydrofurans and tetrahydropyrans when the reaction is carried out in benzene, while tetrahydrofurans are the predominant products (98 %) from 4-penten-1-ol, when pyridine is present.[9] This sensitivity to experimental conditions has been associated with the reaction mechanisms involved; *i.e.* the reaction is likely to proceed by an ionic or concerted mechanism in benzene, while in the presence of pyridine a radical mechanism has been found to be operative.[9] Whether this implies that the diene (12*Z*)-abienol (*22*) reacts by a radical mechanism under both reaction conditions is presently unclear.

Secondly, the reaction of (12*Z*)-abienol (*22*)



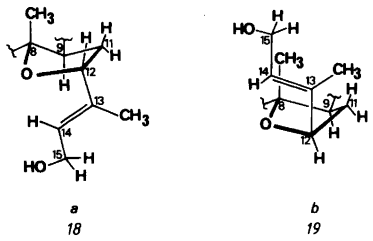
Scheme 1. Proposed routes for the formation of *1* and *2* from (12*E*)-abienol (*3*).



Scheme 2. Formation, assuming a radical mechanism, of *23–26* by LTA oxidation of (12*Z*)-abienol (*22*).

with LTA is stereoselective. The generation of the major acetates *23* and *24* is explained by attachment of the acetoxy radical (or ion) to C–13 from the side shown in Scheme 2, while attack on C–15 would yield the minor acetates *25* and *26*.

Alkaline hydrolysis of acetates *25* and *26* furnished the target alcohols *18* and *19*, respectively. In order to confirm the geometries assigned to the 13,14-double bond in these four compounds (*18, 19, 25, 26*), alcohols *18* and *19* were oxidized using manganese dioxide to the corresponding aldehydes *27* and *28*. Their ^{1}H



a *b*
18 *19*

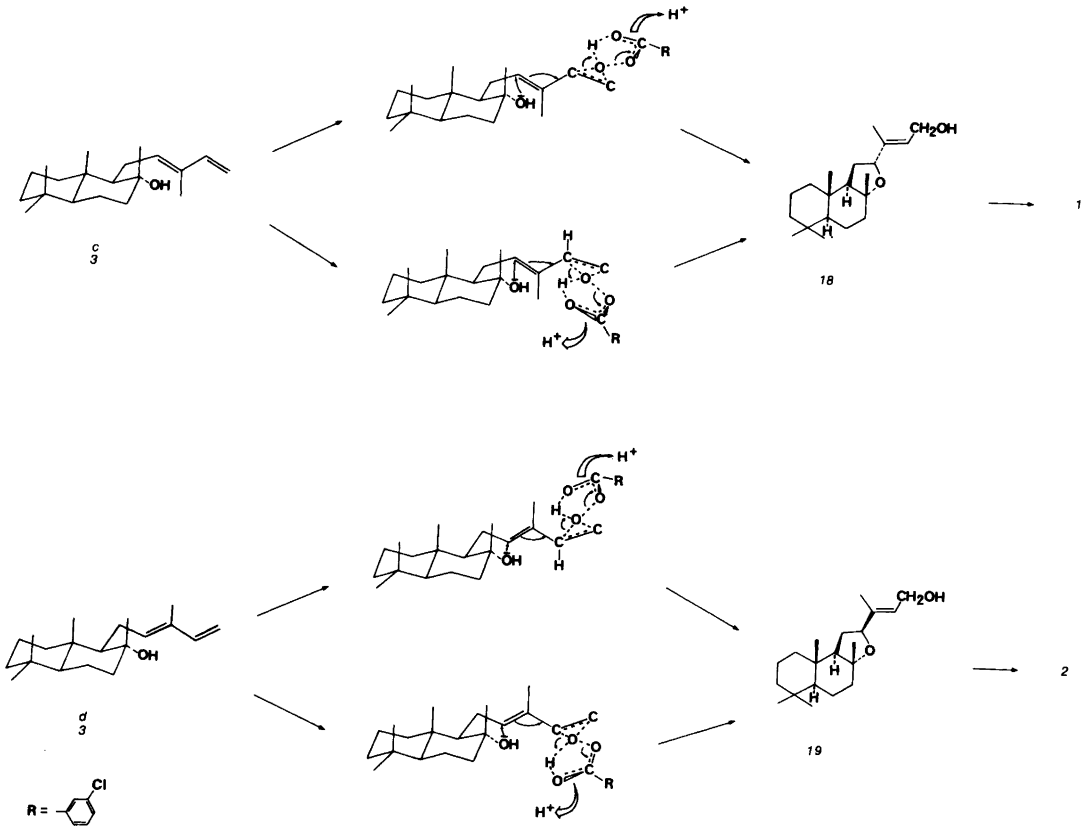
Scheme 3. Reacting conformers in the epoxidation of *18* and *19*.

NMR spectra displayed signals due to H–16 at δ 2.13 and at δ 2.12, respectively, shieldings which are only consistent with *E*-geometries.[10]

The reaction of alcohol *19* with *m*-chloroperbenzoic acid in chloroform proceeded rapidly and virtually stereospecifically with formation of an epoxide, which was identical with (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanol (*2*). Epoxidation of alcohol *18* occurred in a similar manner yielding an epoxide indistinguishable from epoxide *1*. The latter is consequently fully characterized as (12*R*,13*R*,14*R*)-8,12-13,14-diepoxy-15-labdanol.

The outcome of these reactions is explained if conformer *a* of alcohol *18* and *b* of alcohol *19* (Scheme 3), which appear to be energetically favoured, are the reacting species. Their geometries also allow the possibility of anchimeric assistance from the 8,12-ether oxygen and the hydroxyl group at C-15. Such cooperative effects have previously been found to lead to high stereospecificity in the epoxidation of allylic alcohols with peracids.[11]

The experimental results obtained are hence consistent with the formation of alcohols *1* and *2* *via* route B but do not exclude the existence of route C. Further insight into the reaction details



Scheme 4. Mechanisms for the formation of *18* and *19* from (12*E*)-abienol (*3*).

was next sought by treatment of (12*E*)-abienol (*3*) with *m*-chloroperbenzoic acid in a biphasic solvent system consisting of diethyl ether and aqueous sodium bicarbonate.[12,13] No acid-sensitive 1,2-epoxides, *i.e. 16, 17* or *20, 21,* were detected. However, the lower reaction efficiency under these conditions permitted the trapping of alcohols *18* and *19*.

Thus, one route to alcohols *1* and *2* is evidently initiated by attack of peracid on the terminal 14,15-double bond in (12*E*)-abienol (*3*). It is reasonable to assume that the overall process to alcohols *18* and *19* is concerted and since *trans*-products are formed exclusively, the rearrangements are likely to take place in all-*trans* conformers such as *c* and *d* of (12*E*)-abienol (*3*). While the peracid attack has been envisaged in Scheme 4 to occur on both sides of the 14,15-double bond, the steric requirements for the process are evidently very strict in other respects. This follows from the observation that a corresponding process, which would compete with the preferential formation of compounds *29–32 via* epoxidation of the 12,13-double bond, is of little importance in (12*Z*)-abienol (*22*).

In conclusion we wish to comment upon the structural assignments of a few naturally occurring 8,12-epoxy bridged labdanoids. Carterochaetol, a compound isolated from *Carteothamnus anomalochaeta,*[15] has been formulated as (12*R*,13*Z*)-8,12-epoxy-13-labden-15-ol (*33*) mainly on the basis of spectral evidence. A comparison of the published spectral data with those of alcohols *18* and *19* suggests, however, that carterochaetol is identical with the latter, *i.e.* its structure is (12*S*,13*E*)-8,12-epoxy-13-labden-15-ol (*19*). Support for this view is also provided by the spectral congruity of carterochaetol acetate and acetate *26* and by the fact that the ^{1}H NMR data previously reported for the two synthetic C–12 epimers of 8,12-epoxy-13*Z*-labden-15-ol (*33, 34*) [16] are different from our data for alcohol *19*.

16-Hydroxycarterochaetol (*35*), another naturally occurring 8,12-epoxylabdanoid, which has been isolated from a few *Silphium* species,[17] has previously been assigned a 12*R*-stereochemistry. However, its ^{13}C NMR spectrum displays the C-17 signal at δ 24.5, a chemical shift value only consistent with a 12*S*-stereochemistry (*cf.* Table 1 and Ref. 14). As a consequence, the configuration at C-12 may also have to be revised in compounds which have been chemically correlated with 16-hydroxycarterochaetol.

EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos MS 50 Stereo DS 55 SM/DS 55 S mass spectrometer-computer system and some of the ^{1}H NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 18 were used.

Experimental details for the preparation and isolation of the (12*R*,13*R*,14*R*)- and (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanols (*1, 2*) have been given previously.[1]

(12*R*,13*R*,14*R*)-8,12-13,14-Diepoxy-15-labdanol (*1*) had m.p. 126–127 °C; $[\alpha]_D$ –3.7° (*c* 0.30, $CHCl_3$) (Found: $M^{\cdot+}$ 322.2511. Calc. for $C_{20}H_{34}O_3$: 322.2508); IR (CCl_4) bands at 3610 and 3430 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20) and 1.13 (s, H-17) (for other ^{1}H NMR data see partial structure A); MS [*m/z* (%, composition)]: 322 (M, 1), 307 (17), 291 (7, $C_{19}H_{31}O_2$), 277 (1), 262 (8, $C_{18}H_{30}O$), 247 (2, $C_{17}H_{27}O$), 235 (20, $C_{16}H_{27}O$ and $C_{15}H_{23}O_2$), 217 (11, $C_{16}H_{25}$ and $C_{15}H_{21}O$), 206 (5), 191 (100), 175 (5), 163 (3), 149 (6), 137 (38), 123 (22), 109 (28), 95 (30), 81 (24), 69 (37), 55 (22) and 43 (39).

(12*S*,13*S*,14*S*)-8,12-13,14-Diepoxy-15-labdanol (*2*) had m.p. 176–178 °C; $[\alpha]_D$ –15.3° (*c* 0.40, $CHCl_3$) (Found: $M^{\cdot+}$ 322.2512. Calc. for $C_{20}H_{34}O_3$: 322.2508); IR (CCl_4) bands at 3610 and 3440 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.82 (s)/0.84 (s)/0.89 (s) (H-18/H-19/H-20) and 1.15 (s, H-17) (for other ^{1}H NMR data see partial structure B); MS [*m/z* (%)]: 322 (M, 1), 307 (28), 291 (4), 277 (3), 262 (11), 247 (5), 235 (10), 217 (7), 191 (100), 177 (13), 163 (5), 149 (9), 137 (38), 123 (26), 109 (31), 95 (33), 81 (29), 69 (36), 55 (23) and 43 (33).

*Preparation of the (12*R*,13*R*,14*R*)- and (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanoyl acetates* 8 *and* 9. Acetylation using acetic anhydride in pyridine converted *1* and *2* into the (12*R*,13*R*,14*R*)- and (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanoyl acetates *8* and *9*, respectively.

(12*R*,13*R*,14*R*)-8,12-13,14-Diepoxy-15-labdanoyl acetate (*8*) had IR (CCl_4) bands at 1745 and 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20), 1.13 (s, H-17), 1.31 (s, H-16), 2.10 (s, OCOC*H*$_3$), 3.18 (dd, *J*=4 and 7 Hz, H-14), 3.85 (m, H-12), 4.06 (dd, *J*=7 and –12 Hz, H-15a) and 4.37 (dd, *J*=4 and –12 Hz); MS [*m/z* (%)]: 364 (M, 2), 349 (14), 304 (3), 289 (3), 262 (5), 235 (17), 217 (15), 191 (100), 177

(18), 163 (8), 149 (14), 137 (46), 123 (32), 109 (43), 95 (48), 81 (42), 69 (57), 55 (35) and 43 (89).

(12*S*,13*S*,14*S*)-8,12-13,14-Diepoxy-15-labdanoyl acetate (*9*) had IR (CCl_4) bands at 1745 and 1230 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (s)/0.84 (s)/0.87 (s) (H-18/H-19/H-20), 1.14 (s, H-17), 1.39 (s, H-16), 2.10 (s, $OCOCH_3$), 3.11 (dd, J=4 and 7 Hz, H-14), 4.07 (dd, J=7 and –12 Hz, H-15a), 4.10 (m, H-12) and 4.38 (dd, J=4 and –12 Hz, H-15b); MS [*m/z* (%)]: 364 (M, 2), 349 (32), 304 (3), 289 (3), 262 (16), 247 (8), 235 (7), 217 (6), 191 (100), 175 (20), 163 (7), 149 (15), 137 (39), 123 (32), 109 (36), 95 (43), 81 (36), 69 (38), 55 (31) and 43 (83).

*Conversion of (12*R*,13*R*,14*R*)-8,12-13,14-diepoxy-15-labdanol (1) to (12*R*,13*S*)-8,12-epoxy-13-labdanol (12).* An ethereal solution of 35 mg of *1* was refluxed with excess LAH for 1 h. Work-up and chromatography over silica gel afforded 30 mg of (12*R*,13*S*)-8,12-epoxy-13,15-labdanediol (*10*), which had ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.87 (s) (H-18/H-19/H-20), 1.15 (s)/1.22 (s) (H-16/H-17) and 3.9 (overlapping signals, H-12, H-15a and H-15b).

A solution of 25 mg of *10* in 5 ml of pyridine was left with 20 µl of methanesulfonyl chloride at room temperature for 2 h. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed with aqueous H_2SO_4 (10 %), sodium bicarbonate and water. Removal of solvent gave 12 mg of (12*R*,13*S*)-8,12-epoxy-13-hydroxy-15-labdanoyl methanesulfonate (*11*), which had ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20), 1.15 (s)/1.19 (s) (H-16/H-17), 3.02 (s, OSO_2CH_3), 3.90 (m, H-12) and 4.48 (t, J=7 Hz, H-15a and H-15b).

Reduction of 5 mg of *11* with LAH yielded 4 mg of a product, which was indistinguishable from the sample of (12*R*,13*S*)-8,12-epoxy-13-labdanol (*12*) prepared by catalytic hydrogenation of (12*R*,13*S*)-8,12-epoxy-14-labden-13-ol (*4*). Compound *12* had IR (CCl_4) bands at 3590 and 3450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (s)/0.85 (s)/0.87 (s) (H-18/H-19/H-20), 0.92 (t, J=7 Hz, H-15), 1.13 (s, H-16), 1.13 (s, H-17) and 3.91 (m, H-12); MS [*m/z* (%)]: 293 (M-15, 2), 275 (2), 235 (14), 221 (10), 217 (13), 192 (100), 191 (73), 177 (71), 163 (3), 149 (13), 137 (29), 123 (29), 109 (26), 95 (30), 81 (31), 73 (50), 55 (32) and 43 (33).

*Conversion of (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanol (2) to (12*S*,13*R*)-8,12-epoxy-13-labdanol (15).* Reduction of 80 mg of *2* with LAH afforded 35 mg of (12*S*,13*R*)-8,12-epoxy-13,15-labdanediol (*13*), which had ^1H NMR ($CDCl_3$): δ 0.82 (s)/0.85 (s)/0.87 (s) (H-18/H-19/H-20), 1.17 (s, H-17), 1.29 (s, H-16) and 3.8 (overlapping signals, H-12, H-15a and H-15b).

Treatment of 35 mg of (*13*) with 20 µl methanesulfonyl chloride in pyridine at room temperature for 2 h gave 37 mg of (12*S*,13*R*)-8,12-epoxy-13-hydroxy-15-labdanoyl methanesulfonate (*14*), which had IR ($CHCl_3$) bands at 3590 and 3440 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (s)/0.85 (s)/0.87 (s) (H-18/H-19/H-20), 1.17 (s, H-17), 1.25 (s, H-16), 3.01 (s, OSO_2CH_3), 3.73 (m, H-12) and 4.45 (t, J=7 Hz, H-15a and H-15b).

An ethereal solution of 35 mg of *14* was refluxed with excess LAH for 3 h. Work-up and chromatography over silica gel gave 24 mg of a product which was identical in all respects to the sample of (12*S*,13*R*)-8,12-epoxy-13-labdanol (*15*) obtained by catalytic hydrogenation of (12*S*,13*R*)-8,12-epoxy-14-labden-13-ol (*5*). Compound *15* had IR (CCl_4) bands at 3590 and 3440 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.85 (s)/0.87 (s) (H-18/H-19/H-20), 0.93 (t, J=7 Hz, H-15), 1.18 (s)/1.20 (s) (H-16/H-17) and 3.75 (m, H-12); MS [*m/z* (%)]: 293 (M-15, 6), 275 (2), 257 (1), 235 (21), 221 (9), 217 (18), 192 (81), 191 (100), 177 (73), 161 (7), 149 (13), 137 (38), 123 (31), 109 (36), 95 (40), 81 (44), 73 (72), 55 (47) and 43 (59).

Treatment of (12Z)-abienol (22) with lead tetraacetate (LTA). I. To a solution of 1.5 g of (12*Z*)-abienol (*22*) in 30 ml of benzene was added 2.2 g of LTA. The reaction mixture was refluxed for 1 h, diluted with ether, filtered, washed with aqueous sodium bicarbonate (5 %) and water, dried and evaporated. The residue was separated by HPLC using a column packed with Spherisorb/CN and hexane–ethyl acetate (90:10) as an eluent to give (12*R*,13*S*)-8,12-epoxy-14-labden-13-oyl acetate (*23*, 29 %), (12*S*,13*R*)-8,12-epoxy-14-labden-13-oyl acetate (*24*, 37 %), (12*R*,13*E*)-8,12-epoxy-13-labden-15-oyl acetate (*25*, 15 %) and (12*S*,13*E*)-8,12-epoxy-13-labden-15-oyl acetate (*26*, *19 %*).

(12*R*,13*S*)-8,12-Epoxy-14-labden-13-oyl acetate (*23*) was an oil and had $[\alpha]_D$ −15.6° (*c* 1.47, $CHCl_3$); IR (CCl_4) bands at 3090, 1740, 1640 and 1250 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (s)/0.82 (s)/0.87 (s) (H-18/H-19/H-20), 1.13 (s, H-17), 1.56 (s, H-16), 2.03 (s, $OCOCH_3$), 4.17 (m, H-12), 5.21 (d, J=17.5 Hz, H-15a), 5.24 (d, J=11 Hz, H-15b) and 6.05 (dd, J=11 and 17.5 Hz, H-14); MS [*m/z* (%)]: 288 (M-60, 11), 273 (8), 255 (1), 235 (16), 217 (12), 205 (26), 191 (100), 175 (17), 163 (10), 149 (18), 137 (30),123 (21), 109 (40), 95 (30), 83 (41), 69 (39), 55 (59) and 43 (51).

(12*S*,13*R*)-8,12-Epoxy-14-labden-13-oyl acetate (*24*) had m.p. 107–109 °C and $[\alpha]_D$ −21.9° (*c* 1.44, $CHCl_3$); IR (CCl_4) bands at 3090, 1735, 1635 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (s)/0.84 (s)/0.86 (s) (H-18/H-19/H-20), 1.16 (s,

H-17), 1.63 (s, H-16), 2.03 (s, OCOCH_3), 3.83 (m, H-12), 5.14 (d, J=17.5 Hz, H-15a), 5.20 (d, J=11 Hz, H-15b) and 5.95 (dd, J=11 and 17.5 Hz, H-14); MS [*m/z* (%)]: 288 (M−60, 22), 273 (11), 255 (2), 235 (12), 217 (9), 205 (28), 191 (100), 175 (21),163 (12), 149 (20), 137 (30), 123 (25), 109 (40), 95 (32), 83 (68), 69 (40), 55 (69) and 43 (61).

(12*R*,13*E*)-8,12-Epoxy-13-labden-15-oyl acetate (*25*) was an oil and had $[\alpha]_D$ +2.17°(*c* 1.15, $CHCl_3$); IR (CCl_4) bands at 1745, 1675 and 1235 cm^{-1}: ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20), 1.15 (s, H-17), 1.67 (s, H-16), 2.05 (s, OCOCH_3), 4.41 (m, H-12), 4.63 (d, J=7 Hz, H-15a and H-15b) and 5.64 (t, J=7 Hz, H-14); MS [*m/z* (%)]: 288 (M-60, 29), 273 (11), 255 (3), 233 (2), 219 (5), 205 (13), 191 (100), 175 (11), 163 (10), 149 (15), 137 (31), 123 (49), 109 (40), 95 (45), 81 (38), 69 (46), 55 (45) and 43 (65).

(12*S*,13*E*)-8,12-Epoxy-13-labden-15-oyl acetate (*26*) was an oil and had IR (CCl_4) bands at 1740, 1680, and 1235 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.87 (s) (H-18/H-19/H-20), 1.12 (s, H-17), 1.68 (s, H-16), 2.04 (s, OCOCH_3) 4.32 (m, H-12), 4.64 (d, J=7 Hz, H-15a and H-15b) and 5.72 (t, J=6.5 Hz, H-14); MS [*m/z* (%)]: 288 (M-60, 26), 273 (13), 255 (3), 233 (2), 215 (2), 205 (10), 191 (100), 175 (8), 163 (6), 149 (13), 137 (31), 123 (27), 109 (31), 95 (33), 83 (40), 69 (45), 55 (48) and 43 (82).

II. A solution of 60 mg of *20* and 20 mg of $CaCO_3$ in 3 ml of benzene and 32 mg of pyridine was refluxed with 98 mg of LTA for 2 h. Work-up and examination and separation by HPLC yielded *23, 24, 25* and *26* in the ratio 32:38:14:16.

*Hydrolysis of the (12*R,*13*S*)- and (12*S,*13*R*)-8,12-epoxy-14-labden-13-oyl acetates* 23 *and* 24. A solution of 23 mg of *23* and 3 drops of aqueous potassium hydroxide (45 %) in 1 ml of ethanol was refluxed under nitrogen for 2 h. The reaction mixture was diluted with water, acidified and extracted with ether. The residue obtained after removal of the solvent was purified by HPLC (μ-Porasil, hexane–ethyl acetate 95:5) to yield 13 mg of (12*R*,13*S*)-8,12-epoxy-14-labden-13-ol (*4*).

Hydrolysis using the conditions described above converted *24* into (12*S*,13*R*)-8,12-epoxy-14-labden-13-ol (*5*).

*Conversion of (12*R,*13*S*)-8,12-epoxy-14-labden-13-oyl acetate (23) to (12*R,*13*E*)-8,12-epoxy-13-labden-15-ol (18).* A solution of 66 mg of *23* in 5 ml of tetrahydrofuran was stirred with 15 mg of bis(acetonitrile)palladium(II) chloride under nitrogen and at room temperature for 1 h. The reaction mixture was diluted with water, extracted with ether, washed with water, dried and evaporated. The residue was purified by HPLC using a column packed with Spherisorb/CN and hexane–ethyl acetate (90:10) as an eluent to give 45 mg of (12*R*,13*E*)-8,12-epoxy-13-labden-15-oyl acetate (*25*).

A solution of 45 mg of *25* in 5 ml of ethanol and 20 μl of aqueous potassium hydroxide (45 %) was stirred under nitrogen and at room temperature for 3 h. Work-up and purification by HPLC [Spherisorb/CN; hexane–ethyl acetate (60:40)] afforded 35 mg of (12*R*,13*E*)-8,12-epoxy-13-labden-15-ol (*18*), which had m.p. 84−85 °C and $[\alpha]_D$ −3.1° (*c* 0.45, $CHCl_3$); IR (CCl_4) bands at 3610, 3400 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20), 1.16 (s, H-17), 1.66 (s, H-16), 4.20 (d, J=6.5 Hz, H-15a and H-15b), 4.41 (m, H-12) and 5.72 (dt, J=1.2 and 6.8 Hz, H-14): ^1H NMR (CCl_4): δ 0.82 (s)/0.82 (s)/0.88 (s) (H-18/H-19/H-20), 1.10 (s, H-17), 1.60 (s, H-16), 4.04 (d, J=6.6 Hz, H-15a and 15b), 4.28 (m, H-12) and 5.57 (t, J=6.8 Hz, H-14); MS [*m/z* (%)]: 306 (M, 0.4), 291 (3), 275 (40), 206 (18), 191 (100), 150 (12), 137 (35), 123 (65), 109 (40), 95 (49), 82 (49), 69 (64), 55 (45) and 43 (55).

*Conversion of (12*S,*13*R*)-8,12-epoxy-14-labden-13-oyl acetate (24) to (12*S,*13*E*)-8,12-epoxy-13-labden-15-ol (19).* Treatment of 103 mg of *24* with 20 mg of bis(acetonitrile)palladium(II) chloride in 10 ml of tetrahydrofuran for 1 h afforded, after work-up and purification by HPLC [Spherisorb/CN; hexane–ethyl acetate (90:10)], 90 mg of (12*S*,13*E*)-8,12-epoxy-13-labden-15-oyl acetate (*26*).

Hydrolysis of 85 mg of *26* using 20 μl of aqueous potassium hydroxide (45 %) in 10 ml of ethanol, followed by work-up and purification by HPLC [Spherisorb/CN; hexane–ethyl acetate (60:40)] yielded 20 mg of (12*S*,13*E*)-8,12-epoxy-13-labden-15-ol (*19*), which was an oil and had $[\alpha]_{589}$ −13.9°; $[\alpha]_{578}$ −14.7°; $[\alpha]_{546}$ −17.5°; $[\alpha]_{436}$ −29.0° (*c* 0.57, $CHCl_3$); IR (CCl_4) bands at 3620, 3420 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20), 1.14 (s, H-17), 1.66 (s, H-16), 4.21 (d, J=6 Hz, H-15a and H-15b), 4.31 (m, H-12), and 5.79 (dt, J=1.5 and 7 Hz, H-14); ^1H NMR (CCl_4): δ 0.82 (s)/0.82 (s)/0.88 (s) (H-18/H-19/H-20), 1.07 (H-17), 1.61 (s, H-16), 4.05 (d, J=6.6 Hz, H-15), 4.18 (m, H-12) and 5.66 (t, J=6.6 Hz, H-14); MS [*m/z* (%)]: 306 (M, 0.3), 291 (4), 275 (37), 206 (7), 191 (100), 137 (30), 123 (34), 109 (30), 95 (38), 81 (36), 69 (53), 55 (41) and 43 (53).

*Preparation of (12*R,*13*E*)- and (12*S,*13*E*)-8,12-epoxy-13-labden-15-al (27, 28).* A solution of 4.5 mg of *18* in 1 ml of diethyl ether was stirred with 100 mg of manganese dioxide on activated charcoal at room temperature for 5.5 h. The reaction mixture was filtered and concentrated to

give 2.5 mg of (12*R*,13*E*)-8,12-epoxy-13-labden-15-al (*27*), which had IR (CCl_4) bands at 2740, 1678, 1645 and 1618 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (s)/0.84 (s)/0.88 (s) (H-18/H-19/H-20), 1.18 (s, H-17), 2.13 (d, J=0.85 Hz, H-16), 4.49 (m, H-12), 6.20 (d of quintets, J=1.3 and 8.1 Hz, H-14) and 10.05 (d, J=8.1 Hz, H-15); ^1H NMR (CCl_4): δ 0.83 (s)/0.83 (s)/0.88 (s) (H-18/H-19/H-20), 1.13 (s, H-17), 2.09 (d, J=1.2 Hz, H-16), 4.41 (m, H-12), 6.03 (d of quintets, J=1.3 and 7.4 Hz, H-14) and 9.95 (d, J=7.4 Hz, H-15); MS [*m/z* (%)]: 304 (M, 5), 289 (46), 275 (12), 206 (30), 191 (65), 137 (40), 123 (58), 109 (40), 95 (62), 81 (63), 69 (87), 55 (60) and 41 (100).

Treatment of 3.9 mg of *19* in 1 ml of diethyl ether with 100 mg of manganese dioxide on activated charcoal at room temperature for 12 h furnished 1.5 mg of (12*S*,13*E*)-8,12-epoxy-13-labden-15-al (*28*), which had IR (CCl_4) bands at 2740, 1675, 1642 and 1618 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (s)/0.82 (s)/0.88 (s) (H-18/H-19/H-20), 1.11 (s, H-17), 2.12 (d, J=1.1 Hz, H-16), 4.44 (m, H-12), 6.29 (d of quintets, J=1.4 and 8.1 Hz, H-14) and 10.05 (d, J=8.1, H-15); ^1H NMR (CCl_4): δ 0.82 (s)/0.82 (s)/0.88 (s) (H-18/H-19/H-20), 1.08 (s, H-17), 2.09 (s, H-16), 4.34 (m, H-12), 6.14 (d of quintets, J=1.4 and 7.4 Hz, H-14) and 9.95 (d, J=7.4 Hz, H-15); MS [*m/z* (%)]: 304 (M, 9), 289 (21), 275 (11), 206 (11), 191 (82), 137 (21), 121 (20), 109 (31), 95 (58), 81 (45), 69 (100), 55 (61) and 41 (93).

*Epoxidation of (12*R*,13*E*)- and (12*S*,13*E*)-8,12-epoxy-13-labden-15-ols (*18, 19*).* A solution of 30 mg of *18* in 5 ml of chloroform was stirred with 24 mg of *m*-chloroperbenzoic acid at room temperature for 1 h. The reaction mixture was washed with aqueous sodium bicarbonate and water, dried and evaporated. The residue was purified by HPLC using a column packed with Spherisorb and hexane–ethyl acetate (60:40) as an eluent to give 12 mg of (12*R*,13*R*,14*R*)-8,12-13,14-diepoxy-15-labdanol (*1*).

Epoxidation of 20 mg of *19* using 15 mg of *m*-chloroperbenzoic acid in 5 ml of chloroform for 0.5 h afforded, after work-up and purification by HPLC, 8 mg of (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanol (*2*).

*Treatment of (12*E*)-abienol (3) with* m-*chloroperbenzoic acid in a buffered system.* To a stirred solution of 65 mg of *3* in 8 ml of diethyl ether and 4 ml of 0.5 M aqueous sodium bicarbonate was added a solution of 79 mg of *m*-chloroperbenzoic acid in 1 ml of ether. The reaction mixture was stirred at 20 °C for 4 h. The ether phase was washed with water, dried and concentrated. The residue was examined by HPLC using a column packed with Spherisorb/CN and hexane–ethyl acetate (60:40) as an eluent. Polar fractions, which contained (12*R*,13*E*)-8,12-epoxy-13-labden-15-ol (*18*, 1.3 mg), (12*S*,13*E*)-8,12-epoxy-13-labden-15-ol (*19*, 2.6 mg), (12*R*,13*R*,14*R*)-8,12-13,14-diepoxy-15-labdanol (*1*, 0.3 mg) and (12*S*,13*S*,14*S*)-8,12-13,14-diepoxy-15-labdanol (*2*, 2.0 mg), were collected.

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The Reaction between Diazoalkanes and Allylic Halides Carrying Electronegative γ-Substituents. 4. Conformational Equilibria of Highly Substituted 1-Pyrazolines

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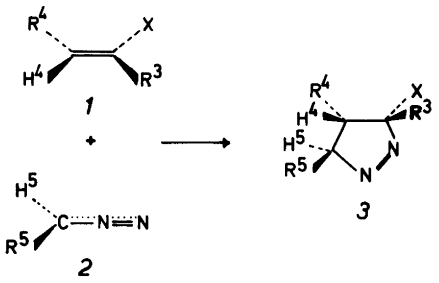
Some di- and trisubstituted 1-pyrazoline-3-carbonitriles were synthesized and the coupling constants between vicinal protons were measured. In those cases where only one large substituent is present, the pseudoequatorial position is preferred. When two large groups are present *trans* to each other in vicinal position, the dipseudoaxial conformation is preferred. The compounds with preference for the dipseudoaxial conformation unexpectedly gave only cyclopropane derivatives when decomposed.

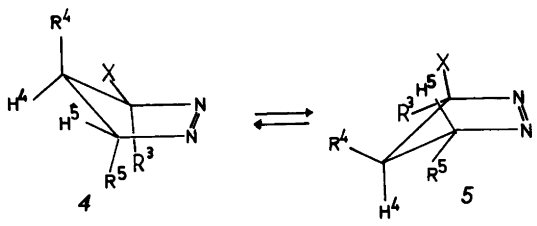
In our studies of the thermal decomposition of 1-pyrazolines substituted with cyano- or ester groups in the 3-position, 1,3-dipolar cycloaddition reactions of diazoalkanes and α,β-unsaturated diesters and dinitriles led to formation of 1-pyrazolines with the same stereochemical relation between the 4- and 5-substituents.[1–3] On the basis of the coupling constants between the vicinal hydrogens, and the sterical restrictions of 1,3-dipolar cyloaddition,[4] the substituents were assumed to be *trans* to each other (Scheme 1).

The *trans* structure of *3d* was verified by X-ray diffraction methods.[5] In the cases where $R^3 \neq X$, the stereochemistry of the starting alkene was assumed to be preserved in the 1-pyrazoline, based on the concertedness of the 1,3-dipolar cycloaddition reactions.[4]

As pointed out earlier,[6–7] the 1-pyrazoline ring system equilibrates in solution between the two conformations with C_s symmetry, where pseudoaxial and -equatorial positions are rapidly interchanging (Scheme 2). In analogy with cyclohexane systems, the relative population of each conformation is controlled by sterical interactions between substituents, and it is generally accepted that the conformer with the highest number in pseudoequatorial positions will be the most



Scheme 1. *1a*, $X=R^3=CN$, $R^4=CBrMe_2$; *1b*, $X=R^3=CN$, $R^4=Bu^t$; *1c*, X=CN, $R^3=Me$, $R^4=CBrMe_2$; *1d*, X=CN, $R^3=Bu^t$, $R^4=CBrMe_2$; *1e*, $X=R^3=CO_2Me$, $R^4=CBrMe_2$; *2a*, $R^5=H$; *2b*, $R^5=Me$; *2c*, $R^5=Ph$; *2d*, $R^5=Bu^t$; *3*, see Table 1.



Scheme 2.

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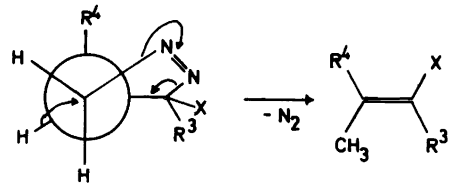
stable.[6] The observed vicinal coupling constants must, as a consequence of the dynamic situation, appear as the weighted average of the coupling constant for each conformer. Based on model substances, coupling constants of 1-pyrazolines have been used to calculate the conformational distribution in solution.[8]

The role of conformational equilibrium of 1-pyrazolines in determining the proportion between alkene and cyclopropane derivatives formed during decomposition is discussed in the literature.[6-7] A general view is that alkenes are formed *via* migration of a pseudoequatorial group (hydrogen or alkyl) from C 4 to replace the leaving nitrogen at C5 (Scheme 3).



Scheme 3.

Consequently, knowledge of the conformational situation for 1-pyrazoline derivatives is essential for prediction of the decomposition products, and for consideration of the mechanism of the pyrolysis reaction.

^{1}H NMR data of 1-pyrazolines prepared according to Scheme 1 are given in Table 1.

The stereochemistry of the alkenes *1c* and *1d* (Scheme 1) deserves some comment. *1d* was synthesized by the Wittig reaction between triphenylphosphonium isobutylide and 3,3-dimethyl-2-oxobutyronitrile followed by allylic bromination with *N*-bromosuccinimide (NBS). ^{1}H NMR of the reaction mixture from the Wittig reaction showed that a stereochemically pure alkene was formed (doublet at 5.8 ppm with splitting of 9.5 Hz). From the nature of the Wittig reaction it is likely that formation of the least sterically hindered alkene is preferred[11] and for that reason the product was assumed to have the

Table 1. Chemical shifts and vicinal coupling constants of ring protons in pyrazolines *3*.

Compound	X	R^3	R^4	R^5	δ_{H^4}	δ_{R^5}	δ_{H^5}	$J_{H_4H_5}$/Hz		Solvent[a]	Temp. °C	Ref.
								cis	*trans*			
3a	CN	CN	Bu^t	H	2.53	5.41	4.47	7.8	11.5	1	-130	*b*
3a	CN	CN	Bu^t	H	2.51	5.18	4.67	8.3	10.7	1	-50	*b, 9*
3b	Me	MeCO	Me	Ph	1.92	–	4.68	–	10.4	2	30	6a
3c	CO_2Me	CO_2Me	Bu^t	H	–	4.83	4.30	8.4	10.0	2	30	8
3d	CO_2Me	CO_2Me	$CBrMe_2$	Ph	3.12	–	5.77	–	10.0	2	30	2, 3
3e	MeCO	Me	Me	Ph	1.60	–	4.90	–	9.8	2	30	6a
3f	CN	CN	$CBrMe_2$	H	3.62	5.71	5.37	8.0	8.0	3	-70	1
3g	CN	CN	$CBrMe_2$	Ph	–	–	6.23		8.7	4	-52	2
3h	CN	CN	$CHMe_2$	H	2.36	5.25	4.43	7.3	8.7	2	-60	10
3i	CO_2Me	CO_2Me	Ph	Me	3.60	–	4.95	–	8.5	2	30	6a
3j	CO_2Me	CO_2Me	$CBrMe_2$	Me	3.20	–	5.62	–	7.8	2	30	*b*
3k	CO_2Me	CO_2Me	Bu	H	–	4.89	4.21	8.3	7.8	2	30	8
3l	CO_2Me	CO_2Me	Et	H	–	4.86	4.24	8.3	7.4	2	30	8
3m	CN	CN	$CBrMe_2$	Me	3.16	–	5.43	–	6.7	3	-60	*b*
3n	CO_2Me	CO_2Me	Me	H	2.82	4.82	4.27	8.0	6.2	2	30	8
3o	CN	Me	$CBrMe_2$	Me	3.00	–	5.11	–	5.4	2	30	*b*
3p	CN	Bu^t	$CBrMe_2$	Me	–	–	5.21	–	4.9	2	30	*b*
3q	CN	Bu^t	$CBrMe_2$	H	2.70	5.28	4.76	9.3	4.1	2	30	*b*
3r	CN	CN	Bu^t	Bu^t	2.74	–	5.48	–	3.2	3	-30	*b*
3r	CN	CN	Bu^t	Bu^t	2.83	–	5.54	–	3.1	3	-60	*b*
3r	CN	CN	Bu^t	Bu^t	2.91	–	5.62	–	2.1	3	-90	*b*
3s	CN	CN	$CBrMe_2$	Bu^t	3.22	–	5.57	–	2.8	4	-60	*b*

[a] Solvents: 1. $CHCl_2F$, 2. $CDCl_3$, 3. acetone-d_6, 4. CCl_3F. [b] This paper.

Z-configuration. The resonance line for the vinylic proton of *1d* was positioned at 6.30 ppm, which corresponds to introduction of a bromine atom in the allylic position with retention of the alkene stereochemistry.[12] The nonbrominated analogue of *1c* was formed as a 1:1 mixture of *Z*- and *E*-isomers, which were separated by fractional distillation. The shifts of the vinylic protons were 5.83 and 6.05 ppm, respectively. Bromination of both isomers resulted in the same allyl bromide with its vinylic resonance line at 6.33 ppm, very close to that of *1d*.

As mentioned above, observed vicinal coupling constants appear as weighted averages according to the conformational equilibria positions (Scheme 2). The torsion angles between *cis* substituents are 20–30° in both conformers,[5,8] and according to the Karplus equation,[13] the *cis* coupling constant will be large independent of the conformational distribution. In contrast, torsion angles between *trans* hydrogens will change from about 85° when situated in diequatorial positions (conformer *4*), to about 145° when situated in diaxial positions (conformer *5*).[5] As a consequence of the Karplus equation, the coupling constant between the *trans* hydrogens will be small when *4* is dominating, and large whenever conformer *5* is the most stable one.[8] In Table 1 the compounds appear in order of decreasing *trans* coupling constants.

At this point one must consider that differences in the substitution pattern may affect the size of the coupling constants in a somewhat unpredictable way.[13] However, these effects are small compared to the differences from the top to the bottom of Table 1 and a change above 1.5 Hz is likely to indicate a change in the conformational equilibrium.

The 4-alkylsubstituted diesters *3n, 3l, 3k* and *3c* show a steady increase in the *trans* coupling constant with increasing size of R^4 (Me<Et<Bu<*t*-Bu).[8] Hence, it is obvious that when other substituents are small, a large R^4 will prefer the pseudoequatorial position (conformer *5*). This effect may be due to the repulsive interaction between the hydrogens of R^4 in axial position and the π-electrons of the azo group. The preference for conformer *5* is also seen for the compounds *3a, 3d, 3f* and *3g*, where only one large substituent is present in the ring (the bromoisopropyl group is regarded as similar in size to a tertiary butyl group). In this connection it should be mentioned that while *3c* is reported to be "locked" in conformation *5* below –15 °C,[8] conformer *4* of the corresponding dinitrile (*3a*) is still significant at –50 °C, as seen by a further increase of J_{trans} when decreasing the temperature to –130 °C.

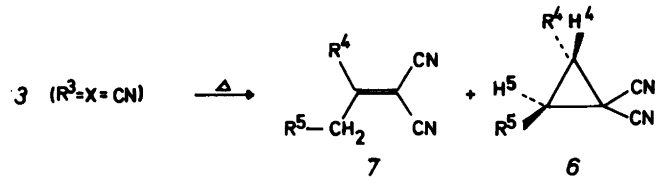
However, the situation changes completely when more than one large group are present. In going from *3f* to *3m* to *3s* the 3- and 4-substituents are kept constant, while the 5-substituent increases from hydrogen through methyl to *tert*-butyl. The result is a drastic reduction of the coupling constant (8.0–6.7–2.8 Hz), which implies a complete change in the conformational equilibrium. In *3s* the bromoisopropyl group and the *tert*-butyl group have a strict preference for the pseudoaxial position, which is in sharp contrast to earlier observations.[6–8] The same situation is seen for compounds *3q* and *3r, i.e.* whenever two large vicinal groups are present in *trans* positions. The slightly higher coupling constant of *3p* relative to *3q* may indicate that the interaction between two pseudoaxial substituents at C3 and C5 (Me, Bu^t) becomes significant, thus reducing the stability of conformer *4*.

In this connection it is interesting to note the temperature dependency in the ^1H NMR spectra of *3r*. When the temperature is reduced from –30 °C to –60 °C and further to –90 °C, the coupling constant drops from 3.2 to 3.1 to 2.1 Hz. Since a reduction in temperature will favor the most stable conformer, this drop in coupling constants represent additional evidence for the two *tert*-butyl groups being in pseudoaxial positions (conformer *4*).

Another interesting feature of the spectra of *3r* is the increased splitting of the lines from the *tert*-butyl groups, indicating hindered rotation about one of the C–CMe_3 bonds at low temperature (see Experimental). Model considerations indicate that this is due to an interaction between the *tert*-butyl group at C5 and the pseudoaxial cyano group at C3.

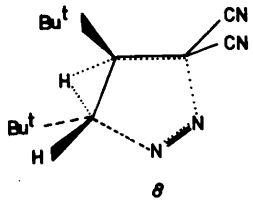
The thermal decomposition into mixtures of cyclopropanes and alkenes of the 1-pyrazoline-3,3-dinitriles appearing in Table 1 is summarized in Table 2 (Scheme 4).

We have earlier suggested that the preference for alkene formation in nonpolar solvents is due to the lack of external stabilization of the developing charge at C5, and that sterical effects are important in this connection. Since we have



Scheme 4.

found that pyrazolines *3r* and *3s* exist entirely in conformation *4* with H^4 in the correct position to replace the leaving nitrogen at C5, one should expect high yields of alkenes from these compounds. However, when decomposed both in polar and nonpolar solvents, *3r* and *3s* gave cyclopropanes as the only products. An explanation for this inconsistency with earlier assumptions may be found in the serious compression of the torsion angle between the large substituents at C4 and C5 in the transition state leading to the alkene. The hypothetically formed alkene will have a neopentyl and a *tert*-butyl group on the same vinylic carbon atom, and if the transition state has some product-like character, serious steric hindrance is to be expected (*8*).



EXPERIMENTAL

General. Melting points (uncorrected) were determined on a micro hot-stage. IR spectra were recorded on a Perkin-Elmer 457 Grating Infrared Spectrophotometer, ^{1}H NMR spectra on a Varian A60A or Varian HA 100 15D spectrometer operating at 98 MHz, ^{13}C NMR spectra on a JEOL FX-60 FT NMR spectrometer and mass spectra on an AEI MS 902 instrument. Elemental analyses were performed by I. Beetz, West Germany.

Diazoalkanes. Diazomethane,[14] diazoethane,[15] phenyldiazomethane[16] and 1-diazo-2,2-dimethylpropane[17] were prepared according to procedures described in the literature. Instead of the usual ethereal solutions, diazoalkanes in $CDCl_3$, CHF_3, $CHCl_2F$ and CCl_2F_2 were prepared in order to study their addition to alkenes in ^{1}H NMR tubes.

Alkenes. Alkylidenemalonate *1e*[18] and alkylidenemalononitrile *1a*[19] were prepared according to literature procedures. *1b* was synthesized by the Knoevenagel condensation between 2,2-dimethylpropanal and malononitrile with Amberlite IR-45 as catalyst.[20]

2,4-Dimethyl-2-pentenoic nitrile (E+Z). Diethyl 1-cyano-ethanephosphonate was prepared in 0.1 mol scale,[21] and this "one-pot olefin synthe-

Table 2. Decomposition of 1-pyrazoline-3,3-dinitriles *3* (R^3=X=CN) to give cyclopropanes *6* and alkenes *7*.[a]

Compound	Solvent: ether		Solvent: methanol		Ref.
	6	*7*	*6*	*7*	
3a	27	73	84	16	[b]
3f	9	91	100	0	1
3g	100	0	100	0	2
3h	–	–	17	83	10
3r	100	0	–	–	[b]
3s	100	0	100	0	[b]

[a] Yield in per cent. [b] This paper.

sis" was completed by addition of 0.1 mol 2-methylpropanal in THF at –40 °C. After 1 h at ambient temperature, the reaction mixture was quenched with aq. ammonium chloride (150 ml, 20 %) and extracted with ether (400 ml). After drying and evaporation, ^{1}H NMR of the residue indicated approx. 1:1 mixtures of the isomeric alkenes. Separated by fractional distillation. *Isomer A:* 2.9 g, b.p.$_{14}$: 39–41 °C. ^{1}H NMR (CCl_4): δ 1.04 (6H,d, J=7.0 Hz), 1.8–1.9 (3H,m), 2.5–2.9 (1H,m), 5.83 (1H, 2m, J=10.0 Hz). *Isomer B:* 3.7 g, b.p.$_{14}$: 48–50 °C. ^{1}H NMR (CCl_4): δ 1.03 (6H,d,J=7.0 Hz), 1.85 (1H,d,J=1.0 Hz), 2.4–2.8 (1H,m), 6.05 (1H,2d, J_1=10.0 Hz, J_2=1.0 Hz). *A+B:* Overall yield: 6.6 g (61 %). Anal. $C_7H_{11}N$: C,H. IR (film): 2240 and 1650 cm^{-1}.

4-Bromo-2,4-dimethyl-2-(Z)-pentenoic nitrile (1c). 2,4-Dimethyl-2-pentenoic nitrile (1:1, *E+Z*) (3.7 g, 0.03 mol), was dissolved in tetrachloromethane (75 ml), added NBS (6.04 g, 0.03 mol) and dibenzoyl peroxide (50 mg) and refluxed for 1 h. After cooling, succinimide was filtered off and the product distilled. B.p.$_{0.3}$: 56–59 °C. 4.2 g (66 %). Anal. $C_7H_{10}BrN$: C,H. ^{1}H NMR (CCl_4): δ 1.97 (3H,d J=1.0 Hz), 2.07 (6H,s), 6.33 (1H, q, J=1.0 Hz). IR (film): 2250, 1650 cm^{-1}. MS: m/e 188+186 (M^+, 30 %), 107 (M^+–Br, 100 %).

*2-*tert*-Butyl-4-methyl-2-(Z)-pentenoic nitrile.* 2-Methyl-propyltriphenylphosphonium bromide (28.5 g, 0.07 mol) was slurried in benzene (360 ml) under nonaqueous conditions and inert atmosphere. A 15 % solution of butyllithium in hexane (46 ml, 0.07 mol) was added at 0 °C. To the reddish solution of the phosphonium ylide 3,3-dimethyl-2-oxo-butyronitrile[22] (8.0 g, 0.07 mol) in benzene (70 ml) was added over a period of 30 min. The mixture was stirred 1 h at ambient temperature, refluxed for 5 h and worked up in the usual manner. Traces of triphenylphosphine oxide were removed by dissolving in pentane and eluting through a short column of alumina. After fractional distillation 2.6 g (24 %) of acceptable purity (GLC>85 %) was obtained. B.p.$_{12}$: 65–68 °C. ^{1}H NMR (CCl_4): δ 1.05 (6H,d, J=10.0 Hz), 1.13 (9H,s), 2.6–3.0 (1H,m), 5.81 (1H,d, J=10.0 Hz).

*4-Bromo-2-*tert*-butyl-4-methyl-2-(Z)-pentenoic nitrile* (1d). The nonbrominated alkene from above (2.1 g, 0.014 mol) was dissolved in tetrachloromethane (25 ml), NBS (2.47 g, 0.014 mol) and dibenzoyl peroxide (25 mg) was added. After 1 h reflux, ^{1}H NMR showed complete bromination. Yield 1.9 g (59 %), b.p.$_{0.5}$: 56–58 °C. Anal. $C_{10}H_{16}BrN$: C,H. ^{1}H NMR ($CDCl_3$): δ 1.20 (9H,s), 2.07 (6H,s), 6.30 (1H,s). IR (film): 2245, 1650 cm^{-1}. MS: *m/e* 231–229 (M^+, 14 %) 150 (M^+–Br, 100 %).

*c-4-(1-Bromo-1-methylethyl)-3,t-5-dimethyl-4,5-dihydro-3*H*-pyrazole-3-carbonitrile* (3o). An ether solution of *1c* (1.0 g, 5.3 mmol) and diazoethane (1 g, 18 mmol) was left in the dark at 5 °C for 9 d. After evaporation the product was recrystallized from ether–pentane. 1.1 g (85 %), m.p. 82–85 °C (dec). Anal. $C_9H_{14}BrN$: C,H. ^{1}H NMR ($CDCl_3$): δ 1.59 (3H,d, J=7.3 Hz), 1.80 (3H,s), 1.90 (3H,s), 1.95 (3H,s), 3.0 (1H,m), 5.11 (1H,2q, J=7.3 Hz, sep. of quartets: 5.4 Hz) ^{13}C NMR ($CDCl_3$): δ 20.5–29.3–31.2–33.3 (4×Me), 62.2 (C4), 63.0 (*C*BrMe$_2$) 85.1 (C3), 91.0 (C5), 117.3 (C≡N). IR (KBr): 2250, 1555 cm^{-1}.

*c-4-(1-Bromo-1-methylethyl)-3-*tert*-butyl-4,5-dihydro-3*H*-pyrazole-3-carbonitrile* (3q). An ether solution of *1d* (0.5 g, 2.2 mmol) and diazomethane (0.42 g, 10 mmol) was left in the dark at room temperature for 15 d. ^{1}H NMR of the reaction mixture showed complete reaction. Yield 0.54 g (91 %). M.p. 81–83 °C (dec.-chloroform–pentane). Anal. $C_{11}H_{18}BrN$: C,H,N. ^{1}H NMR ($CDCl_3$): δ 1.1 (9H,s), 1.78 (3H,s), 1.98 (3H,s), ABX-system: υ_X=2.70, υ_B=4.76, υ_A=5.28, J_{AB}=19.4 Hz, J_{AX}=4.1 Hz, J_{BX}=9.3 Hz. IR (KBr): 2250, 1555 cm^{-1}.

The reaction between 2b *and* 1a. To a solution of *2b* in CCl_3F cooled to –60 °C was added *1a* dissolved in acetone-d_6 until the yellow color of diazoalkane disappeared (approx. 1 mol eqv. required). The ^{1}H NMR spectrum of the solution was recorded immediately at –60 °C and showed the formation of *3m*: δ 1.74 (3H,d,J=6.7 Hz). 2.00 (3H,s),2.16 (3H,s), 3.16 (1H,d, J=6.7 Hz). 5.43 (1H,2q, J=6.7 Hz, sep. of quartets. 6.7 Hz). When heated, nitrogen was evolved, leaving unidentified material behind.

The reaction between 2a *and* 1b. *1b* (0.5 g, 3.7 mmol) was dissolved in min. amounts of ether, cooled to –78 °C, and a 2 M ethereal solution of *2a* (1.9 ml, 3.8 mmol) was added. After 1 h pentane was added and the precipitated white solid was quickly filtered off and stored at –78 °C. The crystals were dissolved in cold $CHCl_2F$, and ^{1}H NMR spectra run at –50 °C and –130 °C, thus identifying the structure *3a*; *–50 °C*: δ 1.20 (9H,s), ABX-system: υ_X=2.51, υ_A=5.18, υ_B=4.67, J_{AB}=18.2 Hz, J_{AX}=8.3 Hz, J_{BX}=10.7 Hz. *–130 °C*: δ 1.21 (9H,s), ABX-system: υ_X=2.53, υ_A=5.41, υ_B=4.47, J_{AB}=18.0 Hz, J_{AX}=7.8 Hz, J_{BX}=11.5 Hz.

Decomposition of 3a. 100 mg *3a* was dissolved in methanol at room temperature under vigorous evolution of nitrogen. The product was shown by ^{1}H NMR to consist of an 84:16 mixture of 2-*tert*-butyl-1,1-cyclopropanedicarbonitrile[23] and 1,2,2-trimethylpropylidenemalononitrile.[24] In

ethereal solution the evolution of nitrogen was slower, giving the same products in 27:73 ratio.

The reaction between 2d *and* 1a. To a solution of *1a* (1.0 g, 5.0 mmol) in ether (5 ml) *2d* (0.5 g, 5.1 mmol) in ether (2.5 ml) was added at –78 °C. The immediate disappearance of the yellow color of diazoalkane without any gas evolution indicated the formation of a pyrazoline, which after some time precipitated from the solution. After filtration, the crystals were quickly dissolved in cold CCl_3F and ^1H NMR spectrum run at –60 °C identified the crystals as *3s:* δ 1.10 (9H,s), 1.90 (3H,s), 2.15 (3H,s), 3.22 (1H,d, J=2.8 Hz), 5.57 (1H,d, J=2.8 Hz).

Decomposition of 3s. *3s* decomposed readily at room temperature, either neat, in methanol or in ether to give a single product which was identified as trans-*2-(1-bromo-1-methylethyl)-3-tert-butyl-1,1-cyclopropanedicarbonitrile.* M.p. 110–111 °C (ether–pentane). Anal. $C_{12}H_{17}BrN_2$: C,H,N. ^{H} NMR ($CDCl_3$): δ 1.32 (9H,s), 1.88 (3H,s), 1.97 (3H,s), 2.07 (1H,d, J=10.0 Hz), 2.47 (1H,d,J=10.0 Hz). MS: m/e 189 (M^+–Br, 2 %), 57 ($C_4H_9^+$, 100 %).

The reaction between 2d *and* 1b. To a solution of *1b* in acetone-d_6 at –78 °C was added an approx. equimolar amount of *2d* in CCl_3F under immediate disappearance of the color of the diazoalkane. ^1H NMR spectra were run at –30 °C, –60 °C and –90 °C, showing the formation of *3r:*

–30 °C: δ 1.06 (18H, broad s), 2.74 (1H,d, J=3.2 Hz), 5.48 (1H,d, J=3.2 Hz).

–60 °C: δ 1.02 (3H,s), 1.07 (12H, broad s), 1.17 (3H,s) 2.83 (1H,d, J=3.1 Hz), 5.54 (1H,d, J=3.1 Hz).

–90 °C: δ 0.86 (3H,s), 1.09 (9H, broad s), 1.17 (3H,s) 1.37 (3H,s), 2.91 (1H,d, J=2.1 Hz), 5.62 (1H,d, J=2.1. Hz).

Decomposition of 3r. The CCl_3F–acetone-d_6 solution of *3r* from the ^1H NMR experiment above was allowed to warm up to room temperature, resulting in evolution of nitrogen. On the basis of the similarity of the ^1H NMR spectrum to that of 2-*tert*-butyl-1,1-cyclopropanedicarbonitrile,[23] the product was assumed to be nearly pure trans *2,3-di-*tert*-butyl-1,1-cyclopropanedicarbonitrile.* ^1H NMR (CCl_3F–acetone-d_6): δ 1.13 (18H,s), 2.00 (2H,s).

The reaction between 2b *and* 1e. To a solution of *1e* (2.0 g, 7.5 mmol) in ether was added *2b* (0.5 g, 8.9 mmol) and the mixture was left overnight at room temperature in the dark. The product (*3j*) was precipitated from the solution by adding pentane. Yield: 1.7 g (70 %). Anal. $C_{11}H_{17}BrN_2O_4$: C,H. M.p. 88–90 °C (ether–pentane). ^1H NMR ($CDCl_3$): δ 1.68 (3H,d, J=7.1 Hz), 1.88 (6H,s), 2.67 (1H,d, J=7.8 Hz), 3.75 (3H,s), 3.90 (3H,s), 4.85 (1H,2q, J=7.1 Hz, sep. of quartets: 7.8 Hz).

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Magnetization-transfer NMR Investigation of the Hydrogen Exchange in Mixtures of *N*-Methylacetamide and Water

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Hydrogen exchange rates in mixtures of *N*-methylacetamide and water are measured by the magnetization-transfer method. The exchange rate is studied as a function of pH, concentration and temperature. In mixtures with the mol ratio of NMA–H_2O=1:5.16, at 351 K, the exchange is catalyzed by protons and by hydroxyl ions; at pH=5.0, where the rate is minimal, the uncatalyzed exchange between the peptide group and H_2O amounts to 75 % of the total exchange measured. The results obtained are discussed with particular reference to the hydrogen exchange in protein solutions.

Measurements of the kinetics of hydrogen exchange in protein solutions are used as a tool to characterize protein conformations. In a recent review[1] on the acquisition and interpretation of hydrogen exchange data, the authors take a realistic attitude to the possibilities and the limitations of the method, and they state that "the interpretation of hydrogen exchange data has lagged somewhat behind the state of data acquisition and remains problematic for all but the simplest peptides". We can only agree with this statement except that we are inclined to include data on simple peptides in the problematic part. The aim of the present study of the hydrogen exchange in mixtures of *N*-methylacetamide (NMA) with water is to elucidate some fundamental aspects of the exchange reaction, and in this way to contribute to a strengthening of the basis of the interpretation of the exchange in protein solutions.

The exchange in NMA–H_2O mixtures is measured by the magnetization-transfer NMR method, as described in detail in Ref. 2. The pH dependence of the exchange rates, and the dependence on the water concentration, are studied at 351 K. This relatively high temperature is chosen in order to make the data directly comparable with available NMR measurements of the hydrogen-deuterium exchange in protein solutions.[3,4] The activation energy of the exchange reaction is estimated from measurements on three NMA-H_2O samples in the temperature range 307–370 K.

MATERIALS AND METHODS

N-Methylacetamide from Fluka (analytical grade) was distilled three times at 95 °C and 14 mmHg, using a Fischer Spaltrohr column with approximately 40 theoretical plates. The water used was glass distilled at atmospheric pressure.

Mixtures of *N*-methylacetamide and water were prepared by pipetting. The density of the components, used in the calculations of the concentrations, was 0.995 g cm^{-3} (H_2O) and 0.956 g cm^{-3} (NMA). The volume change of mixing was ignored in the calculations.

The pH was adjusted by the addition of (small amounts of) 0.5 M HCl or KOH. The pH was measured at room temperature by a Radiometer pH meter PHM 64 with a glass electrode GK 2320C, standardized on aqueous buffer solutions.

NMR EXPERIMENTS

Hydrogen exchange rates in the NMA-H_2O mixtures were measured by the magnetization-

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transfer NMR method using an unmodified Bruker HX-270 NMR spectrometer. The time dependence of the height of both the NH and the water proton signals was monitored after a selective inversion of either one of them, as illustrated in Fig. 1. In each experiment 4–40 accumulations per spectrum were employed, and the delay time between two consecutive accumulations was 4–5 times the longest relaxation time observed. The line width of the NH proton

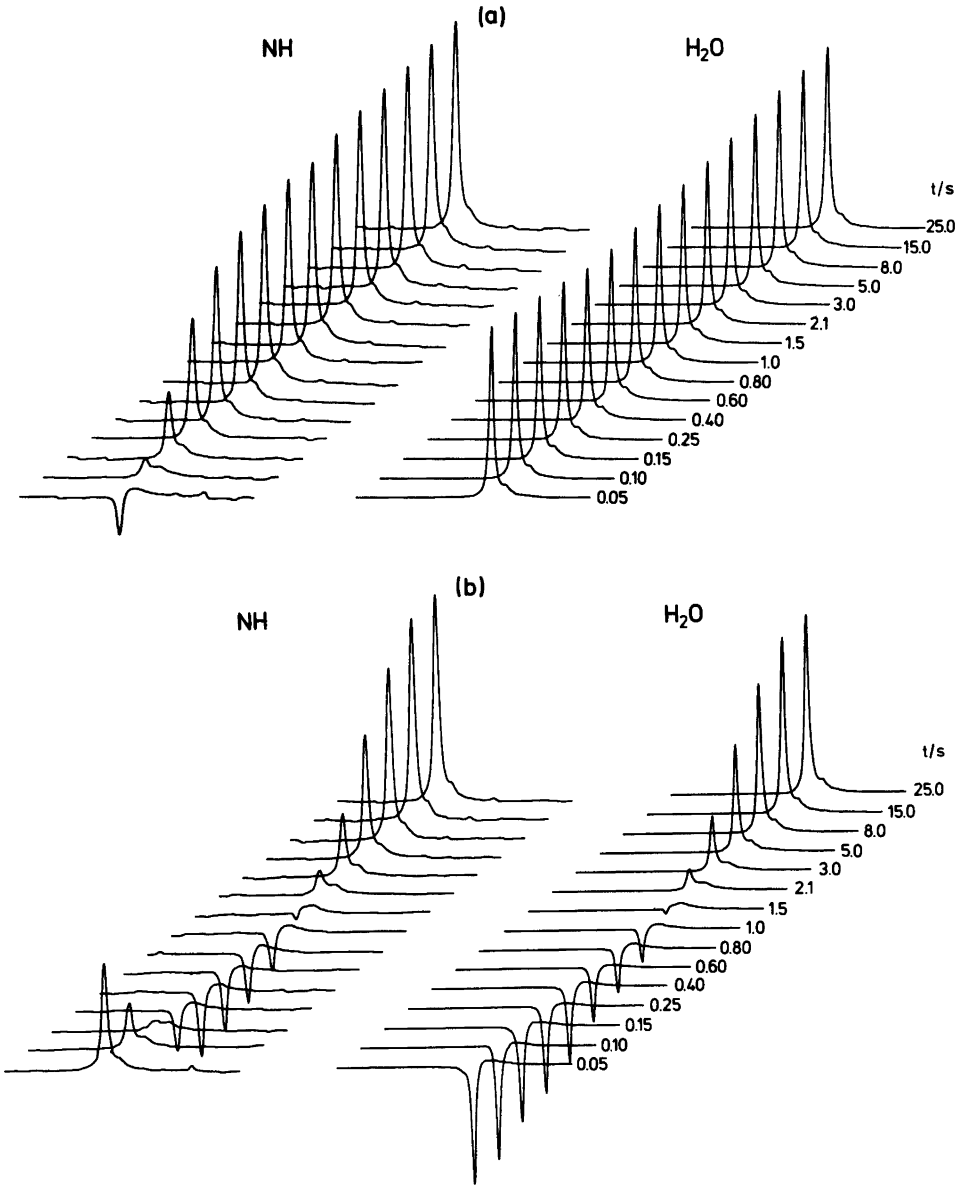

Fig. 1. Inversion transfer NMR spectra of a mixture of NMA and water. The mol ratio of the components is 1:5.16; pH=7.55, and T=339 K. In (a) the NH signal is inverted; in (b) the H_2O signal is inverted. Only every second spectrum of the experiment made is shown. The relative scaling of the NH and H_2O spectrum is ~1:20.

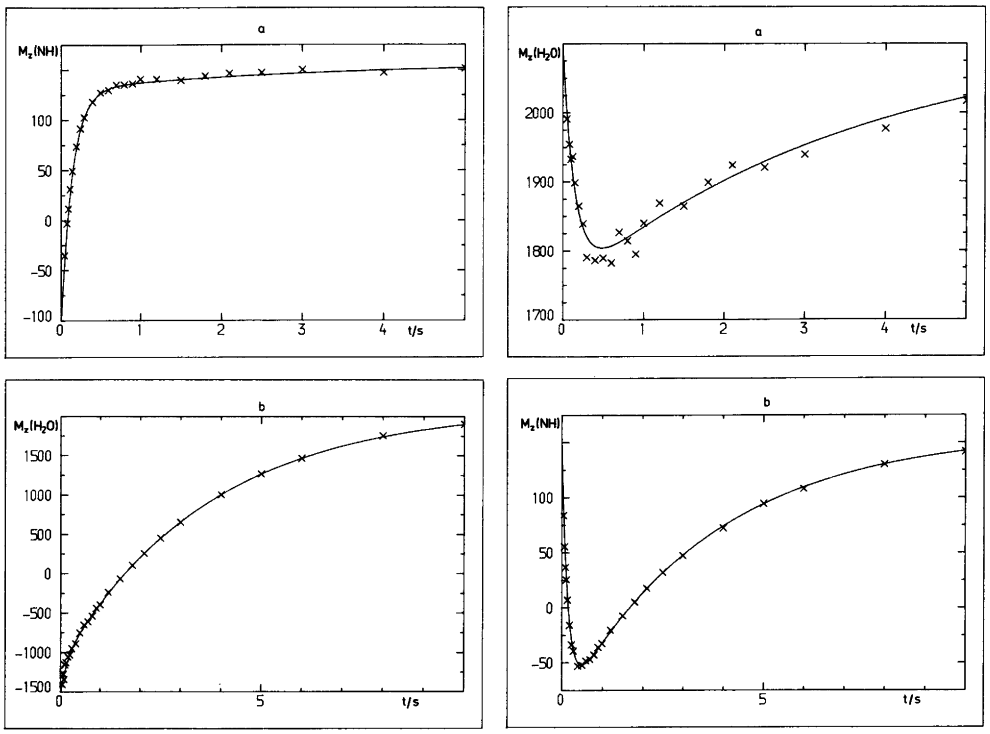

Fig. 2. Plots of the time-dependent peak heights of the set of complementary experiments in Fig. 1. The curves represent the best fit obtained in a simultaneous least squares analysis of the time dependence of the four variables.

signal was substantially decreased by a selective ^{14}N decoupling and the resulting narrowly spaced quartet was effectively averaged to a single signal by applying an exponential multiplication of the FID (free induction decay) signal corresponding to 15 Hz.

The temperature of a sample in the spectrometer was obtained from the chemical shift difference in ethylene glycol;[5] the temperature was measured before and after each experiment.

Further details about the experimental procedure are given in Ref. 2.

Data analysis. The basis of the data analysis was the McConnell equations, modified as previously described.[2] Exchange rates were retrieved by a simultaneous least squares analysis of all the data obtained in a set of complementary inversion-transfer experiments. This procedure allows an independent determination of all the parameters involved.[2] The fitting to the peak heights of the spectra in Fig. 1 is illustrated in Fig. 2.

NOE effects. While a possible static NOE effect caused by the ^{14}N-decoupling is accounted for in the experimental procedure and in the data analysis,[2] a possible transient NOE effect,[6] caused by a mutual dipolar interaction between the NH and H_2O protons, would make the estimated exchange rates smaller than the actual rates. However, in magnetization-transfer experiments performed on samples I, II and III (Table 3) at 293 K, *i.e.* under conditions where the exchange rates are too slow to be measurable, no change in the intensity of the non-inverted signal was observed. A transient NOE effect and NH–H_2O proton dipolar interactions are, therefore, not operative at 293 K. As explained previously[2] this, most likely, also holds at the higher temperatures of the present study.

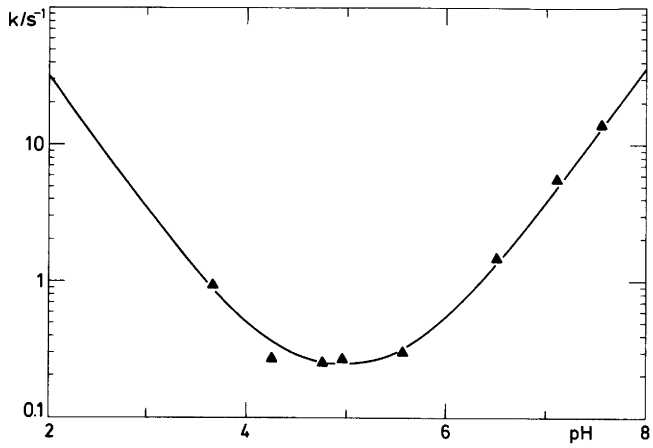

Fig. 3. The data in Table 1 plotted as log k *vs.* pH. The curve corresponds to the values of the parameters given in eqn. (4).

Table 1. Measured rates and 1σ standard deviations of the hydrogen exchange in NMA–H_2O mixtures at 351 K, and various values of pH. [NMA]=5.89(2) M; [H_2O]=30.4(2) M.

pH	k/s^{-1}
3.65	0.92(8)
4.25	0.26(3)
4.75	0.248(12)
4.95	0.267(13)
5.55	0.29(3)
6.49	1.78(18)
7.08	5.3(2)
7.55	13.2(3)

Table 2. Measured rates and 1σ standard deviations of the hydrogen exchange at 351 K in NMA–H_2O mixtures of various mol ratios.

[H_2O]/M	NMA/M	pH	k/s^{-1} (obs.)	k/s^{-1} (calc.)
1.47	12.7	7.55	0.011(2)	0.009
5.02	11.9	7.10	0.018(6)	0.019
11.4	10.4	5.30	0.056(8)	0.084
18.6	8.68	5.17	0.150(6)	0.137
23.8	7.46	5.10	0.182(5)	0.184
30.4	5.89	4.25	0.26(3)	0.25
41.4	3.27	4.25	0.48(4)	0.44
44.2	2.62	4.30	0.51(6)	0.45
48.3	1.64	4.25	0.60(5)	0.50
51.8	0.82	4.32	0.64(2)	0.65

RESULTS

Proton exchange rates, $k=\tau^{-1}$ (s^{-1}), measured at 351 K in NMA–H_2O mixtures with a mol ratio 1:5.16, and at various values of pH, are presented in Table 1 and illustrated in Fig. 3 by a plot of log k *vs.* pH.

Measurements at 351 K of the dependence of the exchange rate of the NMA–H_2O mol ratio are reported in Table 2.

In conformity with general practice, the pseudo first order exchange rate constants measured are expressed as eqn. (1),

$$k=k_{H^+}[H^+]+k_{OH^-}[OH^-]+k_w \quad (1)$$

where k_w is the contribution to k due to the uncatalyzed exchange between a peptide group and water. Assuming that this reaction is of first order with respect to the concentration of water, *i.e.* $k_w=k_{H_2O}[H_2O]$, eqn. (1) can be rewritten as

$$k=k_{H^+}10^{-pH}+k_{OH^-}K_w10^{pH}+k_{H_2O}[H_2O] \quad (2)$$

Here K_w is the ionization constant of water, $K_w=[H^+][OH^-]=K_{H_2O}[H_2O]$, where K_{H_2O} is the dissociation constant.

Values of K_w for pure water are available at temperatures up to 333 K,[7] and extrapolation of these data gives the value $K_w=25.1\ 10^{-14}$ M^2 at 351 K, where [H_2O]=55.25 M. In accordance with Fig. 15-6-1 and Table 15-6-2A in Ref. 8 the

Table 3. Measured rates and 1σ standard deviations of the hydrogen exchange as functions of temperature in three mixtures of NMA and H_2O.
I: $[H_2O]$=11.4 M; [NMA]=10.4 M pH=5.3.
II: $[H_2O]$=48.3 M; [NMA]=1.64 M pH=4.3.
III: $[H_2O]$=30.4 M; [NMA]=5.89 M pH=7.6.

Sample	*T*/K	k/s^{-1}
I	339.2	0.026(8)
	351.5	0.058(2)
	362.0	0.149(4)
	369.6	0.228(5)
II	322.1	0.068(5)
	327.2	0.102(4)
	339.2	0.270(12)
	351.5	0.60(5)
	362.0	1.20(3)
	369.6	2.41(18)
III	307.5	0.163(4)
	313.5	0.466(8)
	322.1	1.274(12)
	329.6	2.40(4)
	339.2	6.07(7)
	351.5	14.1(2)
	362.0	30.0(15)
	369.6	44.9(8)

concentration dependence of K_w at 351 K is expressed as

$$K_w=([H_2O]/55.25)\times25.1\times10^{-14}\exp[a(x_{H_2O}-1)] \quad (3)$$

Here x_{H_2O} is the mol fraction of water, and a is a parameter estimated in the numerical fitting to the experimental data.

The measurements in Table 1 and 2 were analyzed simultaneously using eqn. (2), and with K_w as given in eqn. (3). The result of the least squares analysis of the data is the following

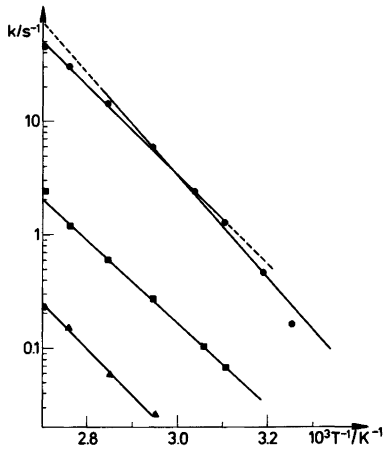

Fig. 4. The data in Table 3 plotted as log k *vs.* T^{-1}. ▲, sample I; ■, sample II; ●, sample III. The specifications of the samples are given in Table 3.

$$k_{H^+}=(2.90\pm0.65)\times10^3\ M^{-1}s^{-1}$$

$$k_{OH^-}=(1.54\pm0.28)\times10^7\ M^{-1}s^{-1}$$

$$k_{H_2O}=(6.00\pm0.43)\times10^{-3}\ M^{-1}s^{-1} \quad (4)$$

$$a=\mathrm{d}\ln K_{H_2O}/\mathrm{d}\,x_{H_2O}=10.85\pm1.2$$

The variation of the exchange rate with the temperature was measured in the temperature range 307–370 K on three samples with the mol ratio NMA–H_2O of 1:1.057 (I), 1:29.67 (II) and 1:5.16 (III), and pH values of 5.30 (I), 4.25 (II) and 7.55 (III), respectively. The rates obtained including the 1σ standard deviations are reported in Table 3 and presented in Fig. 4 as a plot of log k *vs.* T^{-1}. The apparent activation energy of the exchange reaction, estimated as $E^{\#}_{app}=-R\ \mathrm{d}\ln k/\mathrm{d}(T^{-1})$, is given in Table 4.

Table 4. The apparent activation energy of the exchange reaction.

	$E^{\#}_{app}$/kJ mol^{-1}	$E^{\#}_{H^+}$/kJ mol^{-1}	$E^{\#}_{OH^-}$/kJ mol^{-1}	$E^{\#}_{H_2O}$/kJ mol^{-1}
Sample I[a]	80 (6)			
Sample II[a]	70 (2)	97 (18)	25 (1)	48 (8)
Sample III[a]	75 (2) (322–370 K) 88 (1) (307–351 K)			

[a] The specifications of the samples are given in Table 3.

DISCUSSION

In attempts to explain the hydrogen exchange in aqueous protein solutions, it is usually assumed that the exchange in a given peptide group of a protein occurs as a direct proton transfer between the peptide group and water. Exchange rates measured in protein solutions are tentatively taken as a tool to estimate the conditions of solvent exposure of peptide groups in protein molecules, but the mechanisms governing this exposure are at present a subject of some controversy.[9,10] Part of the problems involved in a meaningful interpretation of data on protein hydrogen exchange is related to our limited knowledge of the basic exchange reaction, *i.e.* the hydrogen exchange in a peptide group exposed to water.

The pH dependence of the exchange. Measurements at or below room temperature of the exchange in dilute aqueous solutions of amides or randomly coiled peptides have shown that the exchange of peptide hydrogen atoms is catalyzed by protons and by hydroxyl ions.[11–15] The pH dependence of the pseudo first order rate constants measured is found to be in accordance with eqn. (1), and the data indicates that the uncatalyzed exchange reaction is negligible ($k_w \ll 2(K_w k_{H^+} k_{OH^-})^{1/2}$), so that

$$k \simeq k_{H^+} 10^{-pH} + k_{OH^-} K_w 10^{pH} \qquad (5)$$

This feature of the exchange reaction has been of importance in interpretations of the kinetics of the exchange in protein solutions.[1,9,10,16–18] It seems to be a generally accepted assumption, underlying discussions of the exchange, that under all conditions the pH dependence of the rate of hydrogen exchange in a solvent-exposed peptide group is in accordance with eqn. (5), with pH and K_w being the pH measured in the protein solution, and the ionization constant of pure water, respectively.

However, considerations of dynamic aspects of protein conformations have shown that access of water molecules to interior regions of protein molecules can be made through channels created by "mobile defects",[19] or volume fluctuations of cavities[20] in the conformations. Such channels hold only a limited number of water molecules, and it is to be expected that the ionization constant of water present in the interior of protein molecules may be different from the ionization constant of pure water. In an evaluation of the importance of this mechanism of exposure of protein peptide groups to water it is, therefore, of interest to know the order, with respect to water, of the exchange reaction, and to estimate the ionization constant of water within a protein molecule. Although mixtures of NMA with water are, indeed, imperfect models of protein molecules, measurements of the hydrogen exchange in such mixtures may throw some new light on the mechanisms governing the exchange in protein solutions.

In a preliminary fitting of the parameters in eqn. (1) to the data in Tables 1 and 2 k_w was expressed as $k_w = k_{H_2O}[H_2O]^n$, which resulted in the value of $n = 1.3(2)$. In the subsequent analysis of the data, which led to the results in eqn. (4), it was assumed that $n = 1$.

The values of the parameters in eqn. (4), and Fig. 3, show that under the conditions of the experiments reported, the contribution to the exchange due to the uncatalyzed reaction of NMA with H_2O is substantial. In the mixtures the ionization constant of water, calculated according to eqn. (3), is $K_w = 1.70 \times 10^{-14}$ M^2. The minimal exchange rate is observed at pH=5.0 ($[H^+]_{min} = (K_w k_{OH^-}/k_{H^+})^{1/2}$), and at this pH the uncatalyzed reaction amounts to about 75 % of the total exchange ($k_{H^+}[H^+]_{min} = k_{OH^-}[OH^-]_{min} = (K_w k_{H^+} k_{OH^-})^{1/2} = 2.7 \times 10^{-2}$ s^{-1}, and $k_w = k_{H_2O}[H_2O] = 18 \times 10^{-2}$ s^{-1}).

This result may be of interest in relation to recent NMR measurements of the exchange rates of individual peptide groups of the bovine pancreatic trypsin inhibitor (BPTI),[3,4,18] where pronounced deviations from the "normal" pH dependence, as expressed in eqn. (5), were observed. A comparison of the protein data with the results of the present investigation of the exchange in NMA–H_2O mixtures indicates that the uncatalyzed exchange reaction contributes significantly to the exchange rates observed in the protein experiments.

Since the uncatalyzed reaction is normally assumed to be negligible in peptide hydrogen exchange with bulk water,[3] the data on BPTI may suggest that the observed exchange occurs partly in reactions with "channel" water, characterized by a smaller ionization constant.

The temperature dependence of the exchange. As illustrated in Fig. 4 the exchange rates

measured show a simple exponential dependence on T^{-1}, except for sample III, where the largest temperature range is covered. The apparent activation energy of the exchange reaction, $E^{\#}_{app}$, estimated by a least squares fit of the expression $E^{\#}_{app} = -R \, d \ln k/d \, T^{-1}$ to the experimental data, is reported in Table 4. The values are within the range of the values in the literature,[1] obtained by a similar procedure.

The apparent activation energy may be considered as a sum of three contributions

$$E^{\#}_{app}(pH,[H_2O]) = (k_{H^+}/k)10^{-pH}E^{\#}_{H^+} + (k_{OH^-}/k)K_W \times 10^{pH}(E^{\#}_{OH^-} + \Delta H^{\circ}_W) + (k_{H_2O}[H_2O]/k)E^{\#}_{H_2O} \quad (6)$$

where ΔH°_W is the ionization enthalpy of water. In this expression it is assumed that the individual activation energies and ΔH°_W are independent of the temperature.

At pH=7.6 (sample III) the first two terms of eqn. (6) are negligible, so that $E^{\#}_{app} \simeq E_{OH^-} + \Delta H^{\circ}_W$. The observed deviation from a linear relationship between ln k and T^{-1}, may be due to change in ΔH°_W with changes in the temperature. In pure water ΔH°_W varies only about 10 % [7] in the observed temperature region ($\Delta H^{\circ}_W \simeq 50$ kJ mol^{-1}), but the strong solvation of peptide groups by water [21] may influence the ionization enthalpy of water in the mixtures studied, as well as its temperature dependence.

In an attempt to estimate the individual activation energies, $E^{\#}_{H^+}$, $E^{\#}_{OH^-}$ and $E^{\#}_{H_2O}$, by a numerical analysis based on a combination of eqns. (2) and (6), we have tentatively made the following approximations:

(1) Measurements made below 314 K (sample III) are ignored and (2) the ionization enthalpy of water in the mixtures is assumed to be equal to the ionization enthalpy of pure water at 333.15 K, $\Delta H^{\circ}_W = 52$ kJ mol^{-1},[7] and independent of the temperature. The results of the analysis are presented in Table 4. The relatively small value of $E^{\#}_{H_2O}$, compared with $E^{\#}_{H^+}$ and $(E^{\#}_{OH^-} + \Delta H^{\circ}_W)$, indicates that the uncatalyzed exchange reaction is of increasing importance with decreasing temperature, or that the plateau in a plot of ln k *vs.* pH becomes broader when the temperature is lowered. This behaviour is in contrast to the general view that the plateau is absent at room temperature.[1] It may, however, be noted that the values of $E^{\#}_{H^+}$, $E^{\#}_{OH^-}$ and $E^{\#}_{H_2O}$, reported in Table 4, are based on measurements above 314 K, and that the data on sample III in Fig. 4 suggest that a straight-lined extrapolation of ln k *vs.* T^{-1} below 314 K may not be justified.

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Syntheses and Reactions of Some 5-Vinyl- and 5-Ethynylpyrimidines

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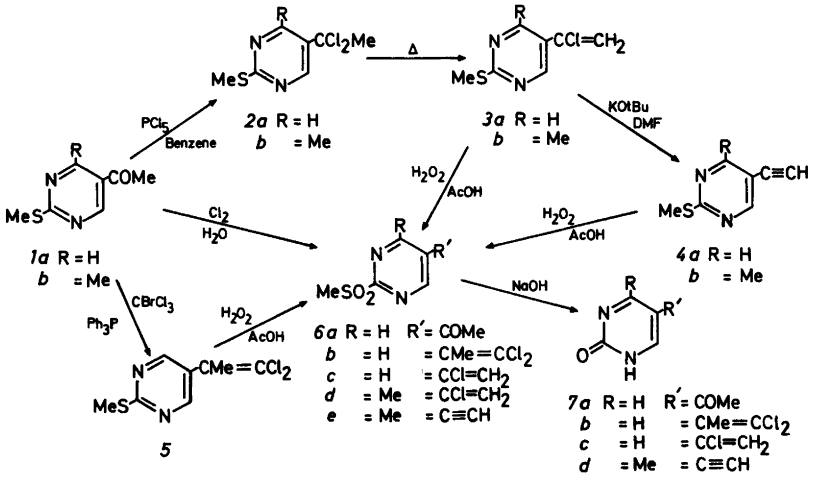
5-β,β-Dichlorovinyl- and 5-α-chlorovinylpyrimidines have been prepared from 5-acetyl-2-methylthiopyrimidines. HCl elimination from the 5-α-chlorovinyl derivatives yields the 5-ethynyl analogues. Peracid or chlorine oxidation of the sulfides yields the sulfones which may be hydrolyzed to the corresponding lactams. The reaction paths are discussed.

Certain 2-alkylsulfonyl-5-substituted pyrimidines possess the ability to inhibit cell proliferation.[1] In this report we describe studies on 2-methylsulfonylpyrimidines carrying unsaturated substituents in the 5-position, in particular vinyl and ethynyl groups.

As starting materials for the syntheses were chosen 5-acyl pyrimidines since simple routes to the preparation of 5-acyl-2-thiopyrimidines are available.[2,3] Furthermore, 5-acylpyrimidines have been converted to olefins by Wittig type reactions,[4] or 5-ketones to α-chlorolefins by treatment with phosphorus pentachloride [5] or by phosphorus oxychloride.[6] We have found that the 5-acetyl derivatives *1* can be converted into the desired 5-α-chlorovinyl derivatives *3* using phosphorus pentachloride in benzene (Scheme 1). Under these conditions replacement of the 2-methylthio substituent was avoided.

The crude reaction product from the phosphorus pentachloride reaction was a mixture (*ca.* 1:1) of the 5-(1,1-dichloroethyl)pyrimidine *2* (^{1}H NMR) and the 5-(1-chlorovinyl)pyrimidine *3*. The former is the intermediate product in the reaction which subsequently eliminates hydrogen



Scheme 1.

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chloride. Elimination went essentially to completion when the mixture was distilled slowly; on rapid distillation both products codistilled. Addition of catalytic amounts of aluminium tribromide before distillation, however, gave the pure vinyl product *3*. The distillate from the 4-methyl homologue *1b* also contained the HCl salt of the base; combination of the base and the gaseous HCl was not observed for *3a*. This may reflect the effect of the 4-methyl substituent on the basicity.

The synthesis of the β,β-dichlorovinyl derivative *5* was initially attempted in the Wittig manner using the phosphorus ylide dichloromethylentriphenylphosphorane generated *in situ* from triphenylphosphine in carbon tetrachloride.[7] The product, however, consisted of a mixture (3:2) of the dichlorovinyl derivative *5* and the α-chlorovinyl derivatives *3a*. Formation of α-chlorolefins have also previously been observed in attempted Wittig reactions with triphenylphosphine in tetrachloromethane, and this course of the reaction has been rationalized by initial formation of dichlorotriphenylphosphorane which reacted further with the ketone.[8] Better yields and cleaner products are claimed by the use of bromotrichloromethane instead of tetrachloromethane.[9] This modification, when applied to the reaction of *1a*, gave 72 % yield of *5*. In addition there was a little of the corresponding monobromo analogue, 5-(2-bromo-2-chloro-1-methylvinyl)-2-methylthiopyrimidine (MS). The latter was readily removed on recrystallization.

A strong base is required for elimination of HCl from the α-chlorovinyl derivative *3* to form the acetylene *4*; potassium *tert*-butoxide in DMF caused the reaction to proceed at room temperature without affecting the 2-methylthio substituent. When attempting the reaction with potassium hydroxide in ethanol, addition products of ethanol to the triple bond were seen together with the desired 5-ethynyl compound.

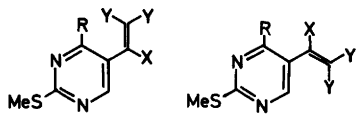
There was a significant difference in the rate of elimination of HCl from *3a* and *3b*; the reaction of *3b* was complete after 4 h at room temperature, whereas only 40 % of the 4-methyl derivative *3a* had reacted after 15 h.

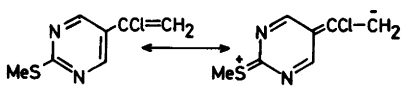
The long-wave absorption of the 4-methyl derivative *3b* is at lower wavelength (MeOH, 264 nm) than for *3a* (278 nm) and its absorptivity is less. This suggests that the non-bonded interaction from the methyl group (*8*, R=Me, X=Cl and Y=H) causes the vinyl group to twist out of the plane of the pyrimidine and thereby reduces resonance interaction with the pyrimidine ring. The electron donating resonance interaction from the *para* methylthio group (*9*) is therefore reduced in *3b* as compared with *3a*. The elimination reaction probably proceeds by an ElcB-type mechanism because of the electron attracting properties of the pyrimidine, the use of a strong base and the relatively poor leaving properties of the chloride ion. The incipient carbanion on the β-carbon of the vinyl group is more destabilized by the electronic effects of the methylthio group in *3a* than in *3b* in accordance with the relative rates of elimination.

The sulfones of the 5-vinyl and 5-ethynyl derivatives were prepared from the respective sulfides using peracetic acid generated *in situ* from 30 % hydrogen peroxide and acetic acid. Under these experimental conditions the unsaturated 5-substituents are deactivated towards oxidation and selective formation of the sulfones was experienced. The 5-acetyl derivative *1a* reacted differently, presumably because the relatively long reaction time (24 h) led to solvolysis of the 2-substituent or reaction on the acetyl function. By using chlorine in aqueous solution as oxidizing agent,[10] the reaction time could be shortened (30 min) and *1a* was converted to its sulfone *6a* in good yield. In ^{1}H NMR the oxidation of a sulfide to a sulfone is seen by a downfield shift of *ca.* 0.8 ppm for the signals of the methyl protons.

The sulfonyl group, in an activated azine position, is readily displaced by nucleophiles. Such sulfones may, therefore, in part exert their biological activity by substitution at the sulfonyl-attached carbon atom by a hydroxy, amino or thiol group at some essential biological site. Tested as inhibitors of cell proliferation,[1] the sulfones *6* were found to possess strong toxic properties.

The relative ease of the displacement of the sulfonyl group of the sulfones *6* is shown by hydrolysis in 0.5 M sodium hydroxide at room temperature. The activation from the acetyl group in *6a* resulted in an almost immediate transformation of *6a* to the corresponding lactam *7a*. *6a* was also hydrolyzed to *7a* under neutral conditions. The reaction time for the β,β-dichlorovinyl derivative *6b* was more than 6 h, whereas the hydrolysis of the α-chlorovinyl de-





Scheme 2.

rivatives *6c* and *6d* was complete in the course of 10–15 min. As opposed to *6a*, *6c* was not hydrolyzed under neutral conditions (5 days, room temperature). The relatively low rate of hydrolysis of the dichloride *6b* is attributed to the conformational preferences of the vinyl group. In the two conformations where the vinyl group lies in the plane of the pyrimidine ring (*8*, R=H, X=Me and Y=Cl) there is a serious non-bonded interaction between an α-methyl or a β-chloro substituent of the vinyl group with the 4- and 6-substituents of the pyrimidine. The vinyl group is, therefore, twisted out of the pyrimidine plane and will have less influence on the electronic properties leading to sulfonyl displacement than if it had been coplanar.

The hydrolytic reaction of the 4-methyl derivative *6d* also led to complete elimination of HCl to give the 5-ethynyl lactam *7d*, whereas no HCl elimination in the hydrolysis of *6c* was observed. The different nature of the products from the hydrolysis of *6c* and *6d* must be attributed to the influence of the 4-methyl group, which may be mainly steric in nature. The experimental findings are then rationalized by assuming initial elimination of HCl from the 4-methyl derivative *6d* with subsequent hydrolysis of the 2-substituent. In contrast, in *6c* the sulfonyl group is initially displaced to give the lactam *7c*. In alkaline solution the latter is negatively charged as a sodium salt, and the negative charge of the pyrimidine ring protects the α-chlorovinyl group against the elimination reaction.

EXPERIMENTAL

5-(1-Chlorovinyl)-2-methylthiopyrimidine 3a. Phosphorus pentachloride (6.2 g, 30 mmol) was added to a solution of 5-acetyl-2-methylthiopyrimidine [3] (3.30 g, 20 mmol) in dry benzene (100 ml). The mixture was refluxed for 3 h before the cooled solution was poured into ice and water (150 ml). The aqueous phase was neutralized with sodium carbonate and extracted with benzene. The combined extracts were dried ($MgSO_4$) and evaporated. The residue was distilled under reduced pressure with a catalytic amount of aluminium tribromide; b.p. 96 °C/0.01 mmHg. The product was recrystallized from light petroleum; yield: 2.1 g (56 %), m.p. 73–75 °C. Anal. $C_7H_7ClN_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.57 (MeS), 5.58 and 5.82 (J 2 Hz, vinyl), 8.72 (H-4, H-6). IR (KBr): 1615 (vinyl), 1580 and 1530 cm^{-1} (pyrimidine). MS [70 eV; *m/z* (% rel.int.)]: 188/186 (35/100, M), 185(20), 151(20), 140(31), 105(33), 86(15), 51(30).

5-(1-Chlorovinyl)-4-methyl-2-methylthiopyrimidine 3b was prepared as *3a* above from 5-acetyl-4-methyl-2-methylthiopyrimidine [2] in 59 % yield, b.p. 129–131 °C/0.2 mmHg. Anal. $C_8H_9ClN_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.55 (Me-4 and MeS), 5.45 and 5.72 (J 1.5 Hz, vinyl), 8.33 (H–6). IR (KBr): 1630 (vinyl), 1570/1530 cm^{-1} (pyrimidine). MS [70 eV; *m/z* (% rel.int.)]: 202/200 (36/100, M), 199(19), 165(21), 154(30), 119(23).

5-Ethynyl-2-methylthiopyrimidine 4a. 1 M potassium *tert*-butoxide (7 ml) was added to a solution of 5-(1-chlorovinyl)-2-methylthiopyrimidine (1.40 g, 6.3 mmol) in DMF (25 ml) over 5 min. The mixture was stirred at room temperature for 48 h before the solvent was distilled off. The residue was triturated with water and extracted into chloroform. The dried solution ($MgSO_4$) was evaporated and the residue sublimed at 30 °C/90 mmHg; yield 8 %, m.p. 46–47 °C. Anal. $C_7H_6N_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.55 (MeS), 3.30 (CH), 8.55 (H–4, H–6). IR (KBr): 3280 (CH), 2120 (C≡C), 1590/1530 cm^{-1} (pyrimidine). MS [70 eV: *m/z* (% rel.int.)]: 150 (100, M), 105(19), 104(31), 77(18), 73(25).

5-Ethynyl-4-methyl-2-methylthiopyrimidine 4b. 1 M potassium *tert*-butoxide (7 ml) was added to a solution of 5-(1-chlorovinyl)-4-methyl-2-methylthiopyrimidine (1.00 g, 5 mmol) in DMF (20 ml) over 10 min. The mixture was stirred at room temperature for 4 h before the solvent was distilled off. The residue was triturated with water and extracted into chloroform. The dried solution ($MgSO_4$) was evaporated and the residue purified on a silica column (silica gel 60, 70–230 mesh, chloroform); yield: 0.70 g (85 %), m.p. 75–76 °C. Anal $C_8H_8N_2S$: C, H. ^{1}H NMR

($CDCl_3$): δ 2.57 (MeS, Me-4) 3.47 (CH), 8.46 (H–6). IR (KBr): 3390 (CH), 2100 (C≡C), 1570/1520 cm $^{-1}$ (pyrimidine). MS [70 eV; *m/z* (% rel.int.)]: 164 (100, M), 163(24), 119(11), 118(44), 77(10), 64(22), 63(19).

5-(2,2-Dichloro-1-methylvinyl)-2-methylthiopyrimidine 5. Bromotrichloromethane (15 ml, 150 mmol) was added to a mixture of 5-acetyl-2-methylthiopyrimidine [3] (1.10 g, 6.5 mmol) and triphenylphosphine (4.00 g, 15 mmol) in dry benzene (15 ml). The mixture was stirred for 2 h at 50 °C under N_2 before the solvent was distilled off. The residue was dissolved in acetone (20 ml) and ether added (300 ml). The solution was filtered and the filtrate evaporated. The residue was dissolved in ether (30 ml), filtered and the filtrate evaporated. The residual material was sublimed at 60–70 °C/0.03 mmHg; yield 1.10 g (72 %), m.p. 61–62 °C. Anal. $C_8H_8Cl_2N_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.20 (CH_3C=) 2.57 (MeS), 8.47 (H–4, H–6). IR (KBr): 1590/1530 cm $^{-1}$ (pyrimidine). MS [70 eV; *m/z* (% rel.int.)]: 238/236/234 (12/66/100,M), 233(29), 191(12), 190(18), 188(27), 153(40), 99(24).

5-Acetyl-2-methylsulfonylpyrimidine 6a. Chlorine was slowly bubbled through a suspension of 5-acetyl-2-methylthiopyrimidine (0.48 g, 2.9 mmol) in water (30 ml) for 30 min at 5 °C. The mixture was neutralized with potassium carbonate and extracted into chloroform. The dried solution ($MgSO_4$) was evaporated and the residue recrystallized from ethanol; yield 0.49 g (84 %), m.p. 132–134 °C. Anal. $C_7H_8N_2O_3S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.72 (CH_3CO), 3.36 ($MeSO_2$), 9.35 (H–4, H–6). IR (KBr): 1700 (CO), 1580/1560 (pyrimidine), 1310/1140 cm $^{-1}$ (sulfone). MS [70 eV; *m/z* (% rel.int.)]: 200 (18,M), 185(11), 137(29), 136(21), 123(28), 121(47), 95(22), 43(100).

Oxidation of the sulfides 3a–5 *with 30 % hydrogen peroxide.* Hydrogen peroxide (30 %, 8 ml) was added to a solution of the sulfide (12 mmol) in acetic acid (25 ml). The mixture was stirred for 2 h and left overnight. The solution was concentrated to ⅓ of its original volume before water (50 ml) was added, and the solution extractred with chloroform (3×50 ml). The extract was washed with a saturated solution of aqueous sodium carbonate and dried ($MgSO_4$). The solvent was distilled off and the residue recrystallized.

5-(2,2-Dichloro-1-methylvinyl)-2-methylsulfonylpyrimidine 6b. Yield: 66 %, m.p. 134–135 °C (MeOH). Anal. $C_8H_8Cl_2N_2O_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.18 (CH_3–C=), 3.37 ($MeSO_2$), 8.86 (H–4, H–6). IR (KBr): 1550 (pyrimidine), 1310/1130 cm $^{-1}$ (sulfone). MS [70 eV; *m/z* (% rel.int.)]: 270/268/266 (5/39/56,M), 231(15), 205(24), 204(18), 203(37), 189(34), 187(54), 171(31), 169(100), 168(52).

5-(1-Chlorovinyl)-2-methylsulfonylpyrimidine 6c. Yield: 50 %, m.p. 111–114 °C (MeOH). Anal. $C_7H_7ClN_2O_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 3.37 ($MeSO_2$), 5.88 and 6.10 (*J* 2.5 Hz, vinyl), 8.85 (H–4, H–6). IR (KBr): 1610 (C=CH_2), 1560/1550 (pyrimidine), 1310/1130 cm $^{-1}$ (sulfone). MS [70 eV; *m/z* (% rel.int.)]: 220/218 (6/16, M), 157(33), 155(100), 141(26), 139(74), 112(31), 100(13), 87(25).

5-(1-Chlorovinyl)-4-methyl-2-methylsulfonylpyrimidine 6d. Yield: 54 %, m.p. 67–68 °C (CCl_4/light petroleum). Anal. $C_8H_9ClN_2O_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.75 (Me–4), 3.34 ($MeSO_2$), 5.62 and 5.92 (*J* 2 Hz, vinyl), 8.74 (H–6). IR (KBr): 1630 (vinyl), 1560/1540 (pyrimidine), 1310/1130 cm $^{-1}$ (sulfone). MS [70 eV; *m/z* (% rel.int.)]: 234/232 (19/53,M). 171(32), 170(27), 169(100), 168(32), 155(32), 153(99), 135(75), 126(50), 118(44), 99(38).

5-Ethynyl-4-methyl-2-methylsulfonylpyrimidine 6e. Yield: 26 %, m.p. 97–98 °C (CCl_4/light pertroleum). Anal. $C_8H_8N_2O_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.78 (Me–4), 3.35 ($MeSO_2$), 3.79 (CH), 8.85 (H–6). IR (KBr): 3250 (CH), 2120 (C≡C), 1310/1140 cm $^{-1}$ (sulfone). MS [70 eV; *m/z* (% rel.int.)]: 196 (14,M), 181(8), 153(10), 134(61), 132(23), 117(100), 106(25), 90(60).

Hydrolysis of the sulfones 6 *with 0.5 M sodium hydroxide.* A mixture of the sulfone (2.5 mmol) and 0.5 M sodium hydroxide (25 ml) was stirred at room temperature till the sulfone had disappeared (TLC). The reaction times were 5 min (*6a*), 18 h (*6b*), 15 min (*6c*) and 10 min (*6d*). The solution was acidified (pH 5) with 1 M hydrogen chloride and extracted with ethyl acetate. The extract was dried ($MgSO_4$), evaporated and the residue sublimed.

5-Acetyl-2-pyrimidinone 7a was sublimed at 140–150 °C/0.03 mmHg; yield 29 %, m.p. 169–171 °C. ^{1}H NMR (DMSO-d_6): δ 2.38 (CH_3CO), 8.74 (H–4, H–6). IR (KBr): 2900–2600 (NH), 1730/1670/1630 cm $^{-1}$ (CO). MS [70 eV; *m/z* (% rel. int.)]: 138 (74,M), 123(100), 96(60), 95(32), 68(15), 53(22), 43(46). High resolution of M: found 138.0429, calc. for $C_6H_6N_2O_2$: 138.0429.

5-(2,2-Dichloro-1-methylvinyl)-2-pyrimidinone 7b. The title compound precipitated upon acidifying in 77 % yield, m.p. 228–229 °C (MeOH). Anal. $C_7H_6Cl_2N_2O$: C, H. ^{1}H NMR (DMSO-d_6): δ 2.12 (Me), 8.33 (H–4, H–6). IR (KBr): 1735 cm $^{-1}$ (CO). MS [70 eV; *m/z* (% rel.int.)]: 208/206/204 (5/30/46,M), 171(32), 169(100), 106(12), 99(10), 63(10).

5-(1-Chlorovinyl)-2-pyrimidinone 7c was sublimed at 110–120 °C/0.03 mmHg; yield: 62 %, m.p. 130–140 °C (decomp.). ^{1}H NMR (DMSO-

d_6): δ 5.44 and 6.01 (J 2.5 Hz, vinyl), 8.56 (H–4, H–6). IR (KBr): 3100–2700 (NH), 1660–1640 cm^{-1} (CO). MS [70 eV; m/z (% rel.int.)]: 158/156 (18/58,M), 155(15), 137(9), 121(100), 101(14), 93(18), 66(17), 51(34). High resolution of M: found 156.0086, calc. for $C_6H_5ClN_2O$: 156.0089.

5-Ethynyl-4-methyl-2-pyrimidinone 7d was sublimed at 130–140 °C/0.01–0.03 mmHg; yield 66 %, m.p. 180 °C (decomp.). ^{1}H NMR (DMSO-d_6): δ 2.34 (Me–4), 4.34 (CH), 8.31 (H–6). IR (KBr): 3250 (CH), 3080–2600 (NH), 1690 cm^{-1} (CO). MS [70 eV; m/z (% rel. int.)]: 134 (100,M), 107(30), 106(27), 79(14), 64(28), 50(37). High resolution of M: found 134.0473, calc. for $C_7H_6N_2O$: 134.0480.

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Short Communications

Separation of Protein and Polyethylene Glycol in Water Solutions by a Desalting Technique

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Various methods for precipitation of proteins have been used as a means for purifying proteins from a mixture. The types of reagents for these precipitations are inorganic salts like ammonium sulfate or sodium sulfate, organic solvents, polyelectrolytes and nonionic hydrophilic polymers. In the latter group, polyethylene glycol (PEG) is most commonly used. This procedure is very useful because rigid control of ionic strength and temperature is not very critical, the proteins are not denaturated and the yields are generally high.

PEG precipitations often lead to very viscous fractions which are difficult to handle. This problem was seen in our laboratory when we isolated albumin from serum by precipitation of the other proteins with PEG after a method of Gambal.[1] Undiluted serum was stirred at 4 °C and powdered PEG 6000 was added slowly to a concentration of 25 % (w/V). The mixture was stirred for 24 h and then centrifuged. Under these conditions albumin is the only protein that can be detected in the supernatant. PEG can be removed from the albumin solution by prolonged dialysis, but Gambal instead recommended gel filtration on a Sephadex G-75 column. However, in our experiments even an extensive dilution of the supernatant before gel filtration leads to a very viscous solution which would clog the Sephadex column. Instead we tried to separate albumin and PEG by a desalting technique and found that addition of, for instance, ammonium sulfate will cause the supernatant to decompose into two phases. This phenomenon may be due to the reduced solubility of PEG in water by addition of salt. The observation that the protein and the PEG appeared unevenly distributed among the two phases motivated us to examine the relative amounts of protein and PEG in the two phases for different types and concentrations of PEG.

Experimental. PEG 1550 pract. (*M* 1300–1600; m.p. 34–41 °C) and PEG 4000 pract. (*M* 3000–3700; m.p. 53–56 °C) were purchased from Serva, Heidelberg, W. Germany, PEG 6000 was from the British Drug

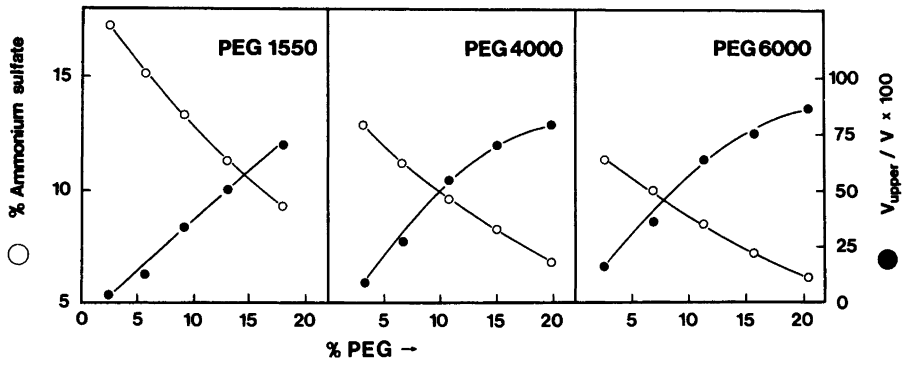

Fig. 1. To 5 ml PEG-solution (PEG 1550, 4000 and 6000) in water is added 3 M ammonium sulfate until onset of turbidity. ○=% ammonium sulfate in final solution, ●=volume of upper phase/total volume ×100 after phase separation. % PEG relates to the final solution.

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Table 1. Phase separation of serum albumin/PEG-solutions by ammonium sulfate.

PEG	% PEG in initial solution	% PEG in final solution	% ammonium sulfate in final solution	$V_{upper}/V \times 100$	% of total amounts in the lower phase		
					PEG	ammonium sulfate	albumin
	4.5	2.1	18.6	3	93	98	67
	9.1	5.0	15.1	7	70	94	83
1550	13.0	6.8	16.1	16	57	93	86
	16.6	precipitation					
	19.9	precipitation					
	4.5	2.8	13.8	11	81	99	98
	9.1	6.3	10.2	18	40	88	90
4000	13.0	8.3	12.0	43	9	67	90
	16.6	12.3	8.7	66	14	47	85
	19.9	15.2	8.1	83	2	28	89
	4.5	3.1	11.9	10	57	94	99
	9.1	6.1	10.8	26	55	74	95
6000	13.0	9.3	9.6	54	20	54	91
	16.6	12.4	8.5	56	2	68	75
	19.9	no visible phase separation					

House. Bovine Serum albumin, crystallized and lyophilized (No. A.4348) was from the Sigma Chemical Company. Ammonium sulfate concentrations were determined by ammonium determination, either by Kjeldahl analysis or by the indophenol method.[2] A Cary 16 spectrophotometer was used to determine protein. Absorbance at 280 nm, in protein solutions without PEG, was determined as an index of protein concentration.

Phase separation in water solutions of pure PEG by addition of ammonium sulfate. To a 5 ml solution of PEG in water, 3.0 M ammonium sulfate is added from a burette until the onset of turbidity. After standing overnight (maximum 20 h) or centrifugation the solution separates into two phases.

Phase separation in water solutions of protein and PEG by addition of ammonium sulfate. To a solution of protein and PEG (3 ml solution of bovine serum albumin plus 3 ml solution of PEG) 3.0 M ammonium sulfate is added from a burette until the onset of turbidity. After centrifugation (3000 G, 30–60 min) the solution separates into two phases. Protein concentration in the lower phase is determined after removal of the PEG: 0.3 ml of the lower phase is applied to a Sephadex G-25 fine column (dimensions: 1×18 cm) and the column is eluted by 0.4 M sodium chloride. (PEG in the concentrations in the lower phase does not clog the column.) Fractions (2–4 ml) were collected and protein determined. Total weights of protein, ammonium sulfate and PEG in upper and lower phase were determined by weighing after lyophilization. Ammonium sulfate concentrations in the upper and the lower phase were determined by ammonia determination on the lyophilized products (corrected for protein in the phases). PEG concentrations in the upper and the lower phase were determined by "difference computing".

Results and discussion. In the experiment shown in Fig. 1 3.0 M ammonium sulfate was added to samples containing 0.25–1.27 g PEG (PEG 1550, 4000 and 6000) in 5 ml water at 22 °C until the onset of turbidity. After standing, the solutions split up into two phases. The figure illustrates that all three kinds of PEG show the same pattern of phase separation. Increasing concentrations of PEG result in decreasing amounts of ammonium sulfate necessary to obtain phase separation. By increasing the PEG concentrations all three kinds of PEG show increasing ratios of upper volumes to lower volumes in the phase separation.

Similar experiments were undertaken for samples containing bovine serum albumin besides PEG. To a mixture of 30 mg serum albumin in 3 ml water and 0.3–1.5 g PEG in 3 ml water, at 22 °C, 3.0 M ammonium sulfate was added until the onset of turbidity. After phase separation the phases were analyzed for protein, PEG and

ammonium sulfate as described above. Table 1 illustrates typical experiments. Addition of ammonium sulfate results in high concentrations of PEG 1550 in the formation of precipitates, whereas no phase separation occurs for PEG 6000 in high concentration. Apart from that, the results are qualitatively similar to those from the experiments without protein, although not reproducible to the same degree.

The table shows that in most experiments nearly all the protein is in lower phase. The recovery may vary from experiment to experiment, by very careful manipulation the protein amount in lower phase can be brought up to about 90–99 % of total protein for all the three PEG's at the different concentrations. The amounts of PEG in the lower phase range from about 2–93 % of total PEG. It is remarkable that a small amount of PEG (2–20 %) in the lower phase is found for PEG 4000 and 6000 in initial concentrations about 13–20 (17) %. Under these conditions the volume of the lower phase is about 25–85 % of the original protein-PEG volume. Thus, separation of protein from PEG 4000 and 6000 by means of ammonium sulfate seems to be a practical tool in protein purification procedures in which PEG has initially been introduced to cause protein precipitation.

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Denaturation and Reactivation of Bovine and Human Cobalt–Carbonic Anhydrases in Guanidine Hydrochloride *

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Bovine and human erythrocyte carbonic anhydrases (carbonate hydro-lyase, EC4.2.1.1) are zinc metalloenzymes which catalyze the reversible hydration of CO_2.[1] Studies of the folding of the bovine and human Zn(II)-carbonic anhydrases in guanidine·HCl and urea have been reported in several papers [2–10] and the presence or absence of Zn^{2+} has been shown to strongly affect the refolding kinetics.[3,6] The zinc ion in native carbonic anhydrase can be removed and a catalytically active Co(II)-enzyme can be made.[11] Coordination of Co^{2+} to the active site gives rise to various absorption bands in the visible wavelength region of the spectrum.[11]

Therefore Co^{2+} can be used as a spectroscopic probe during folding processes of the enzyme. The denaturation of bovine Co(II)-carbonic anhydrase has been investigated, whereas attempts to renature guanidine·HCl denatured Co(II)-enzyme have so far been unsuccessful.[12] In order to elucidate the role of the metal ion in the folding of carbonic anhydrase, we have undertaken an investigation of the denaturation and reactivation of bovine and human erythrocyte Co(II)–carbonic anhydrases.

Experimental. Human carbonic anhydrase B and C were purified from human hemolysate by affinity chromatography according to Khalifah *et al.*[13] For the preparation of bovine carbonic anhydrase C the same conditions as used for the human enzymes were applied except for the elution step. The bovine C enzyme was eluted with 0.1 M Tris-H_2SO_4 buffer, pH 7.0, containing 0.2 M NaN_3. Protein concentrations were estimated spectrophotometrically at 280 nm assuming $A^{1\%}_{280\ nm}$ values of 16.3 cm^{-1}, 18.7 cm^{-1} and 19.0 cm^{-1} for the human carbonic anhydrase B, C and bovine carbonic anhydrase C, respectively.[14]

The homogeneity of the preparations were analyzed by polyacrylamide gel electrophoresis [15] (7.5 % acrylamide, 0.095 M Tris-glycine buffer, pH 9.5).

Bovine Co(II)–carbonic anhydrase was prepared according to Lindskog and Malmström [16] and human Co(II)–carbonic anhydrase B and C were made by the method of Hunt *et al.*[17]

CO_2 hydration activities were measured by a colorimetric method.[18] Corrections for inhibition by guanidine·HCl during the assay were performed as described earlier.[4]

Spectrophotometric measurements were made with a Perkin-Elmer Lambda 3 apparatus equipped with a 10 cm cuvette.

* Communication at the Meeting of the Swedish Biochemical Society in Stockholm, 20–21st December 1982.

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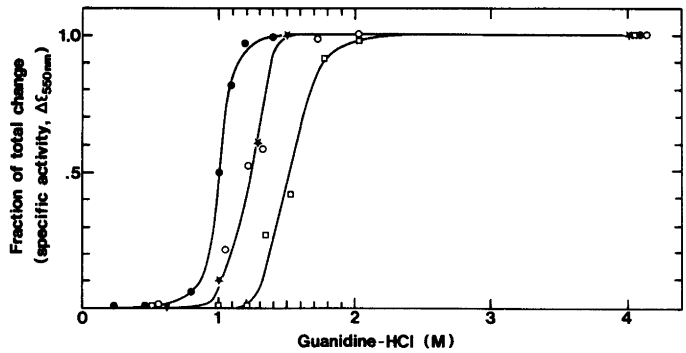

Fig. 1. Effects of guanidine·HCl on the CO_2 hydration activity and absorbance at 550 nm of Co(II)–carbonic anhydrases. Fractional loss of enzyme activity of bovine Co(II)–carbonic anhydrase C, (□); human Co(II)–carbonic anhydrase B, (○); human Co(II)–carbonic anhydrase C, (●). Fractional change in absorbance at 550 nm for human Co(II)–carbonic anhydrase B, (★). Protein concentrations 0.1 mg/ml in 0.1 M Tris-H_2SO_4 buffer, pH 7.5; temp. 23 °C.

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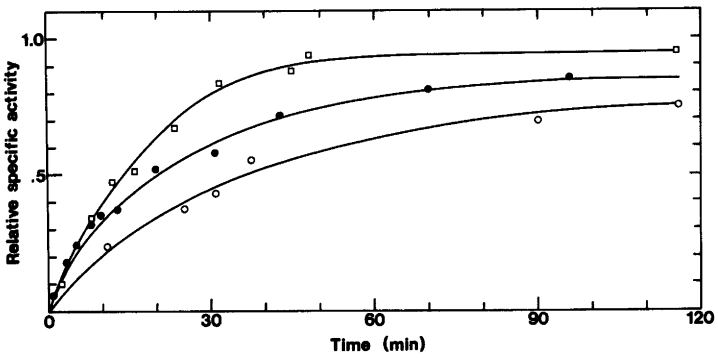

Fig. 2. Time courses for the reactivation of Co(II)–carbonic anhydrases denatured in 5.0 M guanidine·HCl. Final concentrations of protein and guanidine·HCl during reactivation: Bovine Co(II)–carbonic anhydrase C (0.25 mg/ml, 0.5 M), (□); human Co(II)–carbonic anhydrase B (0.025 mg/ml, 0.5 M), (○); human Co(II)–carbonic anhydrase C (0.025 mg/ml, 0.3 M), (●). Buffer: 0.1 M Tris-H_2SO_4, pH 7.5; temp. 23 °C.

Guanidine·HCl was prepared from guanidinium carbonate [19] and recrystallized and the concentration was determined refractometrically.[20]

Results. The stabilities of the bovine and human Co(II)–carbonic anhydrases in guanidine·HCl were investigated by incubation of the enzymes for 2 h in various concentrations of the denaturing agent. After this exposure to the denaturant the CO_2 hydration activity was measured and the results are illustrated in Fig. 1. The
denaturation of the Co(II)–enzymes is a highly cooperative process like that of the Zn(II)–enzymes. The midpoints of the transition profiles are reached at guanidine·HCl concentrations of 1.0, 1.2 and 1.5 M for the human Co(II)–carbonic anhydrase C, B and bovine Co(II)–carbonic anhydrase C, respectively. Corresponding curves for the Zn(II)–enzymes were recorded in parallel for comparative purposes and the following midpoints of denaturation were obtained: 1.0, 1.4 and 1.6 M for the human C, B and bovine C enzyme, respectively. The main absorbance band resulting from the Co^{2+} substitution of Zn^{2+} in the active site of the enzyme has a maximum at 550 nm. For human Co(II)–carbonic anhydrase B the change in absorbance at this wavelength was also monitored as a function of the guanidine·HCl concentration. As shown in Fig. 1 this absorbance decrease coincides with the inactivation curve within experimental error.

The time courses for the reactivation of the bovine and human Co(II)–carbonic anhydrases are shown in Fig. 2. In the reactivation experiments Co(II)-enzymes were first denatured in 5.0 M guanidine·HCl for 2–3 h. The enzymes were then reactivated by rapid dilution of the denaturant with buffer. Optimal reactivation of the human Co(II)–carbonic anhydrases was obtained when the Co(II)-enzymes B and C were each diluted to a protein concentration of 0.025 mg/ml and a concentration of guanidine·HCl of 0.5 M and 0.3 M, respectively. After 24 h of reactivation the human Co(II)–carbonic anhydrases B and C recovered about 80 and 90 %, respectively, of the specific CO_2 hydration activities of the native Co(II)-enzymes. Increased protein concentrations during the reactivation process gave rise to decreasing yields of active enzymes. For the bovine Co(II)–carbonic anhydrase C, a reactivation of 95 % could be obtained in 2 h (Fig. 2) after dilution to 0.5 M guanidine·HCl and a 10-fold higher protein concentration than for the human enzymes. The half-times of reaching the final values of reactivation were 13, 15 and 23 min for the bovine Co(II)–carbonic anhydrase C, human Co(II)–carbonic anhydrases C and B, respectively, (Fig. 2). The corresponding half-times for the Zn(II)-enzymes under identical reactivation conditions were 6, 9 and 4 min.

Discussion. The results of this study indicate that the stability of the tertiary structure of carbonic anhydrase with regards to denaturation in guanidine·HCl is dependant on the metal ion. Substitution of Zn^{2+} in the active site by Co^{2+} seems to some extent lower this stability (Fig. 1). For the bovine Co(II)–carbonic anhydrase C a similar trend has been reported, when the denaturation was followed by various physical parameters.[12] Furthermore, the bovine apoenzyme C has been shown to be considerably less

stable than the holoenzyme.[3,6] The concomitant drop of the visible absorbance and activity with increasing concentrations of guanidine·HCl for the human Co(II)–carbonic anhydrase B (Fig. 1) suggests that the inactivation of the enzyme is accompanied by dissociation of Co^{2+} or a markedly different coordination of the Co^{2+} in an inactive state of the enzyme. Conclusions regarding the existence of different Co^{2+}-intermediates on the folding pathway must wait until kinetic data are available.

The main result of this work is that conditions have been found for practically full reactivation of bovine and human Co(II)–carbonic anhydrase C. For the corresponding human B enzyme, a somewhat lower yield (80 %) of active enzyme was recovered. The reported difficulties in achieving renaturation of the bovine Co(II)–carbonic anhydrase C are probably due to the use of dialysis to remove the denaturant.[12] This relatively slow procedure might favour the formation of "incorrectly" folded states, which have been shown to accumulate in the transition region of the denaturation of human Zn(II)–carbonic anhydrase B.[4] The existence of similar states has also been discussed for the bovine Zn(II)–carbonic anhydrase C.[10] By rapid dilution well below the midpoints of the transition curves, the formation of such dead-end conformations can, however, be minimized.

Since protein concentrations of the order of 0.25 mg/ml can be used for the reactivation of bovine Co(II)–carbonic anhydrase C, it should be possible to employ the Co^{2+} as a spectroscopic probe in kinetic studies of the role of the metal ion during the refolding of this enzyme.

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Evidence for a Chlorocarbenium Ion in Reactions of α-Ethynyl Ketones with Phosphorous Pentachloride

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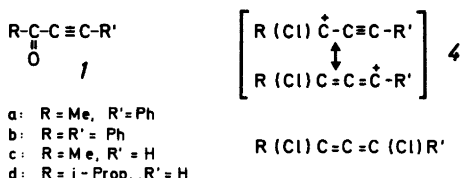

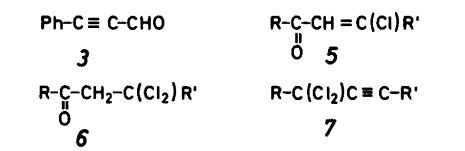

There is evidence for the intermediacy of a chlorocarbenium ion in reactions of ketones with phosphorous pentachloride.[1] The presence of such an intermediate in reactions of α-acetylenic ketones should lead to the formation of dichlorinated allenes among other products. Some years ago, Newman and Ream [2] treated the ketones *1a* and *1b* with phosphorous pentachloride in dichloromethane but no allene *2* was detected in the reaction products; furthermore, a similar reaction of the α-acetylenic aldehyde *3* gave no allenic product. The authors concluded that the absence of any dichlorinated allenes indicated that the reaction did not proceed *via* a chlorocarbenium ion *4*.

In the present note we report that dichlorinated allenes are formed from reactions of α-ethynyl ketones and phosphorous pentachloride, suggesting that in these examples at least, a chlorocarbenium ion is probably an intermediate.

The reaction of 3-butyn-2-one (*1c*) with phosphorous pentachloride in a mixture of pentane–ether as solvent afforded a crude unstable product which exhibited a strong band at 1950 cm^{-1} in the IR spectrum, indicative of an allene; however, all attempts on isolation were unsuccessful. When the reaction was carried out in dichloromethane–ether, the crude product exhibited only weak IR absorption at 1950 cm^{-1}. GLC analysis indicated a mixture of four components. The major and also most volatile component was isolated by preparative GLC and shown to be (*E*)-4-chloro-3-buten-2-one (*5c*).[3,4] The spectral data further revealed that one of the minor products was 4,4-dichloro-2-butanone (*6c*).[5]

The reaction of 4-methyl-1-pentyn-3-one (*1d*) in pentane–ether as solvents was more successful. Some decomposition took place by GLC, but according to the ^1H NMR the product consisted essentially of the allene 1,3-dichloro-4-methyl-1,2-pentadiene (*2d*) and 1-chloro-4-methyl-1-penten-3-one (*5d*). The allene was obtained pure by preparative GLC; besides an IR absorption at 1965 cm^{-1} the allenic hydrogen appeared in the ^1H NMR spectrum as a weakly coupled (J=1.5 Hz) doublet at δ 6.28. The vinyl chloride *5d* was formed essentially as the (*Z*)-isomer but by standing, or during isolation, it was partly converted into the (*E*)-isomer. Both isomers were obtained pure by preparative GLC, and the structures determined spectroscopically. The solvent affects the reaction of *1d* as well; in dichloromethane–ether the product consisted mainly of (*E*)-*5d* and small amounts of the allene *2d* and 1,1-dichloro-4-methyl-3-pentanone (*6d*). By comparing retention times on GLC, the latter compound was most probably also a minor product from the reaction in pentane–ether as solvent.

Finally, the reaction of phenyl ethynyl ketone (*1e*) with phosphorous pentachloride was studied. Due to solubility dichloromethane–ether was used as solvent. The reaction gave no detectable amount of allenic product. Thin-layer chromatography indicated the presence of two compounds, but only one was present in sufficient amount for identification. It was shown to be (*E*)-3-chloro-1-phenyl-2-propen-1-one (*5e*) by comparison of the IR spectrum with that of an authentic sample.[6]

The interesting feature of the present work is that an S_Ni-type mechanism apparently operates in reactions of α-ethynyl ketones with phosphorous pentachloride. The geminal halides *7* were not obtained; these were the main products from reactions of the α-acetylenic ketones *1a* and *1b*.[1] The formation of hydrochloric acid seems unavoidable under the reaction conditions and its addition to initially formed allenes could rationalize the formation of the vinyl chlorides *5*; however, this would demand that protonation should occur at the central carbon of the allenic linkage while protonation at a terminal carbon, with formation of vinyl cations, appears to be

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preferred.[7] Alternatively, the vinyl chlorides may result from addition of hydrochloric acid to the ethynyl ketones, a well-documented reaction;[6,8] *trans* addition with formation of the (*Z*)-isomer is expected. The dichlorides *6* are similarly explained as products from addition of hydrochloric acid to the vinyl chlorides.

Experimental. The instruments used in the present work have been described previously.[9]

The ethynyl ketones were prepared from the corresponding alcohols by oxidation with chromic trioxide.[10,11]

Reactions with phosphorous pentachloride. General method. A solution of the ketone (10 mmol) in 10 ml of ether was added dropwise to a stirred suspension of PCl_5 (10 mmol) in 10 ml of pentane (method A) or in 10 ml of dichloromethane (Method B) at room temperature. Stirring continued overnight and the reaction mixture was poured on ice. The product was extracted with ether, the extract washed with water and dried ($MgSO_4$). The solvents were evaporated under reduced pressure and the product isolated by distillation and preparative GLC.

*Reactions of 3-butyn-2-one (*1c*). Method A.* The IR spectrum of the crude product (0.7 g) exhibited a strong band at 1950 cm^{-1} which gradually diminished in intensity with time. Distillation afforded a liquid, b.p. 40–60 °C (30 mmHg) which by GLC consisted of six components. The product turned rapidly dark by standing.

Method B. The crude product (0.6 g) exhibited medium strong absorption at 1950 cm^{-1} in the IR spectrum. Four components were present according to GLC. The major component, (*E*)-4-chloro-3-buten-2-one (*5c*) [4] was purified by prep. GLC (PEG 4000, 70 °C); IR (CCl_4): 1700, 1685, 1580, 940, 840 cm^{-1}; ^1H NMR (CCl_4): δ 2.21 (3H, s) 6.84 (2H, AB, J 13.5 Hz). A minor component was 4,4-dichlorobutan-2-one [5] (*6c*); IR (CCl_4): 1725, 1645 cm^{-1}; ^1H NMR (CCl_4): δ 2.20 (3H, s), 3.28 (2H, d, *J* 6.0 Hz) 6.05 (1H, t, *J* 6.0 Hz).

*Reactions of 4-methyl-1-pentyn-3-one (*1d*). A.* The crude product (1.3 g) consisted of five components by GLC. Three of these, which represented >90 % of the mixture, were separated by preparative GLC (SE 30, 80 °C). 1,3-Dichloro-3-methyl-1,2-pentadiene (*2d*) (75 % of the mixture). IR (CCl_4): 1965, 860 cm^{-1}; ^1H NMR (CCl_4): δ 1.18 (6H, d, J 6.5 Hz), 2.53 (1H, m), 6.28 (1H, d, J 1.5 Hz).

(*E*)-1-Chloro-4-methyl-1-penten-3-one [4] (*5d*). IR (CCl_4): 1695, 1680, 1580, 940 cm^{-1}; ^1H NMR (CCl_4): δ 1.11 (6H, d, J 6.5 Hz), 2.58 (1H, m) 6.90 (2H, AB, J 13.5 Hz); MS *m/z*: 132, 97, 91, 89, 63, 61, 43, 41.

(*Z*)-1-Chloro-4-methyl-1-penten-3-one (*5d*); IR (CCl_4): 1700, 1675, 1580 cm^{-1}; ^1H NMR (CCl_4): δ 1.11 (6H, d, J 6.5 Hz) 2.78 (1H, m) 6.43 (2H, AB, J 8.0 Hz).

B. The compound *2d* and (*E*)- and (*Z*)-*5d* were present in the reaction product as shown by GLC and ^1H NMR; in addition a fourth compound was isolated by preparative GLC and identified as 1,1-dichloro-4-methylpentan-3-one (*6d*);[5] ^1H NMR (CCl_4): δ 1.12 (6H,d) 2.48 (1H, m) 3.30 (2H, d, J 6.0 Hz) 6.08 (1H, t, J 6.0 Hz); MS *m/z*: 168, 129, 127, 125, 99, 71, 43, 41.

*Reaction of 1-phenyl-2-propyn-1-one (*1e*).* The crude product (1.3 g) exhibited no allenic or acetylenic absorption in the IR. TLC indicated a major component and traces of a second one. The former was isolated by column chromatography (Al_2O_3) and identified as (*E*)-3-chloro-1-phenyl-2-propen-1-one (*5e*) [6]; IR (liq.): 1665, 1600, 1580, 935 cm^{-1}; ^1H NMR (CCl_4): δ 7.30–8.0 (compl. abs.). MS *m/z*: 168, 166, 131, 105, 77.

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Silver Zeolite as Promoter in Glycoside Synthesis. The Synthesis of β-D-Mannopyranosides.

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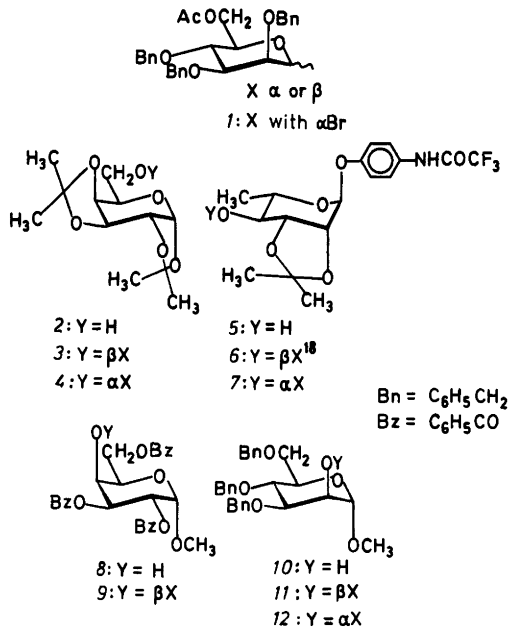

Despite sustained efforts,[1–13] the synthesis of β-D-mannopyranosides remains a problem, particularly in the mannosylation of protected carbohydrates with one free hydroxyl group of low reactivity. β-D-Mannopyranosyl residues occur in a number of natural products, some of which are of biological significance, and the efficient construction of β-D-mannopyranosyl linkages is therefore a matter of some importance. The use of insoluble silver promoters in the complete absence of Lewis acids has been advocated for this purpose.[11–13] Under these conditions, when equilibration at the anomeric centre of the glycosyl halide is suppressed, anomeric inversion of configuration is thought to predominate in the glycosylation reaction.[13] Thus, starting from an α-D-mannopyranosyl halide with a non-participating group in the 2-position, a β-D-mannopyranoside would be produced in the glycosylation. We now report the use of a novel, insoluble silver promoter, which gives improved yields in preparations of β-D-mannopyranosides. In our hands, the promoter, is more readily prepared than is silver silicate.[12]

Starting materials and products are represented by formulae *1–12*. Relevant physical constants for the compounds obtained and other data are given in Table 1. All compounds obtained were syrups. The yields and stereoselectivity for β-D-mannosylation are good for primary hydroxyl groups and for reactive secondary ones (compounds *2* and *5*). The HO-4 of galactopyranosides has low reactivity, nevertheless, an acceptable yield of the β-D-mannoside *9* was obtained. Previous attempts at β-D-mannosylation at the HO-2 of *10* have given poor results.[9,10] In the present work, the β-D-mannoside *11* was obtained with moderate stereoselectivity.

Experimental. General methods were the same as those reported before.[14]

Glycosylation method. Mortared 4Å molecular sieves (Union Carbide, 25 g) were stirred with silver nitrate (12.5 g) in water (50 ml) at room

Table 1. Selected physical data for disaccharide derivatives obtained.

Compound		*3*	*4*	*6* [18]	*7*	*9*	*11*	*12*
Yield (%)		83	15	61	9	33	50	21
$[\alpha]_D^{20}$ (°, $CHCl_3$)		−55	−18	−85	−21	+52	−29	+8
δ_c (p.p.m., $CDCl_3$)	C-1	96.52	–	95.57	95.47	97.38	98.15	99.42
	C-1′	102.66	–	99.76	99.13	102.54	99.52	99.86
$J_{C-1,H-1}$ (Hz)		180.7	–	171.0	168.5	173.4	166.7	170.9
$J_{C-1',H-1'}$ (Hz)		155.0	–	157.5	170.9	153.8	155.6	170.9
δ_H (p.p.m.)	H-1′	4.47(d) [a]	5.00 d [b]	4.94(d) [a]	4.88 d [a]	4.38(d) [a]	4.57(d) [a]	5.20 d [b]
$J_{1',2'}$ (Hz)		<0.5	1.2	<0.5	1.6	<0.5	<0.5	1.4

[a] $CDCl_3$. [b] $(CD_3)_2CO$.

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temperature in the dark for 2 h.[15,16] The mixture was filtered, the residue was washed with water (3×50 ml) then with acetone (50 ml), and kept at 190 °C for one day. The monohydroxy compound to be glycosylated (*2*,*5*,*8* or *10*, 1 mmol) and 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide (*1*, 1.5–2 mmol) in toluene (preparation of compounds *9*, *11* and *12*) or dichloromethane (preparation of compounds *3*, *4*, *6*, and *7*) were stirred at room temperature with silver zeolite (1.5–2 g). The reaction was monitored by TLC. Reaction times varied from a few hours to overnight. The reaction mixture was filtered and the filtrate concentrated. The desired products were obtained after chromatography on silica gel,[17] using toluene–ethyl acetate as irrigant.

Full 400 MHz ^{1}H NMR data for all disaccharides in the Table are available upon request from this Department (PJG).

Acknowledgements. We are indebted to Professor Bengt Lindberg for his interest, to the Swedish Board for Technical Development and the Swedish Natural Research Council for financial support as well as a maintenance grant from the latter (to P.O).

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A Micro-scale Synthesis of (2-^{14}C)- and (methyl-^{14}C)-5-Methyl-2′-deoxycytidine from Radioactive Thymidine Analogues

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Modification of DNA-cytosine by a 5-methyl group is thought to be an important mechanism which regulates the expression of eukaryotic genes.[1] This modification takes place after semiconservative replication.[2] There is very little evidence, if any, that 5-methyl-2′-deoxycytidine (5MedCyd) could be naturally incorporated into mammalian DNA in semiconservative replication. In order to clarify the possibility of incorporating 5MedCyd pharmacologically into human leukemic cells the synthesis of (2-^{14}C)- and (methyl-^{14}C)-5MedCyd has been performed starting from commercially available ^{14}C-thymidine (Thd).

Experimental. (2-^{14}C)-5MedCyd and (methyl-^{14}C)-5MedCyd were prepared from formamide and (2-^{14}C)-Thd or (methyl-^{14}C)-Thd (The Radiochemical Centre, Amersham, England), respectively, essentially according to the procedure of Vorbrüggen and coworkers.[3] Formamide (10 μl, 0.25 mmol), (^{14}C)-Thd (7.4 MBq) and hexamethyldisilazane (100 μl, 0.48 mmol) were heated in a glass ampoule at 140 °C for 76 h. The reaction mixture was then refluxed with absolute methanol (0.8 ml, 20 mmol) for 3 h. The reaction products were rigorously purified using TLC plates. The first purification step was performed on cellulose plates with butanol–water (86:14). The 5MedCyd spots were localized under appropriate UV-light, scraped from the plate and extracted carefully with water. The cellulose particles were centrifuged (9000×*g*, 2 min) and the supernatant was mixed with two volumes of methanol and evaporated to dryness using a nitrogen gas flow. The reaction product was then redissolved in 50 % methanol and chromatographed on cellulose plates using butanol–water–methanol–ammonia (60:20:20:1). The 5MedCyd spots were scraped off, extracted and handled as described above. The final product was dissolved in 20 mM potassium phosphate buffer, pH 7.4, and stored in 2 % ethanol at –20 °C until used. The distribution of radioactivity at both purification steps was analyzed by cutting the chromatography plates and counting the radioactivity using a Wallac scintillation spectrophotometer. The UV-spectrum of the reaction products was recorded with a Varian Cary 118C spectrophotometer.

Results and discussion. The R_f-values of the radioactive derivatives were identical to those of the authentic external markers. The conversion of (^{14}C)Thd to (^{14}C)5MedCyd was performed in the same way independent of starting material. The mother compound and the product counted for more than 80 % of the radioactivity recovered.

The UV-spectra of the purified products and non-radioactive 5MedCyd corresponded. The overall yield of both compounds was 25 %, respectively. The calculated specific activities compared well with the specific activities given for the mother compounds by the manufacturer: (2-^{14}C)-derivatives, 1.8 GBq/mmol (5MedCyd) *vs.* 2.0 GBq/mmol (Thd) and (methyl-^{14}C)-derivatives, 2.0 GBq/mmol (5MedCyd) *vs.* 2.2 GBq/mmol (Thd).

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Application of High-performance Cation-exchange Chromatography for Characterization of Stinging Insect Venoms *

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In recent years clinical and immunological studies have shown that insect venom preparations are reliable for diagnosis and treatment of stinging insect allergy.[1] Venom of common insects such as bees and vespids are complex mixtures of biogenic amines, peptides and proteins (enzymes) with both pharmacological and allergenic activities.[2] The use of modern biochemical separation techniques has made it possible to isolate and characterize individual venom components. The methods used for separation and characterization of venoms from stinging insects of the order Hymenoptera are mainly based upon gel chromatography, ion-exchange chromatography and electrophoresis.[3,4] However, the conventional gel types used for gel filtration and ion-exchange chromatography lack mechanical strength which is a major limitation when operating with high mobile-phase-velocities. In such cases the soft gels cannot be used and there is a requirement of rigid packing materials. We have previously shown that size exclusion chromatography at low pressure on a hydrophilic polyester packing [5] might be useable for rapid characterization of insect venoms. In this communication we report

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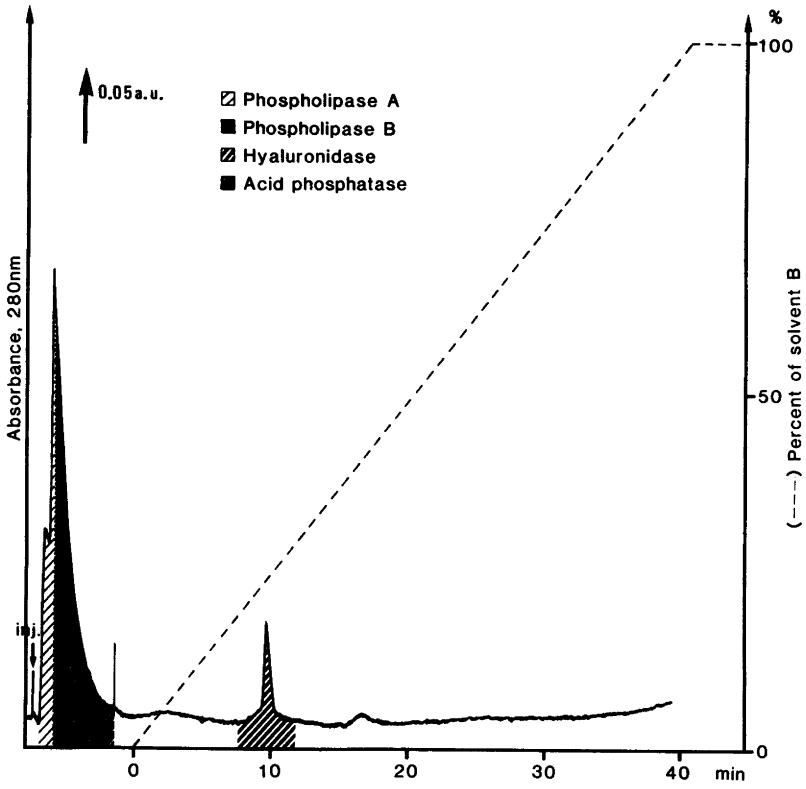

Fig. 1. Ion-exchange chromatographic separation of bee venom. Conditions: Mono S, HR 5/5 column; mobile phase 0.05 M Bicine buffer, pH 8.4; linear salt gradient 0–0.35 M NaCl; flow rate 1.0 ml/min; ambient temperature; UV detection at 280 nm; sample size 7 mg dry weight. The linear salt gradient is illustrated as the dotted line. Identified enzyme activities are indicated in the gradient elution profile.

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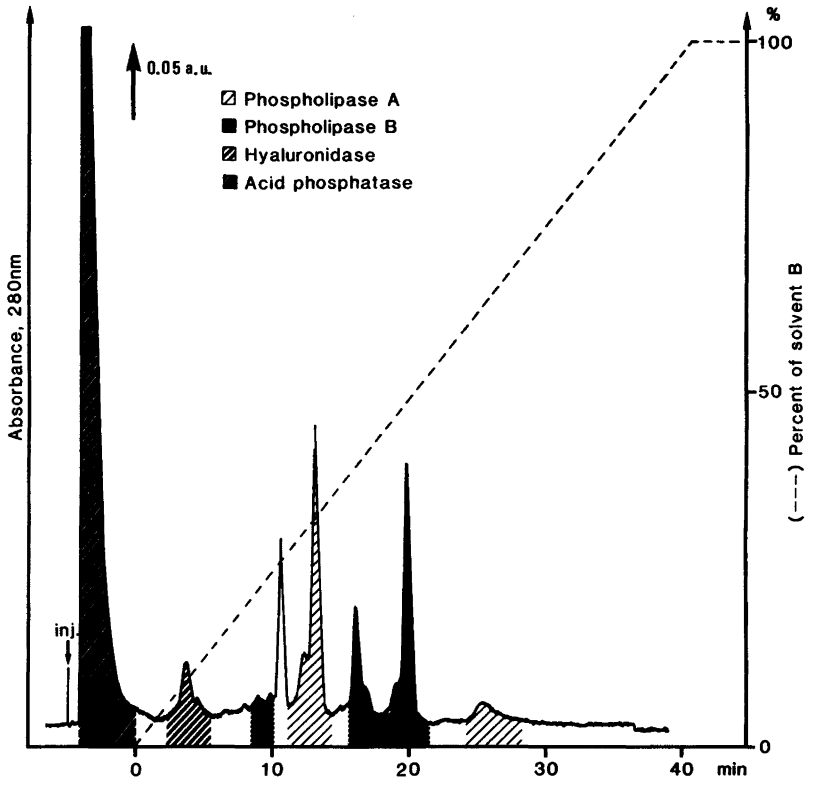

Fig. 2. Ion-exchange chromatographic separation of white-faced hornet. For conditions and details see Fig. 1.

on the use of Mono S™ cation exchanger for studying bee venom and vespid venom. As the composition of the various vespid venoms appears to be similar, white-faced hornet vespid venom has been choosen as a model.

Experimental. Honey bee venom (*Apis mellifera*) obtained by electricaal stimulation was purchased from Mr. C. Mraz, Middlebury, VT, USA. White-faced hornet (*Dolichovespula maculata*) was bought as lyophilized material from Drr. A. W. Benton, Pennsylvania State University, Spring Mills, PA, USA. The vespid venom was extracted from venom sacs.

Ion-exchange chromatography was carried out on an FPLC-system equipped with a cation exchanger Mono S™ (HR 5/5, Pharmacia Fine Chemicals, Uppsala, Sweden).[6] The venoms were dialyzed against starting buffer 50 mM Bicine [*N,N*-bis(2-hydroxyethyl)glycine], pH 8.4 prior to injection on the column. The venoms were eluted from the ion-exchange column by applying a linear salt gradient at constant pH. The eluted material was monitored by UV absorbance at 280 nm.

Enzyme activities were measured on the collected fractions from the ion-exchange chromatography gradient elution profile as previously described.[7]

Results and discussion. Electrofocusing-electrophoresis titration curves [8] were produced on bee venom and white-faced hornet vespid venom in order to establish a suitable pH for choosing proper conditions for cation-exchange chromatography on Mono S™.

Fig. 1 shows a gradient elution profile after ion-exchange chromatographic separation of bee venom components. The insect venom (7 mg) was adsorbed to the cation-exchanger in the starting buffer (50 mM Bicine) and elution was carried out at pH 8.4 with a linear salt gradient of NaCl at a rate of 8.75 mM/min ml. The various fractions from the elution profile were collected and analyzed with respect to the presence of enzyme activity. It is a well-known fact that the

predominant allergens in both bee venom and vespid venoms are enzymes.[2] As can be seen from Fig. 1 phospholipase A and B enzyme activities as well as acid phosphatase activity could not be separated and is detected in the fraction not adsorbed to the cation-exchanger at pH 8.4. After applying the salt gradient hyaluronidase was eluted as a symmetrical sharp peak (retention time 10 min). Obviously the applied experimental conditions give only a limited separation of bee venom components.

Fig. 2 shows a cation-exchange chromatographic separation of white-faced hornet vespid venom. The vespid venom (7 mg) was adsorbed to Mono S™ in 50 mM Bicine buffer at pH 8.4 and eluted with a linear salt gradient (0–0.35 M NaCl) at a rate of 8.75 mM/min, ml. Various fractions were collected and analyzed with respect to the presence of enzyme activities. Acid phosphatase appears not to be adsorbed to Mono S™ at pH 8.4. After applying the salt gradient several well-separated components giving absorbance at 280 nm were eluted. The first eluted peak (retention time approx. 5 min) exhibited hyaluronidase enzyme activity. After that a region with several small partly separated peaks follows (retention time 9 min) showing phospholipase B enzyme activity. The sharp peak (retention time 11 min) was identified as a protein but did not show enzyme activity. This peak might be the previously identified antigen 5, a basic protein in vespids which appears to be a major allergen for vespid venom sensitive individuals.[4] After that a partly separated peak follows (retention time 13 min) giving strong phospholipase A enzyme activity. Phospholipase A enzyme activity was also detected in the late eluted shoulder (retention time 25 min). The two sharp peaks positioned in the region with retention time 18–21 min show phospholipase B enzyme activity.

The elution pattern (absorbance at 280 nm) of white-faced hornet vespid venom and bee venom show quite different peptide/protein profiles. This indicates that the applied ion-exchange system might be a procedure of differentiate between insect venoms and isolated individual venom components. The high resolution and short retention times of the used cation-exchanger (Mono S™) render this system a very powerful tool for rapid discrimination and quality control of Hymenoptera stinging insect venoms.

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Identification of the Isozymes of Glutathione Transferase Induced by *trans*-Stilbene Oxide *

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It is well established that glutathione transferase (EC 2.5.1.18) activity in rat liver can be induced by various chemical agents (see Refs. 1 and 2). One of the most powerful inducers discovered is *trans*-stilbene oxide, which is capable of increasing the total glutathione transferase activity 3–4-fold.[2] Studies involving the use of antibodies for quantitation of enzyme protein demonstrated that the increased specific activities of transferases "A", "B" and "C" were in all cases due to increased concentrations in the cytosol.[2] However, more recent studies have demonstrated that the above transferases are but 3 of the group of 6 isozymes with basic isoelectric points and that binary combinations of 4 different subunits account for the 6 isozymes.[3] The subunits belong to two groups within which they may be combined: One contains subunits B and L, and the other contains subunits A and C. The isozymes are now named according to their subunit composition as glutathione transferase B_2, BL ("B" above), L_2, A_2 ("A" above), AC ("C" above) and C_2. Thus, it has become important to investigate whether the previously observed induction by *trans*-stilbene oxide involves all 4 subunits or only some. The results demonstrate that 2 subunits are selectively induced and that the 4 isozymes containing these subunits increase in amount.

Results and discussion. The technique that made possible the resolution of the basic glutathione transferases in rat liver cytosol was chromatofocusing.[4] By use of this technique, the isozymes in the hepatic cytosol fraction of a rat treated with *trans*-stilbene oxide (400 mg per kg

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Table 1. Activity ratios ($\times 10^3$) of substrates for identification of isozymes of glutathione transferases in rat liver cytosol of control and induced animals.

Animal	Glutathione transferase isozyme (peak No. in Fig. 1)					
	L_2(I)	BL(II)	B_2(III)	A_2(IV)	AC(V)	C_2(VI)
Ethacrynic acid/1-chloro-2,4-dinitrobenzene						
Control	5	23	70			
Induced	5	26	66			
trans-4-Phenyl-3-buten-2-one/1-chloro-2,4-dinitrobenzene						
Control				1	18	80
Induced				2	18	n.d.[a]

[a] n.d., not determined; the amount of glutatione transferase C_2 was too small to give accurate data.

Table 2. Fraction of total glutathione transferase activity of the isozymes separated by chromatofocusing (Fig. 1).

Animal	Glutathione transferase isozyme (peak No. in Fig. 1)					
	L_2(I)	BL(II)	B_2(III)	A_2(IV)	AC(V)	C_2(VI)
Control	0.15	0.29	0.08	0.14	0.30	0.04
Induced	0.32	0.19	0.04	0.27	0.17	0.01

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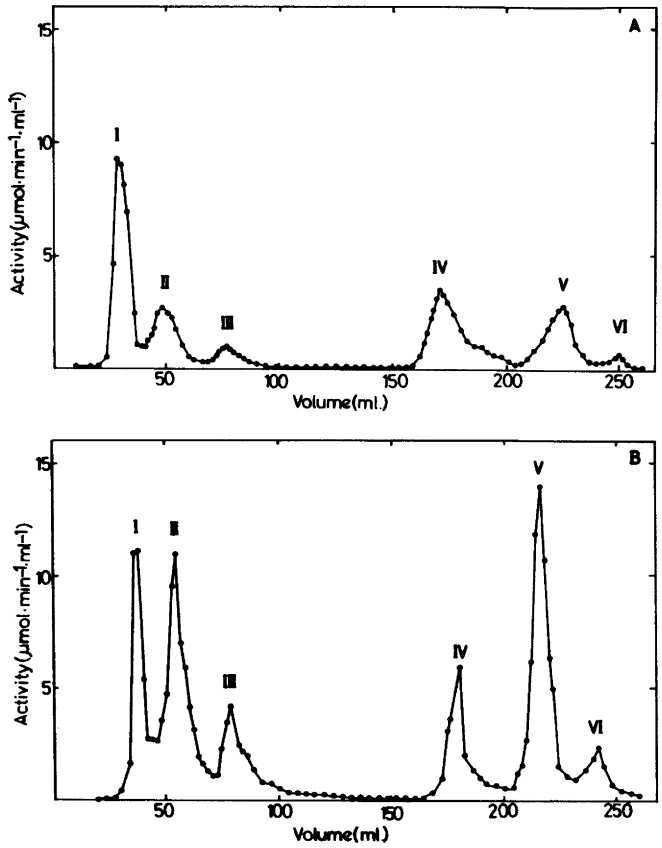

Fig. 1. Separation of basic glutathione transferases from rat liver cytosol of (A) induced and (B) control rats by chromatofocusing. Transferase activity was measured with 1-chloro-2,4-dinitrobenzene as electrophilic substrate. Approximately equal amounts of total transferase activity were loaded onto the chromatofocusing columns, even though the induced rat had a 3-fold higher cytosolic specific activity.

body weight for 5 days) could be separated and compared with those of a control animal. Fig. 1 shows that in the chromatogram of a treated rat peaks I and IV increase whereas the other peaks decrease in relative activity in comparison with the corresponding peaks of the control. Peaks I–VI correspond to transferases L_2, BL, B_2, A_2, AC and C_2 in this sequence. The transferases of the induced rat were identified by, in addition to their elution position, their subunit molecular weights and substrate specificities (*cf.* Ref. 3). Some particularly discriminating ratios of activities with different substrates are listed in Table 1.

Quantitation of the relative amounts of activity in the different peaks obtained by chromatofocusing was made by summing the activities of the individual fractions. Table 2 shows that the relative amounts of transferases L_2 and A_2 increase, whereas those of the other transferases decrease, in the sample from the induced rat, as compared with the control. It may be added that more acidic glutathione transferases,[3,4] which are eluted from the chromatofocusing column by 1 M NaCl, are not induced by *trans*-stilbene oxide.

The simplest, and most likely, explanation of the above findings is that essentially only subunits A and L rise in concentration upon treatment of the rat with *trans*-stilbene oxide. As a result, not only the homodimers, transferases A_2 and L_2, but also the heterodimers, transferases AC and BL, increase in absolute amounts. The formation of the heterodimers takes place at the expense of the homodimers, transferases B_2 and C_2, thus providing an explanation for the decrease of their

relative amounts.

Thus, the induction of glutathione transferase activity in rat liver by *trans*-stilbene oxide [2] can be explained by increases in the levels of transferase subunits A and L resulting in increased amounts of the four transferases L_2, BL, A_2 and AC of the several isozymes identified in the cytosol.

Experimental. Male Sprague Dawley rats (weighing 180–200 g) of the same litter, reared to be free of specific pathogens, were used in the investigation. The cytosol fraction of the liver of control and induced rats was prepared as earlier described; [3,4] one animal was used for each separation. After affinity chromatography on *S*-hexylglutathione Sepharose, a sample was analyzed by chromatofocusing on gel PBE 118 (Pharmacia Fine Chemicals) as described.[3,4] Glutathione transferase activity was measured at 30 °C in 0.1 M sodium phosphate (pH 6.5) with 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene as substrates. Assays with ethacrynic acid or *trans*-4-phenyl-3-buten-2-one as electrophilic substrate were carried out by published procedures.[5]

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Reactions of 1,3-Dithian-2-ylium Tetrafluoroborate with Organocopper, Organolithium and Organomagnesium Derivatives. A Simple Synthesis of 2-Substituted 1,3-Dithianes

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Recently we reported a simple and efficient method for the synthesis of 1,3-dithiolan-2-ylium and 1,3-dithian-2-ylium tetrafluoroborates; acid chlorides are reacted with 1,2-ethanedithiol or 1,3-propanedithiol in the presence of tetrafluoroboric acid.[1] These salts are potentially important synthetic intermediates, and are the cationic equivalents of the widely used ylide synthons of 1,3-thiolanes and 1,3-dithianes.[2] We herein report on the reactions of 1,3-dithian-2-ylium tetrafluoroborates with organometallic reagents.

Treatment of the 2-phenyl-1,3-dithian-2-ylium salt *2* with methylmagnesium iodide gave exclusively the desired adduct *5a*. Five equivalents of the organometallic reagent were required for the reaction to go to completion. If less organometallic reagent was used, the thiol ester *8* was part of the product; the latter arises from the unreacted salt *2* during the hydrolytic work-up of the reaction mixture. In related studies of the behaviour of 1,3-benzoxathiolium and 1,3-benzodithiolium salts towards Grignard reagents, it has also been reported that a large excess of the organometallic reagent must be used to effect complete conversion of the salt.[8] Five equivalents of butylmagnesium bromide were also required for complete reaction of the salt *2*; besides *5b* some of the reduced 1,3-dithiane *9* was formed. Such a reduction is not unexpected in moderately slow reactions when the Grignard reagent contains a hydrogen on its β-carbon.[9] The tendency for reduction was more pronounced using butyllithium when the major product was *9*. The reduction, however, was largely overcome by the use of lithium dibutylcuprate (Table 1).

The 2-butyl salt *3* with benzylmagnesium iodide gave the adduct *6*. Despite the basic properties of the Grignard reagent, proton abstraction to the ketene dithioacetal *11* was not important. The latter, however, is readily formed from *3* on treatment with triethylamine.[1] Having achieved alkylation by adduct formation with the Grignard reagents, the use of the other organometallic reagents was not further elaborated. Attention was instead directed towards derivatives which contain at least one hydrogen atom on an activated exocyclic α-carbon; the 2-benzyl derivative *4* was chosen. With methylmagnesium iodide the main product (64 %) was due to proton elimination (*12*; Table 1), the remaining product was the adduct *7*. The more basic nature of methyllithium was manifested in 90 % yield of the elimination product *12* using this reagent. Elimination, however, is largely overcome by the use of an organocopper reagent which is much less basic than its lithium or magnesium equivalents;[10] the yield of the adduct *7* was 77 %

Table 1. Products from the reaction between 1,3-dithian-2-ylium tetrafluoroborates and organometallic reactants. The reactions were run in diethyl ether. The yields were determined by GLC on the crude products after aqueous decomposition of the reaction mixture.

R^2Met	Substrate	Product yields					
		Comp.	%	Comp.	%	Comp.	%
MeMgI	*2*	*5a*	76				
BuMgBr	*2*	*5b*	85	*9*	9		
BuLi	*2*	*5b*	26	*9*	49		
Bu_2CuLi	*2*	*5b*	48				
$PhCh_2MgBr$	*3*	*6*	73				
BuMgBr	*4*	*6*	26	*10*	12	*12*	58
BuLi	*4*	*6*	9	*10*	15	*12*	74
Bu_2CuLi	*4*	*6*	75	*10*	7	*12*	8
MeMgI	*4*	*7*	30			*12*	64
MeLi	*4*	*7*	6			*12*	90
Me_2CuLi	*4*	*7*	77			*12*	13

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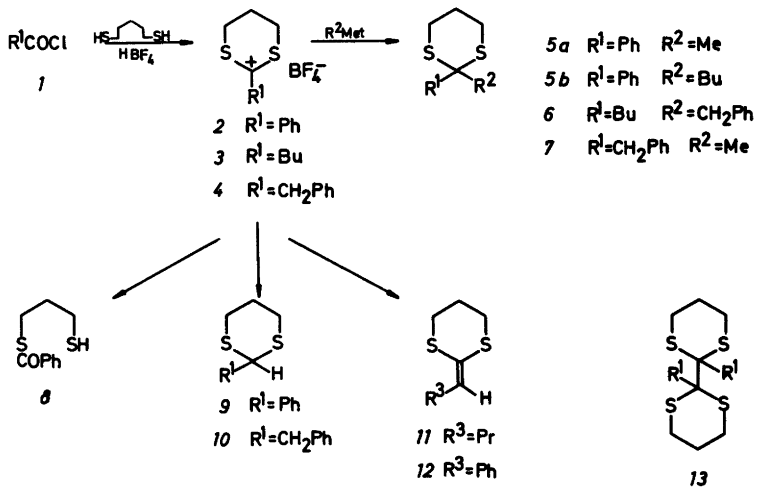

Scheme 1.

using lithium dimethylcuprate (Table 1). Also with butylmagnesium bromide proton elimination from *4* to form *12* is favoured, whereas the corresponding organocopper reagent gives mainly the adduct *6*. In the reactions with butylmagnesium bromide, reduction of the dithianium salt *4* to form *10* also occurs to some extent (7–15 %).

In the above reaction, varying but small amounts of a dimeric product *13* was also observed. This is formed by reductive dimerization of the salts *2–4*; the reductive dimerization is more easily effected using zinc in acetonitrile.[11]

Experimental. All the reactions were carried out under an atmosphere of dry argon. Diethyl ether was dried by distillation from lithium aluminium hydride. The concentration of the butyl- and methyllithium solutions was ascertained by titrations.[12] The copper iodide was purified as described.[13]

GLC was used for analysis of the crude product (Table 1; before purification); the analyses were run on columns of 3 or 10 % SR 2100 Chrom WAV-DMCS 80/100 in the range 100–250 °C at 16 °C/min. Mass spectra were recorded at 70 eV.

The 1,3-dithian-2-ylium tetrafluoroborates were prepared as previously described.[1] When salts had been stored for some time they were washed with diethyl ether before the reaction was started.

The reaction products have previously been prepared by other routes. References to compounds in Table 1 which are not discussed below are: *9*,[14] *10*,[6] *12*.[7]

General procedure for Grignard reactions. The 1,3-dithian-2-ylium tetrafluoroborate (0.01 mol) was added with vigorous stirring at 0 °C to the Grignard reagent (0.05 mol) in ether (70 ml). The cooling bath was removed once the addition was completed, the mixture stirred at room temperature for 30 min and then heated under reflux for 15 min, the cold mixture carefully hydrolyzed with 0.5 M HCl, the organic layer separated, the aqueous layer extracted with ether (3×50 ml), the combined ether solutions washed with saturated aqueous $NaHCO_3$ and a little water and the dried ($MgSO_4$) solution evaporated. The residual material was subjected to GLC analysis; the results are given in Table 1. In preparative runs the crude product was distilled.

2-Methyl-2-phenyl-1,3-dithiane, [3a] *5a,* was thus obtained from *2* in 72 % yield, b.p. 90–91 °C/0.05 mmHg.

2-Benzyl-2-butyl-1,3-dithiane, [5] *6,* was thus obtained from *4* in 46 % yield after chromatography on a silica gel column using toluene–light petroleum 1:1 with subsequent distillation: b.p. 140 °C/0.001 mmHg. ^1H NMR ($CDCl_3$) δ 0.95(Me), 1.1–2.1 ($CH_2CH_2CH_2$ and CH_2-5), 2.7–3.0 (CH_2–4,6), 3.25 (CH_2Ph), 7.3 (Ph). MS [*m/z* (% rel.int.)]: 266(1,M), 209(2), 177(7), 176(7), 175(100), 135(2), 117(5), 91(11).

General procedure for alkyllithium reactions. The alkyllithium solution (16 mmol) was added dropwise with vigorous stirring at −78 °C to a suspension of the 1,3-dithian-2-ylium tetrafluoroborate (10 mmol) in ether (70 ml). The cooling bath was removed when the addition was completed, the mixture allowed to reach room temperature and worked up and analyzed (Table

1) as above.

General procedure for lithium dialkylcuprate reactions. The alkyllithium solution (20 mmol) was added dropwise with vigorous stirring at 0 °C to a suspension of copper iodide (10 mmol) in ether (40 ml). The resultant mixture was stirred for 10 min before the 1,3-dithian-2-ylium tetrafluoroborate (2 mmol) was added. After stirring for an additional 6 h at 0 °C the mixture was poured into 1 M HCl and worked up and analyzed (Table 1) as above.

2-Butyl-2-phenyl-1,3-dithiane, [3b] *5b,* was thus obtained in 17 % yield, b.p. 125 °C/0.05 mmHg. ^{1}H NMR ($CDCl_3$): δ 0.95(Me), 1.1–2.1 ($CH_2CH_2CH_2$ and CH_2-5), 2.75–3.0 (CH_2–C4,6), 7.1–7.9 (Ph). MS [*m/z* (% rel.int.)]: 252(29,M), 205(9), 195(71), 178(31), 163(46), 136(100), 121(34).

2-Benzyl-2-methyl-1,3-dithiane, [4] *7,* was isolated from the reaction between *4* and lithium dimethylcuprate in 64 % yield after chromatografic separation on a silica gel column using benzene–light petroleum 1:1; the other product, 2-benzylidene-1,3-dithiane, [7] *12*, was isolated in 7 % yield.

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Glutathione Transferases in Rat Testis *

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Six glutathione transferases (EC 2.5.1.18) in rat liver cytosol have been characterized as binary combinations of four protein subunits A, B, C, and L.[1] These proteins, which have basic isoelectric points, are responsible for the major part of the glutathione transferase activity in rat liver cytosol as measured with the universal electrophilic substrate 1-chloro-2,4-dinitrobenzene (CDNB).** In addition, three minor forms of glutathione transferase with lower isoelectric points exist in rat liver.

In a survey of glutathione transferases in various rat organs it was noted that the testis has high activity and that a major part of the activity resides in an acidic protein apparently not present in the liver.[2,***] Similar results were independently obtained by other investigators.[3] The present communication documents this finding further, and demonstrates that also the pattern of basic glutathione transferases in rat testicular cytosol differs from that found in liver.

Experimental. Homogenates (20 %, w/v) of rat testes (25 g) were prepared in 0.25 M sucrose, by use of a Potter-Elvehjem teflon-glass homogenizer, and centrifuged at 10 000 *g* for 10 min. The cytosol fraction was obtained by further centrifugation of the supernatant fraction at 105 000 *g* for 60 min. The cytosol fraction (94 ml) was chromatographed on a column of Sephadex G-25 (4×40 cm) packed in 10 mM Tris/HCl (pH 7.8), and the transferase-containing fractions were applied to an affinity column (2×14 cm) of *S*-hexylglutathione linked to epoxy-activated Sepharose 6B, equilibrated with the same buffer. The column was rinsed with 300 ml of 0.2 M NaCl in 10 mM Tris/HCl (pH 7.8) and the transferases eluted with 100 ml of 5 mM *S*-hexylglutathione in the NaCl-fortified buffer. The eluted enzyme-containing fractions were pooled, desalted on Sephadex G-25 (equilibrated with 5 mM Tris/HCl, pH 8.0) and concentrated to 5 ml by ultrafiltration. The subsequent chromatofocusing on gel PBE 118 was performed as described in Ref. 1. After finishing the elution with ampholytes, additional transferase activity was eluted with 1 M NaCl. This last fraction was equilibrated with 25 mM Tris/acetic acid (pH 8.3) by chromatography on a column (2×32 cm) of Sephadex G-50 and applied to a second column (1×25 cm) of chromatofocusing gel PBE 94, equilibrated with the same buffer. The adsorbed transferases were eluted with 300 ml of a mixture of Polybuffer 96 (30 %) and Polybuffer 74 (70 %), diluted 1:10 and adjusted to pH 5.0 with acetic acid. Assays of glutathione transferase activities and other experimental procedures were carried out as previously described.[1,4]

Results and discussion. The cytosol fraction of rat testes was found to have a glutathione transferase activity with CDNB of 1.5 μmol/min per mg protein. This specific activity is somewhat higher than that of rat liver cytosol.[4,5] The transferase activity was separated from the bulk protein by use of affinity chromatography, and various isozymes of glutathione transferases were subsequently resolved by chromatofocusing. A pH gradient of 10.8–8.0 eluted 5 isozymes and further elution with 1 M NaCl released approximately 50 % of the activity that had been applied to the column (Fig. 1). The latter fraction of activity could be resolved into at least 5 additional isozymes with lower isoelectric points by chromatofocusing in the pH interval of 8–5.

The isozyme pattern of glutathione transferase in rat testis differs markedly from that of rat liver, the most conspicuous difference being that a major part of the activity is borne by proteins with acidic isoelectric points. The existence of such acidic transferases has earlier been reported,[2,3] but the resolution of these enzymes into several isozymes has not been reported earlier. The dominating acidic transferase (isoelectric point at pH 5.9) was found to have the highest specific activity (about 100 μmol/min per mg with CDNB) found for any glutathione transferase from the rat. This enzyme is currently being characterized in greater detail.

The pattern of basic transferases in rat testis differs from that of rat liver in that no significant amount of glutathione transferases L_2 and BL is detectable in the testis. The lack of these isozymes (which would be eluted first from the chromatofocusing column) was demonstrated by analysis of protein subunit sizes, reactions with

* Communication at the Meeting of the Swedish Biochemical Society in Uppsala, 20–21st December, 1982.

** Abbreviation: CDNB, 1-chloro-2,4-dinitrobenzene.

*** Data presented at the Meeting of the Swedish Biochemical Society in Stockholm, 26–27th November, 1981.

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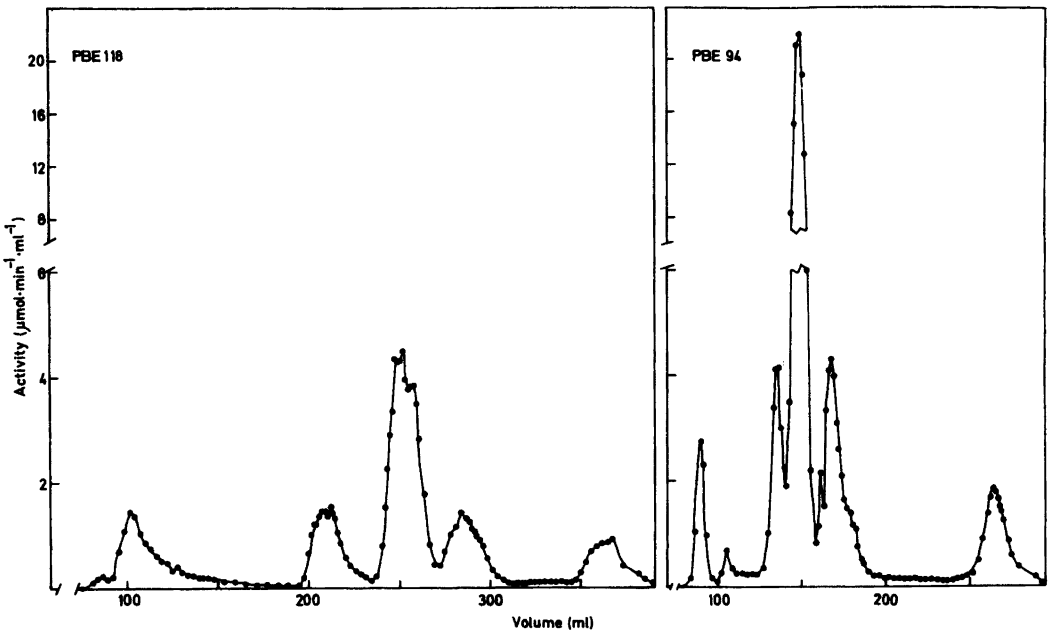

Fig. 1. Separation of glutathione transferase from rat testicular cytosol by chromatofocusing. The left part of the elution profile was obtained by chromatofocusing on gel PBE 118 and the right part by chromatofocusing on gel PBE 94. Transferase activity was measured at pH 6.5 and 30 °C with 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene as substrates.

antibodies, and substrates specificities of the enzymes eluted between pH 10.8 and 8.0. The first enzyme to appear was glutathione transferase B_2, and the fifth (as in liver samples) was transferase C_2. The identification of the isozymes in the intermediate peaks of activity has not been completed, but their properties suggest a relationship to protein subunit A (*cf.* Ref. 1), in that antibodies reacting with this subunit gave precipitin reactions with these isozymes. Significant enzymatic activity with 1,2-dichloro-4-nitrobenzene, which is highest for subunit A,[1] also indicated such a relationship.

Thus, this communication demonstrates that rat testis cytosol not only has high glutathione transferase activity, but that the isozyme pattern differs in significant respects from that of liver.

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The 9-Fluorenylmethoxycarbonyl (Fmoc) Group for the Protection of Amino Functions of Cytidine, Adenosine, Guanosine and Their 2'-Deoxysugar Derivatives

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In the chemical synthesis of DNA and RNA fragments, it is desirable [1] to protect the exocyclic amino functions of cytosine, adenine and guanine residues in view of their susceptibility to attack by electrophiles such as phosphorylating agents. Khorana and his coworkers [2] in their phosphodiester approach have introduced *N*-acyl groups to protect all three base residues and, subsequently, these acyl groups have also been used in the phosphotriester approach.[1] The *N*-acyl groups on cytosine, adenine and guanine residues are relatively stable both under neutral and acidic conditions; however, their rates of removal, under an alkaline condition, are clearly dependent upon the nature of base residues.[1,3] Thus, the removal of *N*-benzoyl groups is complete at room temperature with 5 M aqueous NH_3 in dioxane (1:1; v/v) in 385, 1410 and 4350 min from the corresponding 2'-deoxyribofuranosyl derivatives of cytosine, adenine and guanine residues, respectively.[3] The above periods of deprotection seem to be too long,[4] unless it is carried out at *ca.* 50 °C,[5,6] in view of the fact that the chemical synthesis of a fully protected 10–14 units long DNA sequence on the solid support takes only a day or so.[6] To circumvent this problem, especially in the case of guanine residues, acetyl and isobutyryl groups have been proposed (see Ref. 1). Several other protective groups have also been proposed for this purpose.[4]

Here we report the preparation and properties of 9-fluorenylmethoxycarbonyl-(Fmoc), as in *2* to *7*, as an exocyclic amino protecting group for cytosine, adenine and guanine residues of their corresponding 2'-deoxyribo- and ribonucleosides. The Fmoc group was first introduced [7] for the protection of an α-amino function (pK_a *ca.* 9.7) of an amino acid and it was removable with the help of liquid ammonia in 10–12 h at room temperature. The chemistry of its deprotection centers on the acidic nature of the proton on the β-carbon atom, and hence upon its abstraction by base; the Fmoc group fragments *via* β-elimination liberating the amine as shown in Scheme 1. We reasoned that, in such a β-elimination reaction, the pK_a of the base as a leaving group should have a significant effect on the overall rate of removal of the Fmoc group under the influence of either a nucleophilic or non-nucleophilic basic condition. Thus, it should be expected that the Fmoc group from the corresponding derivatives of cytidine, adenosine, guanosine and their 2'-deoxysugar analogs with pK_a [10] of *ca.* 4.2(4.3), 3.55(3.8) and 2.1(2.4) respectively, should be removable with greater ease. This has led us to prepare the Fmoc derivatives of cytidine, adenosine and guanosine and their corresponding 2'-deoxyribose derivatives, as in *2* to *7*, from their respective parent nucleosides through a "one-pot" synthesis in 88.5, 81, 70, 90, 58 and 70 % yields, respectively, as crystalline compounds. All new compounds have been characterized by UV, ^1H NMR and element analysis. The general procedure for such a "one-pot" synthesis involves trimethylsilylation of a particular nucleoside in dry pyridine solution which is followed by the addition of 9-fluorenylmethyl chloroformate (Fmoc-Cl), 1.2 equiv. with respect to the nucleoside, and then hydrolysis.[9] Thus, Table 1 records some physical and spectroscopic properties of compounds *2* to *7* in support of their chemical structures. We then proceeded to explore the relative rates of removal of these Fmoc groups from the substrates *2* to *7*. Table 2 records the relative rates of removal of the Fmoc group from the corresponding Fmoc

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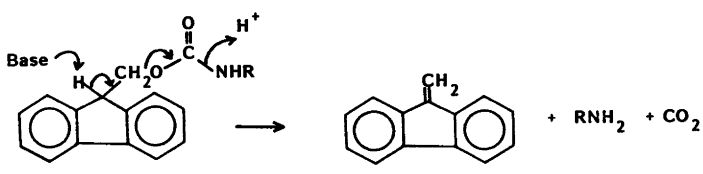

Scheme 1.

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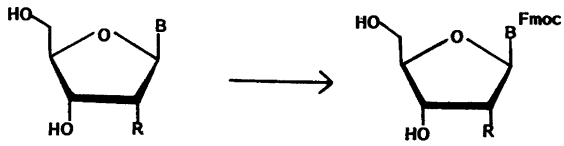
Table 1. The physical and the spectroscopic properties of Fmoc derivatives of cytidine, adenosine, guanosine and their 2′-deoxy analogs.

Compound	M.p. (°C)	Crystallization media	UV absorption properties in ethanol (nm)	^{1}H NMR absorptions in DMSO-d_6+D_2O (δ scale)
2	145	ethanol	λ_{max} (pH 2): 258, 266, 292 (sh.) 301 (sh.); (pH 7): 256, 264, 290 (sh.), 300 (sh.); (pH 13): 259, 266, 293 (sh.), 301 (sh.).	8.71 (d, 4 Hz, 1H); 8.32 (d, 4 Hz, 1H); 7.83 (m, 4H); 7.4 (m, 4H); 6.28 (s, 1H), 4.4 (m, 3H); 3.97 (m, 2H); 3.66 (m, 2H).
3	138–140	toluene	λ_{max} (pH 2): 266, 290 (sh.), 302 (sh.) (pH 7): 266, 290 (sh.), 303 (sh.) (pH 13): 266, 296 and 302.	8.71 (s, 1H); 8.69 (s, 1H); 7.77 (m, 4H), 7.36 (m, 4H); 6.05 (d, 8.5 Hz, 1H); 4.66 (m, 1H);4.4 (m, 4H); 3.82 (m, 2H).
4	167–170	ethanol	λ_{max} (pH 2): 266, 290 (sh.), 301 (sh.) (pH 7): 261, 290 (sh.), 300 (sh.) (pH 13):267, 290 (sh.), 302 (sh.).	8.26 (s, 1H); 7.87 (m, 4H); 7.40 (m, 4H); 5.84 (d, 5 Hz, 1H); 4.46 (m, 3H); 4.19 (m, 1H); 3.91 (m, 1H); 3.66 (m, 2H).
5	145	ethanol–$CHCl_3$ 1:1 v/v	λ_{max} (pH 2): 256, 265, 293 (sh.) 301 (sh.); (pH 7): 256, 264, 292 (sh.), 301 (sh.); (pH 13): 256, 266, 292 (sh.), 300 (sh.).	8.32 (d, 10 Hz, 1H); 7.83 (m, 4H); 7.40 (m, 4H); 6.98 (d, 10 Hz, 1H); 6.12 (t, 8 Hz, 1H); 4.34 (m, 3H); 3.84 (m, 2H); 3.62 (m, 2H).
6	128–130	toluene	λ_{max} (pH 2): 266, 287 (sh.), 302 (sh.); (pH 7): 266, 288 (sh.), 302 (sh.); (pH 13): 266, 292 (sh.), 298 (sh.).	8.69 (s, 1H); 8.14 (s, 1H); 7.69 (m, 4H); 7.36 (m, 4H); 6.38 (t, 10 Hz, 1H); 4.68 (m, 3H); 4.18 (m, 2H); 3.89 (m, 2H).
7	155	toluene	λ_{max} (pH 2): 263, 290 (sh.), 302 (sh.); (pH 7): 261, 290 (sh.), 300 (sh.); (pH 13): 266, 291 (sh.), 301 (sh.).	8.26 (s, 1H); 7.85 (m, 4H); 7.43 (m, 4H); 6.25 (t, 8.5 Hz, 1H); 4.49 (m, 3H); 3.88 (m, 4H).

Table 2. The relative rates of removal of Fmoc group from the corresponding derivatives of cytidine, adenosine, guanosine and their 2′-deoxyribonucleoside analogs.

Fmoc derivatives of nucleosides	Reagents for the removal of the Fmoc group		
	Condition A aq. NH_3 (d 0.85)–pyridine 1:1 (v/v) at 20 °C		Condition B Et_3N (20 eq.) in dry pyridine (10 ml/mmol)
	$t_{1/2}$ (min)	t_∞ (min)	$t_{1/2}$ (min)
2	17	110	180
3	22	150	180
4	120	–	65
5	16	110	330
6	20	130	300
7	105	–	60



General Formula: (1)
R = H or OH
B = 1-Cytosinyl;
9-Adeninyl;
9-Guaninyl;

(2); R= OH; B= 1-Cytosinyl;
(3); R= OH; B= 9-Adeninyl;
(4); R= OH; B= 9-Guaninyl;
(5); R= H ; B= 1-Cytosinyl;
(6); R= H ; B= 9-Adeninyl;
(7); R= H ; B= 9-Guaninyl;

protected derivatives and the data illustrate the pronounced effect of the pK_a of the leaving group on the fragmentation reaction shown in Scheme 1. Thus it is interesting to note that the removal of the Fmoc group, using condition B, from the corresponding derivatives of guanosine and its 2'-deoxy analog. Table 2; compounds: *4* and *7* respectively, is faster than the corresponding cytidine and adenosine derivatives; this is understandably due to the exclusive operation of a β-elimination pathway (Scheme 1). This observation is particularly relevant in the light of the fact that the relative rate of removal of a particular *N*-acyl group, under a usual alkaline conditions (condition: A in Table 2), from the base residues follows the order: guanosine<adenosine<cytidine.

It should also be added that, despite the desired stability of the Fmoc group both under neutral and acidic conditions, it could be completely deprotected within 40 min at room temperature when the compounds *2* to *7* in pyridine (1 part) were treated with liquid ammonia (*d* 0.88) (9 parts, v/v).

We then examined the effect of the Fmoc as 6-amino protecting group of 2'-deoxyadenosine (*6*) on the cleavage of the glycosidic bond (depurination) [1] in view of the recent report in literature [8] which suggests that 6-*N*-benzoyl-2'-deoxyadenosine and 6-*N*-phthaloyl-2'-deoxydenosine have half-lives of depurination of 30 and 120 min, respectively, in 80 % aqueous acetic acid at 30 °C. Under a similar acidic condition the glycosidic bond of the 6-*N*-Fmoc-2'-deoxyadenosine (*6*) was found to be more stable with a half-life of *ca.* 180 min. Thus, it is anticipated that the employment of a building block like *6* in the chemical synthesis of a DNA segment should reduce the formation of the by-products due to depurination during acid hydrolyses steps.

Further work is now in progress in this laboratory on the application of these exocylic amino protected building blocks in DNA and RNA chemistry.

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Carotenoid Sulfates. 2.* Structural Elucidation of Bastaxanthin

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The structural elucidation of bastaxanthin, the major carotenoid of the marine sponge *Ianthella basta,* is reported.

Bastaxanthin, the first known naturally occurring carotenoid sulfate, has been characterized by spectroscopic data (electronic, IR, ^{1}H NMR, ^{13}C NMR, CD and mass spectra) and chemical evidence (20 sulfated and desulfated derivatives) including acidic and enzymatic hydrolysis to bastaxanthol, also encountered as a minor carotenoid in *I. basta*.

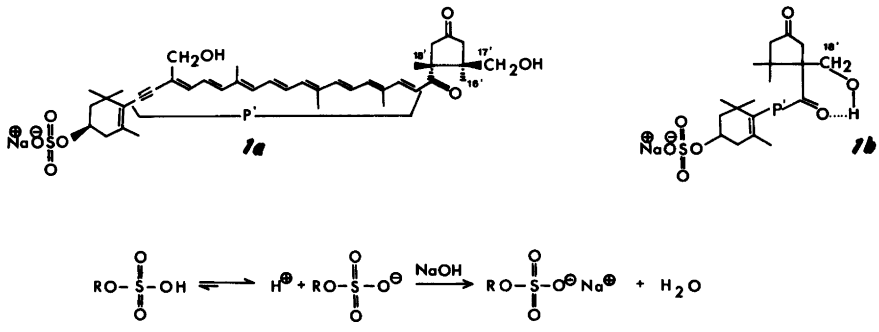
The evidence is consistent with the constitution 3,19,17′-trihydroxy-7,8-didehydro-β,κ-carotene-3′,6′-dione 3-sulfate. The absolute configuration of the three chiral centres is discussed in favour of (3*R*,1′*R*,5′*R*)-configuration.

Besides common phytoplankton type carotenoids the marine sponge *Ianthella basta* contains a group of strongly polar carotenoid sulfates, among which bastaxanthin *c* (*ca.* 40 % of the total carotenoids) is a major constituent.[1] Carotenoid sulfates are so far not encountered in other marine sponges [2] or other natural sources.

Details on the structural elucidation of bastaxanthin *c*, in this paper referred to as bastaxanthin, in favour of structure *1a* are now reported. In preliminary symposium contributions [3–5] the primary, non-allylic hydroxy function was allocated to C-18′ (*1b*), Scheme 1.

RESULTS AND DISCUSSION

Due to the large content of other extractives in the sponge and the high polarity of bastaxanthin and accompanying carotenoids the purification was particularly laborious. Reversed phase chromatography and ion exchange chromatography [6] were employed, but most efficient separation was obtained by pressurized silica columns followed by TLC on silica in combination with fractional precipitation.



Scheme 1.

* No. 1. *Acta Chem. Scand. B 34* (1980) 773.
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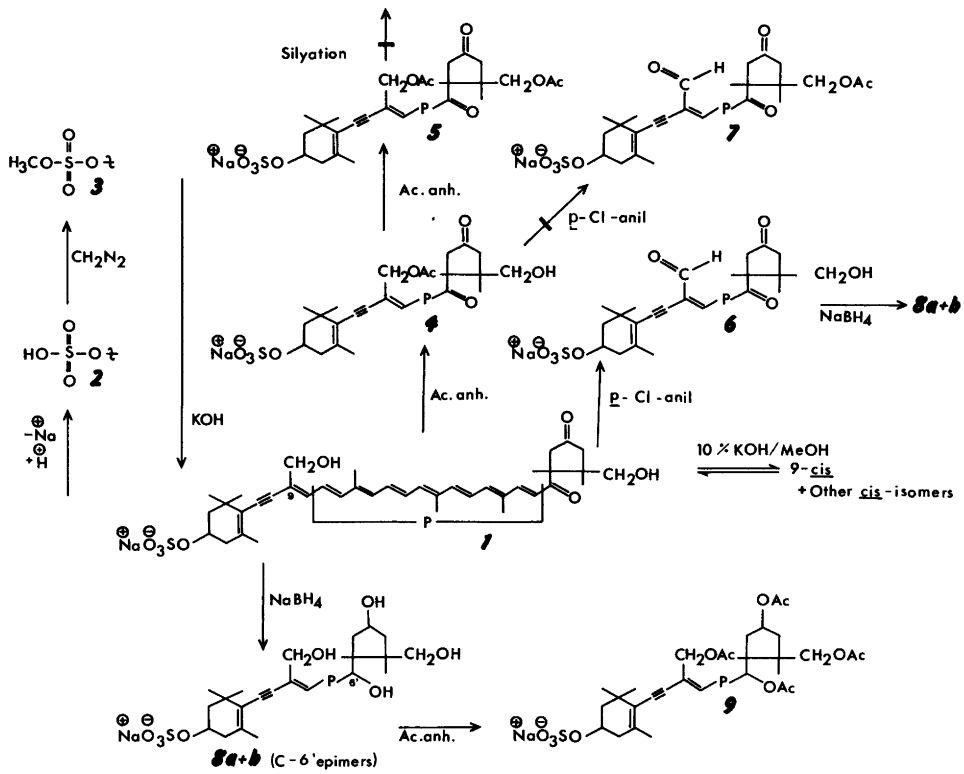
The high polarity and water solubility of bastaxanthin were striking. Previously high polarity of carotenoids has been associated with either carboxylic acids, phenols, enols or sugar derivatives. No such functions were present.

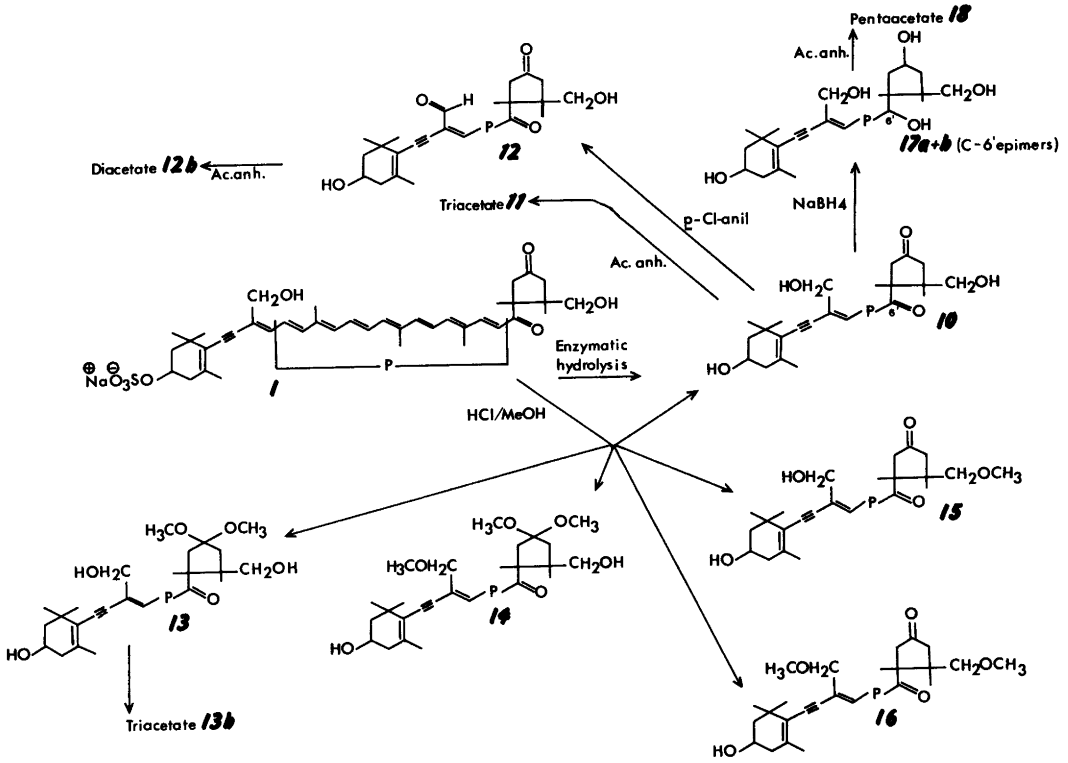
Monoesters of sulfuric acid, alkyl sulfuric acids, are approximately as acidic as sulfuric acid and readily form inorganic salts,[7] Scheme 1. Bastaxanthin was subsequently shown to be a sulfate ester of this type. Being ionized in neutral solution metal alkyl sulfates in general are strongly polar compounds and exhibit water solubility.

The recognition of bastaxanthin as a carotenoid sulfate was hampered by the initial failure of identifying the presumed molecular ion in the mass spectrum as that of a thermal elimination product. Partial syntheses of several model carotenoid sulfates [5,8] subsequently revealed that the thermal elimination of $NaHSO_4$ from their sodium salts prior to ionization is a general phenomenon, *cf.* Scheme 4.

The presence of a sulfate function in bastaxanthin (*1*) was indicated by strong IR absorption at 1240 cm^{-1}.[9] Micro sulfur analysis by X-ray fluorescence spectroscopy confirmed the presence of sulfur. The negative charge was confirmed by its electrophoretic behaviour in comparison with synthetic carotenoid mono- and disulfates. When passed through a suitable ion exchange column the free acid *2*, Scheme 2, was eluted, as confirmed by pH measurement. The acid *2* and the sodium salt *1* had the same R_F-value on adsorption chromatography and were stable in dilute solutions. Methylation with diazomethane gave in 12 % yield the methyl ester *3* of lower polarity. The methyl ester *3* upon electron impact showed a small (<1 %) molecular ion, but a strong M−30 fragment ion, consistent with the reported loss of methanal from the molecular ion of dimethyl sulfate.[10] Upon treatment with various commercial sulfatases bastaxanthin (*1*) was enzymatically desulfated to bastaxanthol (*10*, Scheme 3) of lower



Scheme 2. Sulfated derivatives of bastaxanthin (*1*).

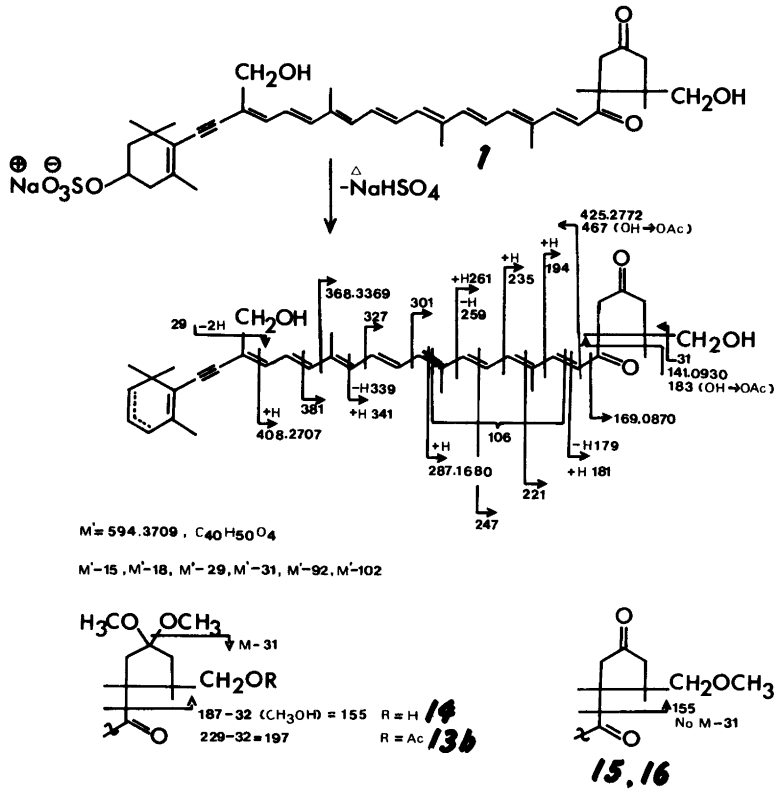


Scheme 3. Non-sulfated derivatives of bastaxanthin (*1*).

polarity, also encountered as a minor carotenoid constituent in *I. basta*. Acid hydrolysis gave the same product (*10*) in addition to secondary products (Scheme 3) to be discussed below. Finally the ^{1}H NMR and ^{13}C NMR spectra in comparison with those of synthetic model sulfates,[5,8] as well as the mass spectra of the therminal elimination products of bastaxanthin and its sulfated derivatives (Scheme 2) are compatible with a sulfate function.

Turning now to a consideration of the chromophore assigned to bastaxanthin (*1*, see Scheme 2) the presence of a disubstituted triple bond followed from IR absorption at 2170 cm^{-1} (KBr) and ^{13}C NMR signals at δ 91.7 and 98.1 (CD_3OD). The electronic spectra of native bastaxanthin (*1*), its reduction product *8* with $NaBH_4$ and allylic oxidation product *6* with *p*-chloranil is consistent with the monocyclic en-yn-octaenone chromophore assigned. Capsanthin [11] has the same chromophore as bastaxanthin (*1*) except for the triple bond, and crocoxanthin [12] has the same chromophore as the $NaBH_4$-reduced derivative *8*. The bathochromic shift (20 nm in methanol) observed upon allylic oxidation is compatible with the formation of a cross-conjugated aldehyde.[13,14] Preference for C-19 location for the allylic, primary hydroxy group follows from ^{1}H NMR, ^{13}C NMR and MS data discussed below (Schemes 5, 6 and 4, respectively). These spectra also support the common methyl substitution pattern of the chromophore. The reduced spectral fine-structure in the electronic spectrum of bastaxanthin (*1*) *versus* capsanthin and of *8* *versus* crocoxanthin is now ascribed to the influence of the hydroxy substituent at C-19, reducing the planarity of the chromophore, *cf.* similar effects for loroxanthin [15] (19-hydroxy-lutein) *versus* lutein.[16]

Isomerization to *Δ9-cis* is known to occur readily in related acetylenic carotenoids [17] and was effected by treatment with base. The presumed *Δ9-cis* isomer could only be isolated on analytical commercial silica plates, and was partly



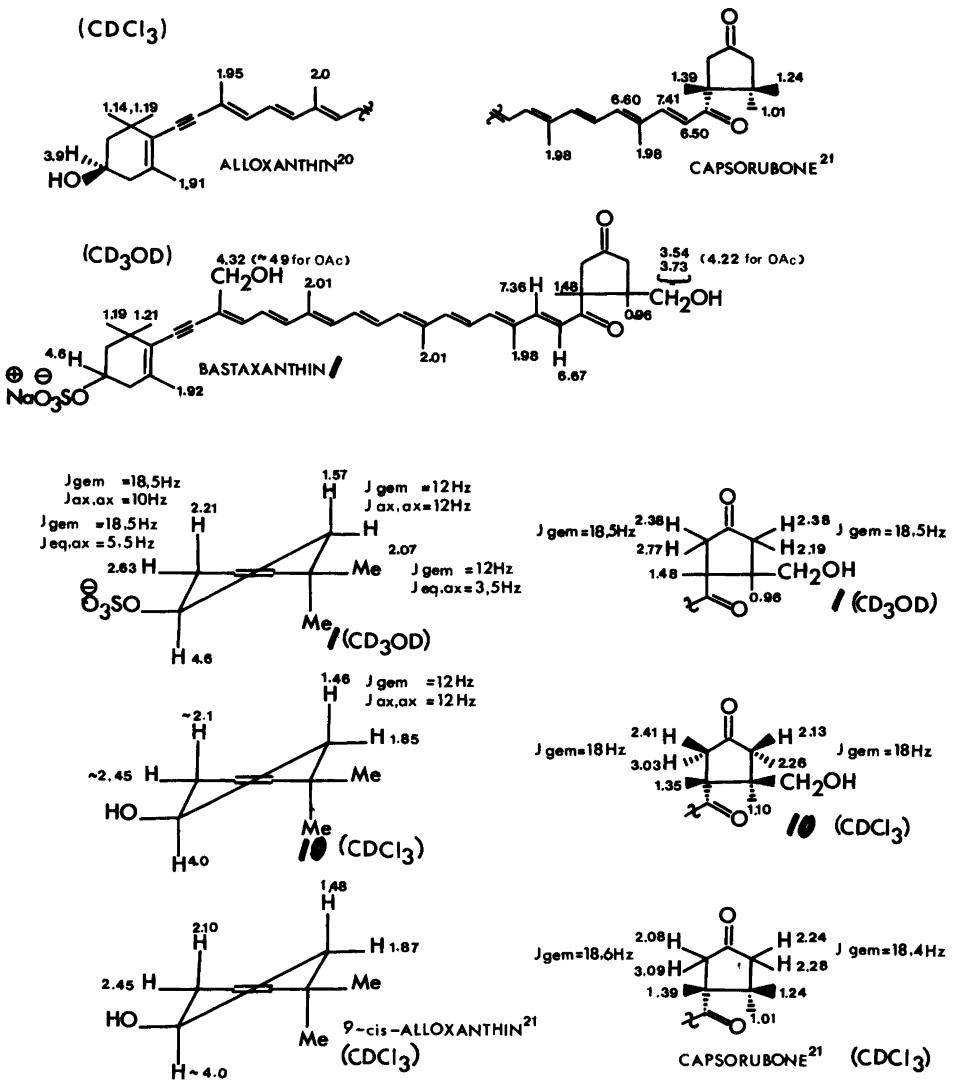
Scheme 4. Mass spectrometric fragmentations of bastaxanthin (*1*) and derivatives.

reversibly isomerized to the all-*trans* isomer in the presence of base. ^{1}H NMR (CD_3OD) of an isomerized mixture showed singlets at δ 4.32 (all-*trans*) and δ 4.18 (9-*cis*) for the $-CH_2OH$ protons at C-19, consistent with previous findings for related in-chain substituted allylic carotenols,[18] and concomitant, small downfield shifts of the CH_3-18 and CH_3-16 or -17 signals.

The mass spectrum of a therminal elimination product, $C_{40}H_{50}O_4$ by precise mass measurements (Scheme 4), obtained from bastaxanthin (*1*), showed characteristic losses of 92 (toluene) and 106 (xylene) mass units from the polyene chain.[19] The loss of 106 mass units defines the C-8′–C-13′ structural element in the non-acetylenic half of the molecule.[19] Other losses in the upper mass region are compatible with the loss of a CHO radical, previously noted for agelaxanthin with analogous C-19 hydroxy substitution,[14] CH_3 and CH_2OH radicals and H_2O. Observed fragment ions are rationalized by the in-chain cleavages indicated in Scheme 4, six of them were confirmed by precise mass measurements. Upon cleavage the charge is nearly exclusively retained on the ketonic part of the molecule. The *m/e* 141.0930 ion ($C_8H_{13}O_2$) assigned by cleavage of the C-5′–C-6′ bond occurs at *m/e* 183 for bastaxanthin diacetate (*5*, Scheme 2), consistent with the presence of one hydroxy group accessible for acetylation in this moiety.

Having now accounted for the presence of a sulfate function, a conjugated keto group, a primary allylic hydroxy group and a second *prim/sec* hydroxy group the final oxygen function in bastaxanthin *1* was, according to IR absorption at 1735 cm^{-1}, compatible with a five-ring ketone. Allocation of this keto group, the sulfate and the second hydroxy function rests on ^{1}H NMR (Scheme 5) and ^{13}C NMR (Scheme 6) data and chemical derivatizations (Schemes 2 and 3).

Assignments of the ^{1}H NMR spectrum (400 MHz) of bastaxanthin (*1*, in CD_3OD for solubil-



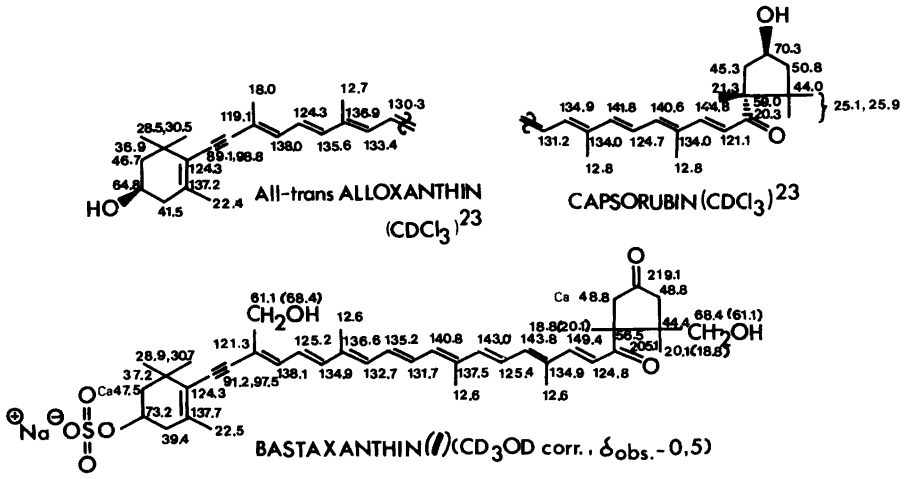
Scheme 5. ^{1}H NMR assignments for bastaxanthin (*1*) and bastaxanthol (*10*).

ity reasons) are aided by comparison with reported data ($CDCl_3$) for alloxanthin [20,1] and capsorubone,[21] Scheme 5.

The sulfate function is clearly assigned to C-3 in an alloxanthin end group by consideration of chemical shifts, coupling patterns and relevant decoupling experiments in comparison with data for alloxanthin [20,21] and related sulfated carotenols.[5,8] The ^{1}H NMR data ($CDCl_3$) for the desulfated bastaxanthol (*10*, obtained by enzymatic or acidic hydrolysis, Scheme 3) support this assignment.

C-3′ location for the five-ring ketone is consistent with the chemical shifts and coupling pattern (J=18 Hz) of four α-methylene protons in bastaxanthin (*1*, CD_3OD) and bastaxanthol (*10*, $CDCl_3$). Treatment with KOD in CD_3OD caused readily D-exchange of all four α-methylene protons, demonstrated by ^{1}H NMR and MS.

An AB system centered at δ 3.54 and 3.73 (2H, J=11.5 Hz), confirmed by spin tickling, is assigned to the diastereotopic methylene protons



Scheme 6. Tentative ^{13}C NMR assignments of bastaxanthin (*1*).

of a hydroxymethyl group attached to C-1′ or C-5′. Upon acetylation a singlet (2H) at δ 4.22 arises. Specific ASIS in pyridine of signals assigned to the two methyl groups and hydroxymethyl group of the cyclopentanone ring support carbonyl functions in their neighbourhood.[22] Preference for C-16′/C-17′ hydroxylation (*1*) *versus* C-18′ hydroxylation (*1b*, Scheme 1) rests on no retroaldol cleavage with formation of methanal upon alkali treatment as expected for a β-hydroxyketone, lacking McLafferty fragmentation with loss of methanal in the MS, no evidence of H-bonding for bastaxanthol (*10*) in ^1H NMR ($CDCl_3$) and the characteristic ^1H NMR chemical shift of CH_3-18′ in capsorubone,[21] bastaxanthin (*1*) and bastaxanthol (*10*). Plausible relative stereochemistry from chemical shift considerations is given for the cyclopentanone group of *10* (Scheme 5).

Tentative ^{13}C NMR assignments made in Scheme 6 by comparison with reported data for all-*trans* alloxanthin and capsorubin [23] and related sulfated carotenols [5] support structure *1* for bastaxanthin. The δ 219 signal is compatible with a five-ring ketone.

Chemical derivatizations of bastaxanthin (*1*) are given in Scheme 2 (sulfated derivatives) and Scheme 3 (non-sulfated derivatives). Scheme 2 summarizes the conversion of bastaxanthin (*1*) to its free acid *2* and the methyl ester *3*, relatively fast acetylation of bastaxanthin to the allylic monoacetate *4*, resistant towards allylic oxidation, and a diacetate *5* which could not be silylated. Allylic oxidation of bastaxanthin (*1*) gave the cross-conjugated aldehyde *6*, which upon acetylation afforded the monoacetate *7* and upon $NaBH_4$-reduction the tetrol *8* as two presumed C-6′ epimers *8a* and *8b*. The same products (*8a+b*) were obtained by complex metal hydride reduction of bastaxanthin (*1*) and were converted to the tetraacetate *9*.

Scheme 3 summarizes the non-sulfated derivatives obtained from bastaxanthin (*1*), including bastaxanthol (*10*) formed by enzymatic hydrolysis accompanied by pronounced 9-*cis* isomerization. Enzymatically produced bastaxanthol (*10*) was identical with natural *10*, characterized as a new, naturally occurring carotenoid. Bastaxanthol (*10*) provided a triacetate *11* upon acetylation, the cross-conjugated carotenal *12* upon allylic oxidation (*12* further gave the diacetate *12b* upon acetylation) and the pentol *17* (*a+b*, C-6′ epimers) upon $NaBH_4$-reduction. Unexpected for a carotenoid, bastaxanthin (*1*) did not decompose with strong acid and provided upon treatment with 0.1 N HCl in methanol for 30 min (70 % pigment recovery) in addition to unreacted bastaxanthin (*1*, 20 % of total) five less polar products of unchanged chromophore. The functional group modifications were determined by MS and acetylation. The most polar product bastaxanthol (*10*) was preceded by the triol

dimethyl ketal *13* and its allylic methyl ether *14*. Dimethyl ketal formation was demonstrated by MS, see also characteristic fragmentation in Scheme 4 for *13b* and *14*. Unusual, facile formation of a non-cyclic ketal may be explained by release of ring strain in the substituted cyclopentanone end group. It is also noteworthy that bastaxanthol dimethyl ketal (*13*) was isolated as a minor carotenoid from *I. basta* (Batch 4+5) and probably represents an artefact of bastaxanthol (*10*) and produced during manipulations with methanol. Low yield of a dimethyl ketal was also obtained in parallel experiments involving treatment of capsanthinone [26] with HCl–methanol. Two less polar, minor products had polarity and MS properties (Scheme 4) compatible with the monomethyl ether *15* and the diether *16*, respectively. Nucleophilic attack of methanol on a protonated *prim.* hydroxy function (C-17′) by S_N2 mechanism is considered more likely than intramolecular attack of the *prim.* hydroxy group on the 5-ring ketone to a cyclic hemiketal followed by methyl ketal formation, since the intermediary cation would not be planar.

Regarding the chirality of bastaxanthin (*1*), natural *1*, bastaxanthin diaceatate (*5*) and bastaxanthol (*10*) exhibited similar CD spectra with a positive peak at 285 nm (with measured $\Delta\varepsilon$= +10.2, +12 and +2.5, respectively). Alloxanthin (half-structure, Scheme 5) has a weak, negative Cotton effect.[25] Capsorubin [26] (half-structure, Scheme 6) has a positive peak at 300 nm (methanol, $\Delta\varepsilon$=*ca.* +10) and also capsorubone (half-structure, Scheme 5) has a positive peak at 304 nm (dioxane, $\Delta\varepsilon$=+6.3; Dr. H. Mayer, personal communication), consistent with previously reported ORD data.[11] The positive Cotton effect seems therefore largely to be governed by the chirality at C-5,5′, and the same chirality at C-5′ for bastaxanthin (*1a*) and capsorubone is assumed. The additivity hypothesis has previously been used successfully for carotenoids with κ end groups.[11] On biogenetic grounds [27] bastaxanthin is expected to exhibit the same chirality at C-3 as alloxanthin. Guided by CD and ^{1}H NMR the tentative stereochemical assignment *1a*, Scheme 1, for bastaxanthin (3*R*,1′*R*,5′*R*) is considered.

EXPERIMENTAL

Methods. If not specified, these were as commonly employed for carotenoid work in our laboratory.

Electronic spectra were recorded on a Coleman Hitachi 124 or Beckmann-DB spectrophotometer, using $E^{1\%}_{1\,cm}$=2500 at λ_{max} for calculation of concentrations, % III/II as a measure of spectral fine-structure and D_B/D_{II} as a measure of *cis*-peak intensity;[28] IR spectra on a Perkin Elmer 257 or 580 B instrument in KBr disc; ^{1}H NMR spectra on a Jeol JNM-FX 100 (100 MHz) FT instrument or a Bruker WM (400 MHz) spectrometer; ^{13}C NMR spectra on the above Jeol instrument (25.1 MHz); MS on an AEI MS902 instrument with direct inlet and CD spectra on a Roussel-Jouan Dicrograph. MS peak intensities are quoted for selected spectra. Diagnostically useful ions only (often without intensities) are cited for less purified derivatives. X-Ray fluorescence spectroscopy was carried out by cand.real. S. Melsom, Central Institute for Industrial Research, Blindern, Oslo, on a Philips 1410 X-ray spectrometer using the Kα-radiance of S as a measure of concentration. R_F-values for sulfated carotenoids are not well reproducible and only meaningful in relation to a reference sulfate.

Biological source. The marine sponge *Ianthella basta* (Porifera, class Desmospongiae, subclass Ceractinomorpha, order Verongida, family Ianthellideae),[1] RRIMP Museum specimen FN 1784/01/000, was collected by Roche Research Institute of Marine Pharmacology, Dee Why, Australia, on the Great Barrier Reef off the coast of Queensland.

In total 5 batches, each up to 6 kg sponge, were examined. No marked difference in the content of polar carotenoids [1] were noted for lyophilized or frozen sponge material.

Extraction was effected with MeOH or acetone–MeOH at room temperature, for Batches 4 and 5 followed by a partition into epiphasic (non-polar) and hypophasic (polar) carotenoids in ether–H_2O 2:1. Hypophasic carotenoids were transferred to EtOAc from H_2O.

Chromatography. Suitable systems for separation of the sulfated carotenoids were: Sephadex LH20 (MeOH), ion exchange chromatography,[30] kieselgel G60 Merck Labor Ferligsaüle (Art. No. 10401) (column packed wet in acetone, EtOAc or 5–10 % MeOH/EtOAc, for preliminary purification), pressurized (0.3–2 atm.), flow *ca.* 15 ml/min) kieselgel G60 (40–63 μm) columns (20 % MeOH in EtOAc, for further purification), cellulose columns (Linters No. 124 or

Avicel, eluant MeOH-acetone), preparative TLC (SiO_2, MeOH-EtOAc) and analytical TLC (Merck No. 5553 DC-Alufolien Kieselgel 60, 0.2 mm). Unsuitable adsorbents were acetylated polyamide and $CaCO_3$ columns.

Several different combinations were used for the various batches depending on quantities and the presence of non-carotenoid contaminants.

Precipitation of contaminants from crude extracts and chromatography fractions were effected at −20 °C from (i) acetone and (ii) MeOH/EtOAc. The colourless precipitates were removed by centrifugation and the process repeated up to 8 times.

Saponification. Particularly oily fractions were submitted to standard saponification (5 % KOH in EtOH−ether overnight) after it had been demonstrated that such alkali treatment caused no other modification of bastaxanthin than *cis*-isomerization.

Yield. The yield was greatly reduced by repeated chromatography and precipitations. Chromatographically purified bastaxanthin available for further studies were *ca.* 3.5+20+10+13+22 mg from the five batches.

Bastaxanthin (*1*)

Bastaxanthin, as salt (1). 1 was isolated as an unspecified salt by extraction and chromatography, as the Na-salt by standard saponification with NaOH in methanol−ether of the diacetate *5* below or as the Ba-salt by precipitation with Ba-acetate in aqueous methanol.

Crystallization of tiny samples was effected from acetone−hexane or EtOH−ether; m.p. (corr., evacuated tube) *ca.* 190 °C.

Sulfur analysis by X-ray fluorescence spectroscopy gave 44 μg S in 0.51 mg *1* (calc. 22.5 μg S).

VIS λ_{max} (MeOH) 360, 474 nm, % D_B/D_{II} <16; (acetone) 474 nm.

IR ν_{max} (KBr) 3430 (vs, OH), 3040 (w, CH=), 2975, 2930 and 2880 (s, CH), 2170 (w, C≡C), 1735 (s, 5-ring C=O), 1660 (s, conj. C=O), 1550 and 1520 (vs, C=C), 1490 (w), 1460 (vw, CH_2), 1420 (vw), 1400 (w), 1240 (vs, S=O), 1160 (vw), 1120 (vw), 1065 (vs, S−O?), 1050 (vs, C−O in CH_2OH), 1010 (w, allylic CH_2OH), 1000 (w). 965 (vs, *trans* CH=CH), 910 (vw), 835 (m, R_2C=CHR) and 790 (w) cm^{-1}.

^1H *NMR, cf.* assignments Scheme 5, δ (CD_3OD, fresh solution, 400 MHz) 0.96 s (3H, CH_3−16′), 1.19 s (3H) and 1.21 s (3H, CH_3−16, 17), 1.48 s (3H, CH_3−18′), 1.57 t (J=12 Hz, 1H, H−2_{ax}), 1.92 s (3H, CH_3−18), 1.98 s (3H, CH_3−19′), 2.01 s (6H, CH_3−20,20′), 2.07 dd (J_{gem}=12 Hz, $J_{ax,eq}$=3.5 Hz, 1H, H−2_{eq}), 2.19 d (J_{gem}=18.5 Hz, 1H, H−2′), 2.21 dd (J_{gem}=18.5 Hz, $J_{ax,ax}$=10 Hz, 1H, H−4_{ax}), 2.38 d (J_{gem}=18.5 Hz; 2H, H-2′, H-4′), 2.63 dd (J_{gem}=18.5 Hz, $J_{eq,ax}$=5.5 Hz, 1H, H-4_{eq}), 2.77 d (J_{gem}=18.5 Hz, 1H, H-4′), 3.54 d (J=11.5 Hz, 1H, H_a-17′), 3.73 (J=11.5 Hz, 1H, H_b-17′), 4.18 s (trace, H-19 in Δ9-*cis*), 4.32 s (2H, H-19), 4.6 m (1H, H-3), 6.67 d (J=14.5 Hz, 1H, H-7′), 7.36 d (J=14.5 Hz, 1H, H-8′), 6.3−6.8 m (10H, olefinic). Homonuclear spin decoupling was effected. The first figure cites frequency of irradiation in ppm and the second figure observed change at ppm: 7.36−6.67 (d→s), 6.68−7.36 (d→s), 4.6−1.57 (t→d), 2.07−2.21 (dd→d) and 2.63 (dd→d), 2.63−2.21, 2.6−4.61.

Spin tickling confirmed the relationship between the δ 3.54 and δ 3.73 doublets. Storage in CD_3OD or treatment with KOD/CD_3OD caused disappearance of the δ 2.19, 2.38 and 2.77 signals.

δ (D-pyridine) 1.13 s (3H, CH_3−16′), 1.22 s (3H) and 1.30 s (3H), CH_3−16,17, 1.63 s (3H, CH_3−18′), 1.90 s (6H, CH_3−18,19′), 2.01 s (6H, CH_3−20,20′), 2.34−3.28 m (*ca.* 6H, CH_2), 3.78 d (J=12 Hz, H_a−17′), 4.00 d (J=12 Hz, H_b−17′), 4.80 s (2H, H-19), 5.2 m (1H, H-3), 6.4−7 m (olefinic H), 7.68 d (J=15 Hz, H-8′).

^{13}C *NMR, cf.* tentative assignments, Scheme 6, δ (CD_3OD, 10 mg *1*): 13.15 (C-20,19′,20′), 19.35 (C-18′ or C-16′), 20.58 (C-16′ or C-18′), 22.97 (C-18), 29.41 and 31.28 (C-16,17), 37.72 (C-1), 39.94 (C-4), 44.91 (C-1′), *ca.* 48 (C-2), *ca.* 49.3 (C-2′ and C-4′), 56.96 (C-5′), 61.59 (C-19 or C-17′), 68.96 (C-17′ or C-19), 73.75 (C-3), 91.71 (C-7), 98.09 (C-8), 121.84 (C-9), 124.83 (C-6), 125.35 (C-7′), 125.76 (C-11), 125.94 (C-11′), 132.20 (C-14′), 133.25 (C-14), 135.47 (C-12 and C-9′), 135.77 (C-15), 137.11 (C-13), 138.05 (C-13′), 138.22 (C-5), 138.57 (C-10), 141.27 (C-14′), 143.49 (C-12′), 144.31 (C-10′), 149.92 (C-8′), 205.62 (C-6), 219.60 (C-3′).

MS (210 °C, 70 eV), *m/e*: 594.3721 (M′, calc. 594.3709 for $C_{40}H_{50}O_4$, 21 %), 579 (M′−15, 2 %), 576 (M′−18, 9 %), 565 (M−29, 1 %), 563 (M−31, 1 %), 558 (M′−18−18, 1 %), 530 (M′−64, 2 %), 502 (M′−92, 1 %), 488 (M′−106, 8 %), 470 (M′−124=M′−106−18, 5 %), 455 (M−139, 2 %), 428 (M′−166, 4 %), 425.2772 (calc. 425.2843 for $C_{31}H_{37}O$, 3 %), 410 (M−184, 2 %), 408.2707 (calc. 408.2664 for $C_{27}H_{35}O_3$, 2 %), 407 (2 %), 368.3369 (calc. 368.3389 for $C_{24}H_{32}O_3$, 3 %), 341 (3 %), 339 (3 %), 313 (2 %), 301 (2 %), 287.1680 (calc. 287.1648 for $C_{18}H_{25}O_3$, 4 %), 235 (17 %), 221 (1 %), 169.0870 (calc. 169.0864 for $C_9H_{13}O_3$, 16 %), 141.0930 (calc. 141.0915 for $C_8H_{13}O_2$, 55 %), 119 (45 %), 91 (100 %), 69 (60 %), 43 (100 %).

CD (MeOH): nm ($\Delta\varepsilon$) 240 (−3.1), 250 (−2.8), 258 (0), 290 (+10.2), 325 (0), 373 (−3.2).

For comparison capsorubin [26] had (MeOH): 240 (0), 249 (2.0), 299 (+9.9), 325 (0), 370 (−2.5).

R_F-value: TLC (SiO_2, 15 % MeOH−EtOAc) *ca.* 0.21; (SiO_2, 10 % MeOH−EtOAc) 0.14.

Electrophoretic behaviour. Cellulose acetate and Whatman papers were unsuitable due to irreversible pigment absorption. Acetate buffer, 0.05 M pH 7.0 containing 30 % isopropanol (for solubility reasons) was employed for (i) glass fiber sheets and (ii) polyacrylamide gel tubes at 2 m.a. Electrophoretic mobility (i) zeaxanthin 0.0 cm, zeaxanthin monosulfate 1.8 cm, bastaxanthin 2.0 cm, zeaxanthin disulfate 2.4 cm, (ii) zeaxanthin monosulfate 0.6 cm, bastaxanthin 0.7 cm, zeaxanthin disulfate 1.3 cm.

Solubility. Bastaxanthin dissolves well in MeOH, H_2O (if solid material first moistened with MeOH) and DMSO, is partly soluble in pyridine and EtOAc and badly soluble in tetrahydrofurane, ether and acetone.

Partition behaviour. Bastaxanthin was completely hypophasic when partitioned between hexane−50 % aq. MeOH.

Iodine catalyzed stereomutation in MeOH with traces of I_2 dissolved in benzene caused over longer periods no change in the electronic spectrum or formation of new zones on TLC (SiO_2).

Stereomutation by alkali treatment. Treatment of *1* (1 mg) with 5−10 % KOH in MeOH overnight caused no change in *vis.* absorption, ^{1}H NMR, MS or R_F-value (TLC, SiO_2).

Separation on Merck No. 5553 DC-Alufolien Kieselgel 60 (0.2 mm) in 15 % MeOH–EtOAc with prolonged development gave four zones:

All-*trans* bastaxanthin (*1*), major, λ_{max} (MeOH) 360, 474 nm, % D_B/D_{II}=16; MS (200 °C), *m/e* 594 (M′), 576 (M′−18), 565, 502 (M′−92), 488 (M′−106), 470 (M′−106−18), 141.

Neo A bastaxanthin (*1*, 9-*cis*?), major, λ_{max} (MeOH) 360, 470 nm, % D_B/D_{II}=20; MS (200 °C) *m/e* 594 (M′), 576 (M′−18), 488 (M′−106), 470 (M′−106−18), 141.

Neo B bastaxanthin (*1*, unspecified di-*cis*?), minor, λ_{max} (MeOH), 360, 471 nm, % D_B/D_{II}=20, MS (200 °C) *m/e* 594 (M′), 488 (M′−106), 470 (M′−106−18), 141.

Neo C bastaxanthin (*1*, unspecified mono-*cis*), major, R_F-value as bastaxanthin diacetate (*5*), λ_{max} (MeOH) 471 nm, % D_B/D_{II}=22; MS (200 °C) *m/e* 594 (M′?), spectrum contaminated with brominated metabolites; IR (KBr) 3400, 2930, 2860, 2170, 1735, (1660), 1635, 1575, 1470, 1375, 1240, 1090, 1050, 970, and 830 cm^{-1}. Acetylation provided a product with MS very similar to that of bastaxanthin diacetate (*5*) below. Prolonged treatment of the acetylated product with $NaBH_4$ in EtOH gave a reduced product λ_{max} (EtOH) 445 and 472 nm, inseparable from all-*trans* *8a* and *8b*, see below.

Slow reversible isomerization in 1 % KOH–MeOH (not readily in I_2-MeOH) of all-*trans* to Neo A, neo A to all-*trans*, neo B to neo A and of neo C to all-*trans* was demonstrated chromatographically.

Evidence for the presence of the 9-*cis* isomer in alkali-treated bastaxanthin (*1*) followed from ^{1}H NMR (CD_3OD): δ 4.18 s (=C−CH_2OD in 9-*cis*), up to 30 % of the δ 4.32 s (in all-*trans*) signal. Likewise Δ9-*cis* isomerization caused a shift of the δ 1.21 methyl signal to δ 1.30 and of the δ 1.92 methyl signal to δ 1.90, *cf.* Scheme 5.

Treatment with diazomethane. 1 (0.17 mg) in MeOH (1 ml) was treated with CH_2N_2 in ether (5 ml) for 5 min. No new products were formed according to *vis.* spectrum, MS and TLC (SiO_2).

Bastaxanthin, as acid (2). A solution of bastaxanthin (*1*, 0.95 mg) in MeOH/H_2O 1:1 was ion exchanged on a column (0.8×15 cm) packed with Dowex 50 (Fluka 44445). The eluate (10 ml) had pH 1.88. *2* had λ_{max} (MeOH) 474 nm, R_F (SiO_2, 15 % MeOH−EtOAc) 0.21 as *1*, was stable in dilute ether solution, but decolourized upon concentration.

Bastaxanthin methyl ester (3) was prepared from *2* (0.65 mg) and freshly prepared CH_2N_2 [29] in ether and isolated by TLC (SiO_2). *3* had R_F 1.00 (25 % MeOH−EtOAc), 0.24 (40 % acetone−hexane), λ_{max} (MeOH) 474 nm, MS (190 °C) *m/e* 706 (M<1 %), 676 (M−30, 5 %), 648 (3 %), 548 (5 %), 523 (6 %), 429 (40 %), 410 (60 %), 325 (7 %), 281 (12 %), 221 (18 %), 191 (24 %), 151 (50 %), 119 (21 %), 91 (60 %), 69 (65 %), 43 (100 %).

Acetylation of bastaxanthin (1). The acetylation of *1* (0.1 mg) in dry pyridine (1 ml) with acetic anhydride (0.1 ml) at 0 °C was monitored by TLC (SiO_2, 10 % MeOH−EtOAc). *1* (R_F=0.14) was converted *via* the monoacetate *4* (R_F=0.18) to the diacetate *5* (R_F=0.26). The following ratios were estimated: 5 min 50 % *1* +50 % *4*, 10 min. 20 % *1* +70 % *4* +10 % *5*, 15 min. 5 % *1* +80 % *4* +15 % *5*, 30 min, 0 % *1* +50 % *4* +50 % *5*, 45 min. 0 % *1* +30 % *4* +70 % *5*.

The mono- (*4*) and diacetate (*5*) were isolated in preparative experiments by acetylation of *1* (1−7 mg) at room temperature.

Bastaxanthin monoacetate (4), ca. 0.1 mg λ_{max} as *1*, was submitted to allylic oxidation with *p*-chloroanil [30] in EtOH for 1 h. No new, more pink products were formed judged by TLC.

Bastaxanthin diacetate (5), total yield *ca.* 10

mg; had λ_{max} (MeOH) 474 nm; IR (KBr) ν_{max} 3400 (s, OH), 3015 (w,=CH), 2960, 2910 and 2860 (s, CH), 2170 (w, C≡C), 1740 (s, acetate and 5-ring ketone), 1655 m (conj. C=O), 1555 (s), 1510 (m), 1460 (m), 1360 (m), 1230 (vs, S=O and ester), 1065 (s), 1040 (s, C–O), 970 (s, *trans*-CH=CH–), 835 (m, CR_2=CHR), and 795 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, after prolonged storage in CD_3OD, resulting in complete exchange of the four 2′,4′ protons): δ 1.04 s (3H, CH_3–16′), 1.19 s (3H) and 1.20 s (3H, CH_3–16,17), 1.47 s (3H, CH_3–18′), 1.92 s (3H, CH_3–18), 1.57 t (J=12 Hz, 1H, H-2_{ax}), 1.98 s (3H, CH_3–19′), 2.00 s (6H, CH_3–20,20′), 2.01 s (3H, OAc), 2.02 s (3H, allylic OAc), 2.21 dd (J_{gem}=18.5 Hz, $J_{ax,ax}$=10 Hz, 1H, H-4_{ax}), 2.61 dd (J_{gem}=18.5 Hz, $J_{eq,ax}$=5.5 Hz, 1H, 4-H_{eq}), 4.22 s (2H, H-17′), 4.9 s (=C–CH_2OAc), 6.3–6.9 m (olefinic H), 7.36 d (J=16 Hz, 1H, H-8′), irradiation at δ 7.34 caused the doublet at δ 6.65 (J=14.5 Hz) to collapse to a singlet; δ (100 MHz CD_3OD, fresh solution) exhibited extra CH_2 signals at *ca.* 2.35 m (*ca.* 2H, H-2,4) and 2.76 d (J=18 Hz, 1H, H-4); δ (pyridine-d_5) 1.11 s (3H, CH_3–16′), 1.25 s (lipid and CH_3–16,17), 1.46 s (3H, CH_3–18′), 1.95 s (*ca.* 3H, CH_3–18), 2.02 s (CH_3–20,19′,20′ and Ac), 2.2–3.4 m (CH_2), 4.40 s (2H, CH_2OAc), 5.12 s (=C–CH_2OAc), 6.4–7.5 m (olefinic H); MS (200 °C) *m/e* 678 (M′, 7 %), 663 (M′–15, <1 %), 618 (M′–60, 3 %), 572 (M′–106, 3 %), 512 (M–166, 1 %), 497 (2 %), 407 (2 %), 183 (8 %), 105 (90 %), 91 (90 %), 69 (49 %) and 43 (100 %); CD (MeOH) nm ($\Delta\varepsilon$) 232 (–3.8), 247 (–3.8), 258 (0), 290 (+12), 325 (0), 380 (–2.8).

Attempted silylation of bastaxanthin diacetate (5). *5* (0.15 mg) was submitted to standard silylation conditions [31] at room temperature. No new products were formed according to TLC.

Alkaline hydrolysis of bastaxanthin diacetate (5) at standard conditions [31] in 5 % KOH–methanol provided bastaxanthin (*1*) according to *vis.* spectrum, ^1H NMR, MS, and TLC (SiO_2).

Allylic oxidation of bastaxanthin (1). *1* (0.3 mg) in abs. EtOH (0.5 ml) and benzene (4 ml) was reacted with *p*-chloranil (1.5 mg) for 3 h. Additional *p*-chloranil (1.5 mg) and traces of I_2/benzene were added [30] and the reaction interrupted after 10 h at room temperature; pigment recovery 50 %. TLC revealed the formation of a slightly less polar, pink product *6*, R_F=0.22 (SiO_2, 15 %, MeOH–EtOAc); λ_{max} (MeOH) 495 nm; no MS could be obtained.

Acetylation of the allylic oxidation product 6 at 0 °C was monitored by TLC. *6* (0.1 mg) was converted to the monoacetate (*7*, R_F=0.29 on SiO_2, 15 % MeOH–EtOAc). The following ratios were estimated: 3 min 98 % *6* +2 % *7*, 10 min. 90 % *6* +10 % *7*, 15 min. 80 % *6* +20 % *7*, 30 min 70 % *6* +30 % *7*, and 4 h 100 % *7*.

$NaBH_4$ reduction of allylic oxidation product 6. Treatment of *6* (0.05 mg) with $NaBH_4$ in EtOH caused reduction to *8a+b* (1:1), λ_{max} (MeOH) (325), 338, (418), 443, and 471 nm, inseparable from *8a* and *8b* characterized below.

Complex metal hydride reduction of bastaxanthin (1). Treatment at 0 °C for 10 min of bastaxanthin (*1*, 0.1–0.5 mg aliquots) with (i) excess $NaBH_4$ in EtOH or (ii) $LiAlH_4$ in dry tetrahydrofurane or of bastaxanthin diacetate (*5*) with excess $NaBH_4$ in EtOH at room temperature gave the same reduction product *8*.

All-*trans* bastaxanthin (*1*, not previously alkali treated) gave on the commercial kieselgel plates two products (*8a* and *8b*) in 1:1 ratio, considered as all-*trans* C-6′ epimers.

Previously alkali-treated bastaxanthin gave four products, considered as mainly all-*trans* *8a* and *8b* (1:1) and mono-*cis* *8a* and *8b* (1:1), the latter pair being slightly more strongly adsorbed. Upon storage the mono-*cis* isomers were partly converted to the all-*trans* isomers. The isomerization occurred more rapidly in the presence of 5 % KOH in MeOH.

Reduced bastaxanthin 8. All-*trans* reduction product *8a+b*, R_F=0.18 (SiO_2, 15 % MeOH–EtOAc), had λ_{max} (MeOH) (325), 335, (418), 443, and 472 nm, % D_B/D_{II}=15 and % III/II=24. Mono-*cis* reduction product *8a+b* had R_F=0.22 (SiO_2, 15 % MeOH–EtOAc), λ_{max} (MeOH) (325), 335, (418), 443 and 472 nm, % D_B/D_{II}=16 and % III/II=24.

8 had λ_{max} (MeOH) (325) 338, (418), 443, and 471 nm % D_B/D_{II}=15, % III/II=22, IR (KBr) ν_{max} 3400 (s, OH), 3015 (w, =CH), 2960, 2920 and 2860 (s, CH), 2170 (w, C≡C), 1460 (m), 1230 (s, S=O), 1065 (m), 965 (s, *trans* CH=CH), and 835 (w, R_2C=CHR) cm^{-1}; ^1H NMR (CD_3OD) δ 0.95 s (3H, CH_3–16′), 1.19 s (3H) and 1.22 s (3H, CH_3–16,17), 1.28–1.6 (imp. and CH_3–18′), 1.95 s (6H, CH_3–18,19′), 1.98 (6 H, CH_3–20,20′), 3–4 (imp. and H-17′), 4.33 s (2H, =C–CH_2OH), and olefinic H; MS (220 °C) *m/e* 598 (M′, 1 %), 596 (M′–2, 1 %), 583 (M′–15, 5 %), 492 (M′–106, 1 %), 477 (M′–106–15, 2 %), 455 (2 %), 256 (19 %), 145 (16 %), 143 (24 %), 105 (40 %), 91 (60 %), 69 (50 %), 43 (100 %).

Standard acetylation of *8* (0.7 mg) provided after purification by TLC the tetraacetate *9* (0.5 mg).

Tetraacetate 9 *of reduced bastaxanthin.* *9* had R_F=0.67 (SiO_2, 25 % MeOH–EtOAc); λ_{max} (MeOH) 420, 444 and 470 nm, % III/II=23; ^1H NMR (CD_3OD) δ 1.09 s (lipid and CH_3-16′), 1.18 s (3H) and 1.20 s (3H, CH_3-16,17), 1.26 s

(lipid and CH_3-18'), 1.92 s (*ca.* 6H, CH_3-18,19'), 1.98 s (*ca.* 9H, CH_3-20,19',20'), 2,02 s (3H, Ac), 2.03 s (3H, Ac), 2.08 s (6H, two Ac), 2.1–3.0 m (CH_2), 4.07 d (J=11 Hz, 1H, H_a-17'), *ca.* 4.1 m (H-3), 4.23 d (J=11 Hz, 1H, H_b-17'), 4.83 s (2H, =C–CH_2OAc), and 6.2–6.8 m (olefinic H); MS (210 °C) *m/e* 776 (M', <1 %), 183 (13 %), 119 (41 %), 91 (67 %), 60 (80 %), 43 (100 %).

Semisynthetic bastaxanthol (*10*)

Enzymatic hydrolysis of bastaxanthin (1). Enzymes used were purchased from Sigma Chemical Company, St. Louis, Missouri, and were isolated from (i) *Helix pomatia* or (ii) *Patella vulgaris*. Equal weights of carotenoid and enzyme were used. Experiments carried out in 0.2 M acetate buffer or 0.2 % NaCl solution were unsuccessful due to salting out of the carotenoid.

Bastaxanthin (0.5–1 mg) was dissolved in 1 drop of MeOH, the solution diluted with 1.5 ml H_2O and treated with the enzyme at 37 °C for *ca.* 24 h. After transfer to EtOAc the pigment recovery was 90–100 % with 20–30 % conversion to bastaxanthol.

Bastaxanthol (10), total yield from enzymatic hydrolysis *ca.* 2 mg, R_F=0.87 (SiO_2, EtOAc), R_F=0.40 (SiO_2, 40 % acetone–hexane); λ_{max} (acetone) 362, 469, (495), (hexane) 360, 470 (495), (MeOH) 360, 470 and (benzene) 373, 486 nm; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.07 s (3H, CH_3-16'), 1.15 s (3H) and 1.22 s (3H, CH_3-16,17), 1.42 s (3H, CH_3-18'), 1.94 s (3H, CH_3-18), 1.96 s (9H, CH_3-20,19',20'), 2.13 d (J=18 Hz, 1H, H_a-2'), 2.26 d (J=18 Hz, 1H, H_b-2'), *ca.* 2.45 (H-4), 2.42 d (J=18 Hz, 1H, H_a-4'), 3.02 d (J=18 Hz, 1H, H_b-4'), 3.59 d (J=12 Hz, H_a-17'), 3.81 d (J=12 Hz, H_b-17'), 4.04 m (1H, H-3), 4.22 s (=C–CH_2OH, Δ 9-*cis*, *ca.* 35 % rel. *trans*), 4.38 s (=C–CH_2OH, *trans*), 6.2–6.8 m (olefinic H), 7.52 d (J=14 Hz, 1H, H-8'); δ (100 MHz, CD_3OD, protons at 2',4' partly exchanged) δ 0.97 s (*ca.* 3H, CH_3-16'), 1.19 s (*ca.* 3H) and 1.22 s (*ca.* 3H, CH_3-16,17), 1.48 s (*ca.* 3H, CH_3-18'), 1.92 s (*ca.* 3H, CH_3-18), 2.00 s (*ca.* 9H, CH_3-20,19',20'), 2.1–3.0 m (CH_2), 3.50 d (J=12 Hz, 1H, H_a-17'), 3.72 d (J=12 Hz, 1H, H_b-17'), 4.18 s (=C–CH_2OH, in Δ9-*cis*, *ca.* 40 % of *trans* signal), 4.36 (=C–CH_2OH, *trans*) 6.2–6.8 m (olefinic H), and 7.38 d (J=14 Hz, 1H, H-8'); δ ($CDCl_3$, 400 MHz) 1.10 s (*ca.* 3H, CH_3-16'), 1.15 s (CH_3-16 or 17 in all-*trans*), 1.20 s (*ca.* 3H, CH_3-17 or 16), 1.29 s (CH_3-16 or 17 in Δ9-*cis*), 1.35 s (3H, CH_3-18'), 1.46 t (J_{gem}=12 Hz, $J_{ax,ax}$=12 Hz, 1H, H-2_{ax}), 1.86 dd (J_{gem}=12 Hz, $J_{eq,ax}$=*ca.* 3 Hz, H-2_{eq}), 1.93 s (<3H, CH_3-18 in all-*trans*), 1.95 s (6H, CH_3-19,20'). 1.97 s (CH_3-18 in Δ9-*cis*), 1.99 s (3H, CH_3-19'), 2.13 d (J=18 Hz, 1H, H-2'$_a$), 2.26 d (J=18 Hz, 1H, H-2'$_b$) 2.41 d (J=18 Hz, H-4'$_a$), *ca.* 2.10 m (*ca.* 1H, H-4_{ax}),[21] *ca.* 2.45 m (*ca.* 1H, H-4_{eq}),[21] 3.03 d (J=18 Hz, 1H, H-4'$_b$), 3.49 dd (J=11 Hz, J_2=10 Hz, 1H, H_a in –CH_2OH at C-17'), 3.61 s broad (1H, OH), 3.80 d (J=11 Hz, 1H, H_b in CH_2OH at C-17'), 4.00 s broad (1H, H-3), 4.22 s (=C–CH_2OH, Δ 9-*cis*, *ca.* 40 % of *trans* signal) 4.38 s (=C–CH_2OH in all-*trans*), 4.42 s broad (1H, OH, signal disappears upon D_2O addition), 6.3–6.9 m (*ca.* 11 H, olefinic) with tentative assignments, 6.31 d (1H, J=9 Hz, H-10), 6.34 d (1H, J=12.5 Hz, H-7'), 6.39 d (1H, J=10 Hz, H-14), 6.41 d (1H, J=12.5 Hz, H-12), 6.46 d (1H, J=14 Hz, H-12'), 6.52 d (1H, J=9 Hz, H-14'), δ 6.58–6.73 m (*ca.* 5H, H-15,15',11,11',10'), 6.88 dd (<1H, J_1=8 Hz, J_2=13 Hz, H-11 in Δ 11 *cis*?); 7.52 d (J=15 Hz, 1H, H-8); signals ascribed to impurities δ 0.87 and 1.25 (lipid), 1.56 (H_2O), 2.59 dd (J_1=8 Hz, J_2=16 Hz, *ca.* 1H), 2.79 t (J=8 Hz, <1H), 2.86 t (J=8 Hz, <1H), 4.07 dd (J_1=8 Hz, J_2=16 Hz, *ca.* 1H), 5.05 s (<1H, signal remains after D_2O addition), 6.98 s (*ca.* 1H, not H-bonded OH since present also in CD_3OD spectrum of *10*) and signal did not disappear upon addition of NaOD/D_2O; MS (200 °C) *m/e* 612.3801 (calc. 612.3815 for $C_{40}H_{52}O_5$), 594 (M–18), 576 (M–18–18), 506 (M–106), 141, 105, 91, 69 and 43.

D-exchange of bastaxanthol (10). After D-exchange in NaOD/CD_3OD/D_2O followed by TLC (SiO_2) and elution with CH_3OH d_{1-4}-bastaxanthol (*10*) had MS (200 °C) corresponding to that of *10* with *m/e* 616, 615, 614, 613 (M) *etc.*

In a parallel experiment, capsanthinone (M=582) was treated in the same manner and showed for d_{1-4}-capsanthinone MS *m/e* 586, 585, 584, 583 (M).

Bastaxanthol triacetate (11), prepared by standard acetylation of bastaxanthol (*10*, 0.1 mg) had R_F=0.31 (SiO_2, 10 % acetone–hexane), R_F=0.87 (SiO_2, 40 % acetone in hexane); λ_{max} (hexane) 362, (445), 470 and 498 nm, % III/II=5, (MeOH) 360, 470 nm; MS (200 °C) *m/e* 738 (M), M–60, M–106, M–60–60, M–136, 143, 105, 91, 69, 43.

Allylic oxidation of bastaxanthol (10) was effected with *p*-chloranil [30] for 3 h and resulted in a deeper pink oxidation product (*12, ca.* 30 % of recovered pigment), which could not be properly separated from *10*. Acetylation gave the presumed diacetate *12b*, R_F=0.90 (SiO_2, EtOAc); λ_{max} (MeOH) 497 nm.

Acid hydrolysis of bastaxanthin (1). To bastaxanthin (*1*, 0.56 mg cryst.) in MeOH (3 ml) was added 0.3 N HCl in MeOH (1.5 ml). The mixture

was kept at 30–40 °C for 30 min, pigment recovery after transfer to EtOAc 0.4 mg (70 %). TLC (SiO_2, 10 % MeOH–EtOAc) revealed the presence of unreacted *1* (*ca.* 20 % of total) and in order of decreasing adsorption (SiO_2) the products *10* and *13* (together *ca.* 60 % of total) and *14, 15,* and *16* (together 20 %). The more polar products *10* and *13* were further characterized after standard acetylation.

Bastaxanthol (10) from acid hydrolysis, characterized as the triacetate *11* had R_F=0.31 (SiO_2, 10 % acetone–hexane) and was inseparable from the triacetate *11* derived from bastaxanthol (*10*) from the enzymatic hydrolysis, had λ_{max} (hexane) 472 and 500 nm, (acetone) 470 nm and (MeOH) 470 nm; MS (190 °C) *m/e* 738 (M), 678 (M–60), 632 (M–106), 618 (M–60–60), 572 (M–106–60), 512 (M–106–60–60), 452 (M–106–60–60–60), 407, 183, 141, 123, 105, 91, 69, 43.

Bastaxanthol dimethyl ketal (13) from the acid treatment, characterized as the triacetate *13b*, had λ_{max} (acetone) 467 nm; MS (200 °C) *m/e* 784 (M), 753 (M–31), 752 (M–32), 710 (M–32–42), 692 (M–92), 678 (M–106), 664 (M–60–60), 650 (M–32–42–60), 633 (M–60–60–31), 618 (M–106–60), 604 (M–60–60–60), 590, 558, 257, 197 (100 %), 137, 105 (100 %), 91 (100 %), 43 (100 %), 32 (100 %).

Bastaxanthol dimethyl ketal 19-methyl ether (14) from the acid treatment had λ_{max} (acetone) 466, (495) nm; MS (200 °C) *m/e* 672 (M), 641 (M–31), 640 (M–32), 622 (M–32–18), 577 (M–31–32–32), 566 (M–106), 215 (cleavage of Δ 7′), 155 (100 %), 138, 105 (100 %), 91 (100 %), 69 (100 %), 32 (100 %).

Bastaxanthol methyl ether (15) from the acid treatment had λ_{max} (acetone) 465, (493) nm; MS (200 °C) *m/e* 626 (M) 577, 551, 520 (M–106), 155 (100 %), 91 (100 %), 69 (100 %), 32 (100 %).

Bastaxanthol dimethyl ether (16) had λ_{max} 465, (493) nm, MS (200 °C) *m/e* 640 (M), 520 (M–106), 155 (100 %), 105, 91, 69, 55, 44, 43, 32 (all 100 %).

Capsanthinone dimethyl ketal-d_{3-4}. Capsanthinone-d_{3-4} (0.26 mg) was kept in 0.1 N HCl/MeOH (3 ml) at 30–40 °C for 1.5 h; pigment recovery 75 %. TLC (SiO_2, 40 % acetone in hexane) showed unreacted capsanthinone (80 % of total) and the less polar dimethyl ketal (15 %), λ_{max} (acetone) 358, 464 nm; MS *m/e* 631, 632 (d_{3-4}, M), M–32, 141, and 142 (strong, corresponding to *m/e* 155 for ketal *14* and *m/e* 197 for ketal *13b*).

Natural bastaxanthol (*10*)

Bastaxanthol (10), total yield *ca.* 1 mg, was found as a minor carotenoid amongst the non-polar carotenoid fractions of Batches 4 and 5. Natural *10* had R_F=0.87 (SiO_2, EtOAc), 0.40 (SiO_2, 40 % acetone in hexane); λ_{max} (acetone) (360), 468, (490) nm, (MeOH) 468, (490) nm; IR (KBr,weak) ν_{max} 3400 (vs, OH), 2900–3000 (m, CH) *ca.* 2100 w (C≡C), 1735 (m, 5-ring C=O), 1660 (s, conj. C=O), *ca.* 1550 (s, C=C), 1210 (m), 1120–1140 (m), *ca.* 1050 (m, C–O), 985 (m, *trans* CH=CH), 835 (w, CR_2=CHR), 700 (w) cm^{-1}; ^1H NMR (CD_3OD) δ 0.96 s (3H, CH_3–16′), 1.18 s (3H) and 1.23 s (3H, CH_3–16,17), 1.48 s (3H, CH_3–18′), 1.92 shoulder (CH_3–18), 1.96 s (*ca.* 3H, CH_3–19′), 2.00 s (*ca.* 6H, CH_3–20,20′), 2–3 (CH_2), 3–4.2 (imp. and H-17′) 4.32 s (=C–CH_2OH), 6.3–6.8 m (olefinic H) and 7.32 d (*J*=14 Hz, 1H, H-8′); MS (190 °C) *m/e* 612 (M), 594 (M–18), 576 (M–18–18), 506 (M–106), 488 (M–106–18), 141; CD (MeOH) nm Δε 220 (–9), 260 (0) 287 (+2.5), 310 (0).

Acetylation of natural 10. The acetylation at standard conditions at 0 °C, monitored by TLC (SiO_2), showed three intermediary acetates, presumably the allylic monoacetate (A), two allylic diacetates (B and C) and a final triacetate (*11*). The following ratios were estimated 5 min. 50 % *10* +50 % *A; 10 min. 40 % 10,* 40 % A and 20 % *B,* 15 min. 30 % *10,* 50 % A and 20 % B, 30 min. 0 % *10,* 50 % A, 25 % B, 25 % C; 45 min. 0 % *10,* 20 % A, 50 % B, 20 % C and 10 % *11*, 90 min. 0 %*10,* 10 % A, 40 % B, 10 % C and 40 % *11*.

Bastaxanthol triacetate (11) had R_F=0.31 (SiO_2, 10 % acetone in hexane) and λ_{max} (MeOH) as *10*; MS (200 °C) *m/e* 738 (M), 678 (M–60), 576, 572 (M–60–106), 183.

$NaBH_4$-reduction of natural bastaxanthol (10). Reduction of *10* (0.1 mg) in MeOH with $NaBH_4$ gave the presumed pentol *17* as two epimers (*a* and *b*) with R_F=*ca.* 0.5 (SiO_2, 10 % MeOH–EtOAc), each with λ_{max} (MeOH) (415), 441 and 469 nm, % III/II=28. Standard acetylation of *17* (0.05 mg) provided the less polar presumed pentaacetate of unchanged *vis.* spectrum; MS (210 °C) *m/e* 766 (M–60), 660 (M–60–106).

Artefact bastaxanthol dimethyl ketal (13). *13,* yield *ca.* 0.3 mg, was isolated from Batch 4+5. *13,* R_F=0.6 (SiO_2, 40 % acetone in hexane), less strongly adsorbed than bastaxanthol (*10*) had λ_{max} (MeOH) 470 (490) nm; MS (190 °C) *m/e* 626.3971 (calc. 626.3941 for $C_{41}H_{54}O_5$, M–32), 608.3866 (calc. 608.3837 for $C_{41}H_{52}O_4$, M–32–H_2O), 594 (M–32–32), 520

(M−32−106), 155, 141, 91, 69, 43, 32. Acetylation provided the triacetate *13b* R_F=0.5 (SiO_2, 10 % acetone in hexane), VIS λ_{max} (acetone), 470 nm; MS (200 °C) *m/e* 784 (M), 752 (M−32), 740 (M−44), 724 (M−60), 710 (M−32−42), 678 (M−106), 660 (M−60−32−32), 197 strong, 91, 69, 60, 43, 32.

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DNA Concentrations in the Human Cerebellum. Computation from Kinetics of Deoxyribose Extraction in Hot Acid

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DNA can be measured in mammalian tissues by extracting deoxyribose from unfixed, lyophilized tissue specimens with 0.5 N perchloric acid at 90 °C. Deoxyribose concentrations in the extract are determined photometrically by reaction with diphenylamine. Inevitably, some deoxyribose is destroyed during exposure to the hot acid. A computer program has been written which corrects photometric absorbance data for such loss of deoxyribose. When extrapolated to infinite duration of extraction, the corrected absorbances yield a measure of the DNA content of the specimen.

This method was used to estimate DNA concentrations in human cerebellar cortex and white matter. The results are discussed in relation to stable carbon isotope ratios of human cerebellar DNA.

Several photometric methods for estimating tissue DNA are based on measurements of extractable deoxyribose, because almost all deoxyribose in animal tissues exists as the pentose moiety of DNA.[1] Deoxyribose can be almost completely extracted from mammalian tissues within a few hours using 0.5 N perchloric acid at 90 °C. To compensate for the concomitant partial destruction of deoxyribose during its exposure to hot acid, Løvtrup and Roos devised a reiterative graphical procedure (LRP) for extrapolating the corrected absorbance, A_o^*, from the sequence of absorbances observed after the reaction of diphenylamine with sequential aliquots of the extract.[2–4] They found that A_o^*, the corrected absorbance which would have been observed after prolonged extraction had there been no concomitant destruction of deoxyribose, correlated well with independent microbiological assays of tissue DNA.

Although other techniques are available for determining DNA in tissue, photometric methods based on the extraction of deoxyribose from tissue powder by hot acid,[5–7] the so-called Schneider procedure, are convenient and are used widely. It has been found that DNA concentrations measured by such extractions vary in accuracy with the type of tissue being analyzed and with the physiological state of cells in a specimen, apparently due to differences in the extractability of deoxyribose or to variable rates of deoxyribose destruction by hot acid.[8–11] In 1966, Munro and Fleck [12] suggested that automated analysis may restore the attractiveness of the Schneider procedure in spite of inherent errors in extraction. The computation method described in this communication fulfills Munro and Fleck's criterion of full automation of data analysis, while taking into account not only the kinetics of deoxyribose extraction and destruction but also the presence of acid-resistant interfering substances in the extract. Although the principle of the calculation is straightforward, a small computer is required to carry it out.

THEORY

In the LRP each absorbance was corrected by adding the estimated loss of absorbance attributable to prior destruction of deoxyribose in the hot acid extract. Program CORABS (Tables 1 and 2) obtains the corrected absorbance, A_o^*, using a

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similar technique. Correction is discontinuous, as in the LRP, until the time of maximum absorbance, t_m. Thereafter, correction is continuous by integration of a function which fits absorbance values in the range $t \geq t_m$, where t is the duration of extraction. Suppose

$$A(t) = K + A_u(t) \quad (1)$$

where $A(t)$ is the absorbance observed after t min of extraction, $A_u(t)$ is the portion of observed absorbance attributable to deoxyribose, and K is the absorbance attributable to acid-resistant interfering substances. Assume that, for $t > t_o$, extraction and destruction kinetics are exponential. That is, if

$$t' = t - t_o \quad (2)$$

$$dA_u(t')/dt' = k_u D e^{-k_u t'} - k_e A_u(t') \quad (3)$$

where k_u and k_a are the apparent time constants for extraction and destruction of deoxyribose, respectively, and D is the tissue deoxyribose pool at $t'=0$. Such kinetic behavior implies

$$A(t') = K + S_1 e^{-k_e t'} - S_2 e^{-k_u t'} \quad (4)$$

where S_1 and S_2 are constants of integration.

Actually, absorbance values are found to fit similar equations of the form

$$A(t-t_o) = V + S e^{-k_e(t-t_o)} - S e^{-k_u(t-t_o)} \quad (5)$$

where $t \geq t_m$ and V, S, k_u, k_e and t_o are empirical constants. Thus, if A_m^* is the absorbance at $t=t_m$ corrected by the LRP for deoxyribose loss prior to t_m,

$$A_o^* = A_m^* + \int_{t'_m}^{\infty} (A(t') - V) \cdot k_e \cdot dt' \quad (6)$$

where

$$t'_m = t_m - t_o \quad (7)$$

These equations yield A_o^*, the absorbance corrected for loss of deoxyribose and extrapolated to infinite duration of extraction:

$$A_o^* = A_m^* + k_e \cdot S \cdot (e^{-k_e t'_m}/k_e - e^{-k_u t'_m}/k_u) \quad (8)$$

Eqn. (5) is similar to the following equation of the LRP.

$$A(t) = A_\infty + (A_o - A_\infty) \times 10^{-kt} \quad (9)$$

V is the analogue of A_∞ and S is the analogue of $(A_o - A_\infty)$. The analogue of k is k_e where

$$k_e = k(\ln 10) \quad (10)$$

Since eqn. (9) has no parameter analogous to k_u, it cannot fit absorbances at or near the maximum absorbance.

COMPUTER PROGRAM

Program CORABS determines empirical values of t_o, V, S, k_e and k_u which minimize the deviation of observed absorbance values from isochronous absorbance values on the fitted curve (eqn. (5)). It is postulated that the probability of relating absorbance values with deoxyribose concentrations decreases with time exponentially, with the same time constant as that of deoxyribose destruction. Thus, in line 150 of the curve-fitting algorithm of CORABS, each absorbance point, $A(t)$, is assigned the statistical weight $e^{-k_e t} \cdot |A(t) - K|$.

The calculation of A_m^* in eqn. (8) is based on the LRP. In the LRP, the average absorbance between the start of extraction and t_1, the time of the first observed absorbance, is estimated as 0.75 $A(t_1)$ if $t_1 = t_m$ and as 0.50 $A(t_1)$ if $t_1 \neq t_m$. A quantitative basis for such approximations was developed by observing that the initial rapid increase in absorbance is roughly exponential. Then, if

$$x = (A(t_1) - K)/(A(t_m) - K) \quad (11)$$

it can be shown that the ratio to $A(t_1) - K$ of the average net absorbance prior to t_1 is

$$y \simeq x^{-1} + (\ln(1-x))^{-1} \quad (12)$$

In the range $0.90 < x < 0.99$, y varies from 0.68 to 0.79, and averages 0.72, the factor used in line 220 of CORABS for $t_1 = t_m$. This is close to the factor 0.75 used in the LRP. If $t_1 \neq t_m$, eqns. (11) and (12) are used to estimate the average absorbance in the range $0 < t < t_1$. In CORABS,

absorbances at or below $A(t_m)/5$ are automatically neglected in the calculation of A_o^*. This minimizes errors in A_o^* which otherwise could be attributed to acid-resistant interfering substances extracted slowly from tissue powder.

The curve-fitting algorithm of CORABS makes use of the following relationships among k_u, k_e, t_m, and t_o. If

$$f = (t_m - t_o)/w \tag{13}$$

where w min is the time interval between aliquot samples of the extract, and if

$$g = k_u/k_e \tag{14}$$

it may be shown from the first derivative of eqn. (5) that

$$\ln g = wfk_e(g-1) \tag{15}$$

The solution of eqn. (15) is

$$g = \lim\,[1+\ln(1+\ln(1+\ln\ldots (1+\ln(1+\ln g_o/(wfk_e)))\ldots))] \tag{16}$$

where g_o is any real number greater than one. Eqn. (16) is used in line 120 of CORABS. Eqns. (13) through (16) are used in CORABS to calculate provisional values of k_u from tentative values of t_o and k_e, as indicated by the first command of line 130 in CORABS. Provisional values of k_e are calculated from absorbance values at $t \gg t_m$ using Guggenheim's algorithm,[13] as in the LRP.

The particular version of program CORABS displayed in Table 1 is written in a BASIC computer language specified for the Casio FX-702P pocket computer.[14] In addition to standard BASIC functions, the following special statistical functions of this portable (186 g) minicomputer are used in CORABS.

STAT x;y assigns the statistical weight y to the number x in a series of numbers during calculation of the standard deviation.

Table 1. List of program CORABS.

```
 1Ø X=Ø:Z=9E99:INP"W",W,"P",P:FOR I=1 TO P:PRT" A";W*I;
 2Ø INP A(I):PRT"A";W*I;"=";A(I);:IF A(I)>X;X=A(I):M=I
 3Ø NEXT I:L=P:FOR I=M+1 TO P:IF A(I)≥.7*A(M);Q=I:PRT"!";
 4Ø IF A(I)≤A(M)/5;L=I-1:I=P
 5Ø NEXT I:FOR N=M TO Q:PRT"WAIT FOR A=AØ*";Q-N+1;"/";Q-M+1;
 6Ø J=INT((L-N)/2):G=3Ø:H=9E99:F=Ø:Y=Ø
 7Ø SAC:FOR I=Ø TO L-N-J:X=A(N+I)-A(N+I+J):IF X≤Ø THEN 9Ø
 8Ø STAT W*(N+I),LN X
 9Ø NEXT I:B=LRB:X=EXP(B*W):C=EXP LRA/(1-X↑J):SAC
1ØØ FOR I=Ø TO L-N:STAT A(N+I)-C*X↑(N+I):NEXT I:K=MX:J=.4
11Ø F=F+J:IF F≤Ø THEN 11Ø
12Ø X=G:G=1-LN X/(F*W*B):IF ABS(X-G)>.ØØ5 THEN 12Ø
13Ø O=G*B:D=C*EXP(B*W*(M-F)):SAC:FOR I=M TO L
14Ø X=W*(I-M+F):X=EXP(B*X)-EXP(O*X)
15Ø STAT((K+D*X)/A(I)-1)↑2;ABS(A(I)-K)*EXP(B*W*I)
16Ø NEXT I:IF F=Y THEN 21Ø
17Ø IF MX≤H;H=MX:G=3Ø:GOTO 11Ø
18Ø IF ABS(1-MX/H)≤1E-Ø4 THEN 2ØØ
19Ø F=F-5*J/2:J=J/2:G=3Ø:H=9E99:GOTO 11Ø
2ØØ F=F-3*J/2:Y=F+J:G=3Ø:GOTO 11Ø
21Ø A(Ø)=Ø:A(L+1)=Ø:FOR I=1 TO M:IF M*I>1 THEN 23Ø
22Ø A(L+2)=A(1)-B*W*.72*ABS(A(1)-K):GOTO 27Ø
23Ø IF I≠1 THEN 26Ø
24Ø Y=ABS((A(1)-K)/(A(M)-K)):Y=1/Y+(LN(1-Y))↑-1
25Ø A(L+2)=A(1)-B*W*Y*ABS(A(1)-K):GOTO 27Ø
26Ø A(L+I+1)=A(L+I)+A(I)-A(I-1)-B*W*(A(I)+A(I-1))/2
27Ø NEXT I:T=A(L+M+1)-B*D*(EXP(O*W*F)/O-EXP(B*W*F)/B)
28Ø IF MX≤Z;Z=MX:E=B:V=K:U=O:R=W*(M-F):S=D:P=A(L+M+1):A=T
29Ø NEXT N:PRT" AØ*";A:END
```

MX calculates the weighted arithmetic mean of data entered during standard deviation analysis.

STAT x,y;z assigns the statistical weight z to the point (x,y) in a series of points during calculation of the regression line through those points.

LRA and *LRB* calculate the y-intercept and the slope of the regression line, respectively.

SAC clears the statistical processing registers.

The commands, STAT, MX, LRA, LRB, and SAC can be replaced by subroutines if CORABS is modified for a computer which does not happen to use such statistical functions directly. When running program CORABS, the time interval in minutes between removal of aliquots of the extract and the total number of such aliquots are entered and stored in registers W and P, respectively. The 600 nm absorbances determined from these aliquots are entered sequentially and stored in the array A(1), A(2),...,A(P). These absorbance values correspond to W, 2W,..., and PW minutes of extraction, respectively. A_o^* is then computed, stored in register A, and displayed automatically. Registers B through Z are used for other parameters of CORABS, as shown in Table 2.

Table 2. Parameters of program CORABS.

A	Computed value of A_o^*, eqn. (8)
B	Provisional values of $-k_e$
C	Intermediate parameter, analogous to A_o-A_∞ of the original Løvtrup-Roos procedure
D	Provisional values of S
E	Computed value of $-k_e$
F	Intermediate parameter f, eqn. (13)
G	Intermediate parameter g, eqns. (14)–(16)
H	Intermediate variable
I	Integral variable
J	Intermediate parameter; W*J corresponds to τ of Guggenheim's algorithm, Ref. 13
K	Provisional values of V
L	Array integer for the last observed absorbance greater than $A(t_m)/5$
M	Array integer for the maximum observed absorbance, $A(t_m)$
N	Integral variable
O	Provisional values of $-k_u$
P	Number of observed absorbance points; finally, computed value of A_m^*
Q	Array integer for the last observed absorbance greater than or equal to $0.7A(t_m)$
R	Computed value of t_o, eqn. (5)
S	Computed value of S, eqn. (5)
T	Provisional values of A_o^*
U	Computed value of $-k_u$
V	Computed value of V, eqn. (5)
W	Constant time interval between aliquots of acid extract, minutes
X	Intermediate variable
Y	Intermediate variable
Z	Weighted mean square fractional deviation of absorbance points from the fitted curve, eqn. (5)

The following separate three-command program can be used to generate points on the fitted curve, eqn. (5).

```
1 INP "T", T:PRT V+S*(EXP(E*(T-R))-
EXP(U*(T-R)));:GOTO 1
```

Examples of fitted curves together with the absorbance points from which they are derived are shown in Fig. 1, where absorbance data are from case No. 1.

EXPERIMENTAL

Lateral lobes of four normal human cerebella were removed *post-mortem*, then frozen at −70 °C pending DNA analysis. Information concerning the autopsies is shown in Table 5. Each cerebellum was normal, and the decedents showed no evidence of primary neurological disease. Cadavers were refrigerated prior to the autopsy.

Each cerebellar specimen was divided into white matter and cortical portions by macroscopic dissection, and the portions were lyophilized separately. Weighed portions of these tissue powders and known quantities of calf thymus DNA were treated concurrently with 5 % perchloric acid at 90 °C, as described previously.[2-4] Aliquots (1 ml) of the extracts were removed every 5 min. The aliquots were treated with diphenylamine, and the resulting 600 nm absorbance readings were tabulated for entry into the array A(1), A(2),..., A(P). The results of processing these data with program CORABS are shown in Tables 3 and 4.

DISCUSSION

Only a few investigators [15,16] have reported using the Løvtrup-Roos correction procedure, presumably because the original procedure is difficult. Since the computation of A_o^* is now

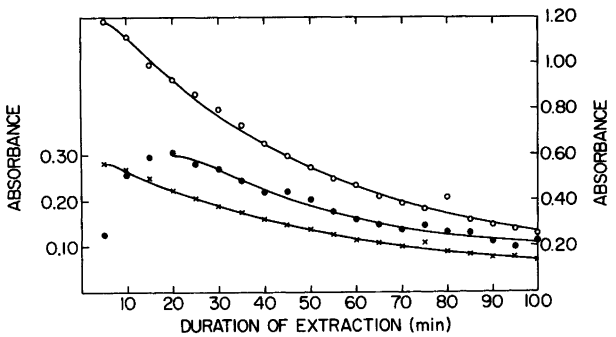

Fig. 1. Absorbance data and fitted curves for case No. 1. The upper curve (open circles, cerebellar cortex) corresponds to the ordinate on the right (scale: zero to 1.20). The middle (closed circles, cerebellar white matter) and lower (small x's, DNA control) curves correspond to the ordinate on the left (scale: zero to 0.30).

automated, the Løvtrup-Roos correction should be more easily applied.

The computer program presented here (Tables 1 and 2) is based on the original Løvtrup-Roos procedure (LRP). It uses an algorithm which fits an empirical kinetic equation to absorbance values beginning at the time of extraction which yields maximum absorbance. In the LRP, A_o^* is obtained by extrapolation from absorbances observed during the first few hours of extraction. The program presented here uses a formula to calculate A_o^* by extrapolation to infinite duration of extraction with continuous compensation for destruction of deoxyribose.

Tissues analyzed were *post-mortem* specimens of human cerebellar cortex and cerebellar white matter. It can be calculated from the results of analyses listed in Tables 3 and 4 that average concentrations of DNA in cerebellar cortex and white matter are 0.72±0.02 μg per mg dry tissue and 0.05±0.01 μg DNA per mg dry tissue, respectively. It was also found that the average concentration of DNA in the macroscopically dissectable human cerebellar leptomeninges and blood vessels is less than 0.20 μg per mg dry weight. Comparison of Tables 4 and 5 shows no correlation between age, time interval between death and autopsy, and cerebellar DNA concen-

Table 3. Results obtained using the program CORABS.

Case No.	Specimen	A_m^*	S	V	t_o	k_u	$k_e \times 10^2$
1	Cerebellar cortex	1.260	1.285	0.108	−1.72	0.4921	2.0736
1	Cerebellar white matter	0.419	0.354	0.081	8.61	0.1990	2.8571
1	Standard DNA	0.300	0.287	0.032	−0.28	0.6879	2.0231
2	Cerebellar cortex	1.237	1.045	0.098	6.70	1.2884	1.9652
2	Cerebellar white matter	0.510	0.625	0.055	−2.84	0.1786	2.4805
2	Standard DNA	0.301	0.283	0.032	0.38	0.8203	2.0418
3	Cerebellar cortex	1.335	0.751	0.149	20.00	4137.7	2.2501
3	Cerebellar white matter	0.377	0.292	0.059	9.98	0.7135	2.2269
3	Standard DNA	0.312	0.365	0.044	−7.32	0.2015	2.2103
4	Cerebellar cortex	1.109	1.649	−0.390	−0.59	0.3318	1.1099
4	Cerebellar white matter	0.419	0.366	0.005	14.61	0.6162	2.5520
4	Standard DNA	0.320	0.263	0.032	5.00	8808.5	2.1936

Table 4. Results obtained using the program CORABS.

Case No.	Specimen	Dry weight	Corrected absorbance A_o^*	DNA concentration μg/mg dry tissue
1	Cerebeller cortex	209.8 mg	2.376	0.702
1	Cerebellar white matter	895.2 mg	0.669	0.046
1	Standard DNA	34.6 μg	0.558	–
2	Cerebellar cortex	185.0 mg	2.216	0.741
2	Cerebellar white matter	904.8 mg	0.955	0.065
2	Standard DNA	34.6 μg	0.559	–
3	Cerebellar cortex	170.9 mg	2.086	0.719
3	Cerebellar white matter	929.5 mg	0.638	0.040
3	Standard DNA	34.6 μg	0.587	–
4	Cerebellar cortex	210.7 mg	2.574	0.725
4	Cerebellar white matter	914.7 mg	0.737	0.048
4	Standard DNA	34.6 μg	0.583	–

Table 5. Post-mortem diagnoses.

Case No.	Age, sex	*Post-mortem* interval to autopsy	*Post-mortem* diagnoses [a]
1	64 yr, male	<10 hr	Anaplastic carcinoma of the urinary bladder with local and systemic metastases. Microscopic metastases in the cerebral dura and the superior sagittal sinus; brain otherwise normal. Left hydronephrosis. Recent peptic ulcer of the duodenum.
2	60 yr, male	17 hr	Severe panacinar emphysema. Severe right ventricular hypertrophy. Ascites, peripheral edema, bilateral hydrothorax and pericardial effusion. Fibrinous pericarditis. Chronic passive congestion of the liver. Multiple organizing pulmonary arterial thrombi, probably embolic. Tracheostomy. Large sacral decubitis ulcer. Small left parietal meningioma and two small, organized cerebral infarcts, both asymptomatic; brain otherwise normal.
3	73 yr, male	8 hr	Squamous cell carcinoma of the lung with local and systemic metastases. Severe coronary artery atherosclerosis with left ventricular myocardial scarring and hypertrophy. Acute purulent bronchopneumonia. Brain normal.
4	62 yr, male	27 hr	Squamous cell carcinoma of the esophagus with local and systemic metastases. Esophageal perforation with focal subacute mediastinitis. Two small carcinoid tumors of the ileum, asymptomatic. Acute bronchopneumonia and pulmonary edema. Brain normal.

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trations.

It has been estimated that the convoluted cerebellar cortex occupies 90 % of the volume of the cerebellum.[17] Assuming conservatively that not more than 5 % of the cerebellum is occupied by leptomeninges and blood vessels, it is inferred that the non-vascular moiety of the human cerebeller cortex constitutes about 85 % of the cerebellum as a whole. Since 95 % of cerebellar cortical nuclei are in the granular layer of the cortex [18] and since, in the mammal, over 99 % of granular layer nuclei are neuronal,[19] it follows that approximately 94 % of all cerebellar cortical nuclei are neuronal. Using the values of DNA concentrations presented here, it is calculated that 92 % of whole cerebellar DNA is situated in neurons of the cerebellar cortex. These calculations are in accord with observations on nuclear fractions of whole cerebellar homogenates from dogs and rats, which show that about 90 % of cerebellar nuclei are neuronal.[20,21]

These measurements of cerebellar DNA lend support to the postulate that there is significant turnover of cerebellar neuronal DNA during the human lifetime.[22] That postulate is based on stable carbon isotope ratio measurements on the carbon moiety of DNA isolated from unfractionated homogenates of human cerebellar tissue from which only minor portions of the cerebellar white matter and leptomeninges had been removed prior to homogenization for DNA extraction. The foregoing calculations indicate that 90–92 % of such carbon is derived from cerebellar neuronal DNA. Human tissue $^{13}C/^{12}C$ ratios are significantly lower in Europeans than in Americans.[23] Thus, the finding that $^{13}C/^{12}C$ ratios in whole cerebellar DNA from European-born Americans tend to approach ratios which are typical of American tissues is interpreted as evidence that a substantial proportion of neuronal DNA in the cerebellum may undergo slow turnover during adult life.

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Conformational Analysis. XXIII. ^{13}C and ^{1}H NMR Conformational Study of 2-Oxo-1,3-dioxane and Its Methyl Derivatives

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The ^{13}C NMR chemical shifts for 2-oxo-1,3-dioxane and its methyl derivatives are reported. Substituent effects on them are derived and their type and magnitude shown to be closely related to the exact ring geometry and other conformational aspects. Together with some ^{1}H NMR data the results point out the usefulness of the ^{13}C NMR chemical shifts in conformational and configurational analysis.

Relatively little attention has been previously paid to the conformational analysis of 2-oxo-1,3-dioxane (*1*) and its methyl derivatives (*2–24*). Since the early work of Arbuzov *et al.*[1,2] Katzhendler *et al.*[3] have studied the ^{1}H NMR spectra of a few 2-oxo-1,3-dioxanes and explained them in favour of a predominant chair form. Pihlaja and coworkers [4] concluded from the ^{1}H NMR spectra of *1*, *5–7*, and *10* (Table 1) that they exist in a chair conformation where the $C_{4\text{-}5\text{-}6}$ region of the ring is flattened when compared with cyclohexanes but more puckered than that of the 1,3-dioxane ring. Using the values of the vicinal coupling constants they estimated [4] that *r*-4,*cis*-5,*trans*-6-trimethyl-2-oxo-1,3-dioxane (*14*) contains about 58 % of the 5-equatorial (eea) conformer. Also Hellier and Webb [5] note that substituted derivatives of *1* prefer the chair form and Samitov and Aminov [6] state that *2* is predominantly in the equatorial chair form.

In the present work we report the ^{13}C NMR chemical shifts for 2-oxo-1,3-dioxane (*1*) and all of its methyl derivatives (Table 2). From them the substituent effects on the ring carbon atoms are estimated to clarify the ring conformation and possible conformational equilibria in detail.[7] To get further insight into compounds with possible conformational equilibria the ^{1}H NMR spectra of *2*, *3* and *14* were also recorded and analyzed.

EXPERIMENTAL

The 2-oxo-1,3-dioxanes were prepared by the method described before.[4] Their boiling or melting points and refractive indices are presented in Table 1. The ^{1}H NMR spectra of *2* and *3* were recorded on a Jeol FX-200 and that of *14* on a Bruker 360 MHz NMR spectrometer using $CDCl_3$ as solvent (Table 6). The spectrum of *2* was analyzed using a LAME program whereas the spectra of *3* and *14* were practically first order at 200 and 360 MHz, respectively.

RESULTS

The ^{13}C chemical shift data for compounds *1–24* are collected in Table 2 along with the corresponding assignments. The chemical shift parameters (Table 3–5) were derived by a multiple linear regression analysis of the shift data using eqn. (1),[7–9]

$$\delta C(x) = \delta_p C(x) + \Sigma SE(x) \qquad (1)$$

where $\delta C(x)$ is the chemical shift of the xth carbon in any given derivative, $\delta_p C(x)$ that of the

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Table 1. 2-Oxo-1,3-dioxanes prepared in this study.

	2-Oxo-1,3-dioxane	B.p./K kPa^{-1} or M.p./K	n_d^{293}
1	Parent	314	
2	4-Me	436/2.3	1.4453
3	5-Me (24 % a+76 % e)	363–364/0.1	1.4492
4	4,4-Me_2	348	
5	5,5-Me_2	370	
6	*cis*-4,6-Me_2	435/2.8	1.4413
7	*trans*-4,6-Me_2	432–433/2.8	1.4459
8	*trans*-4,5-Me_2	422–428/1.5 [a]	1.4417 [a]
9	*cis*-4,5-Me_2 (5 % ae+95 % ea)		
10	4,4,6-Me_3	369	
11	*r*-4,*cis*-5,*trans*-6-Me_3 (33 % aae+67 % eea)	429/1.7	1.4451
12	*r*-4,*trans*-5,*cis*-6-Me_3	431/1.9	1.4476 [b]
13	*r*-4,*cis*-5,*cis*-6-Me_3		
14	4,4,5-Me_3 (12 % 5a+88 % 5e)	327	
15	4,5,5-Me_3	364	
16	4,4,5,5-Me_4	458	
17	4,4,6,6-Me_4	351	
18	*cis*-4,5,5,6-Me_4	331 [c]	
19	*trans*-4,5,5,6-Me_4		
20	*trans*-4,4,5,6-Me_4	376 [d]	
21	*cis*-4,4,5,6-Me_4		
22	4,4,5,6,6-Me_5	341	
23	4,4,5,5,6-Me_5	376	
24	4,4,5,5,6,6-Me_6	441	

[a] For a mixture of *8* and *9*. [b] For a mixture of *12* and *13*. [c] For a mixture of *18* and *19*. [d] For a mixture of *20* and *21*.

same carbon atom in the parent compound (*1*) and $\Sigma SE(x)$ the sum of the different substituent effects influencing it.[7–9] The chemical shift parameters were first calculated using the shift data for compounds *1, 2, 4–8, 10, 12, 13, 15, 16, 18–21,* and *23* with anancomeric or two equivalent chair conformations. The values of the shift parameters so obtained were then used to estimate the mol fractions of the two unequal chair conformations in the case of compounds *3, 9, 11,* and *14* (Scheme 1). We also came up with numerically the same parameters before and after including the shift data for compounds *3, 9, 11* and *14* weighed by the best fit values of the mol fractions (Scheme 1).

Compound *2*, however, seems to be predominantly in the equatorial chair form as can be concluded also from its ^{1}H NMR spectrum (Table 6). The data for compounds *17, 22* and *24* were not used for deriving the chemical shift parameters (Tables 3–5) since they are the only compounds with independent 4a6a, 4a5e6a, and 4a5a6a substitution patterns, respectively.

PARAMETRIZATION

In addition to the primary (α, β, γ, δ), geminal (G_i), and vicinal (ee, ea, ae; Tables 3–5) effects we have introduced some long range and polysubstitution ($n \geqq 3$) parameters to explain further perturbation of the basic ring geometry for reasons stated before.[7,8]

The long range and polysubstitution parameters are considered significant if they are larger than 0.2 ppm and/or at least twice their standard deviations. All substituent effects which have been involved in the multiple linear regression analysis are present at least three times (Tables 3–5). Of course, it can be argued that given enough parameters a given set of data can be made to fit a pattern but we feel, however, that this is not possible without forgetting the chemistry behind our experiments. Our conclusion is drawn from the observation that independent of the initial parametrization we always ended up with the same set of best parameters. Furth-

Table 2. ^{13}C chemical shifts of 2-oxo-1,3-dioxanes.

	2-Oxo-1,3-dioxane	C-2	C-4	C-5	C-6	Methyl carbons
1	Parent	148.66	68.09	21.70	68.09	
2	4-Me	149.18	75.95	28.52	67.18	21.05(4e)
3	5-Me (24 % a+76 % e)	148.72	73.29	26.18	73.29	11.83(5e) [a]
4	4,4-Me_2	149.18	81.35	32.68	64.84	27.74(av.)
5	5,5-Me_2	148.20	77.45	28.33	77.45	20.92(av.)
6	*cis*-4,6-Me_2	149.44	75.37	36.38	75.37	21.12(4/6e)
6	*trans*-4,6-Me_2	149.57	72.70	33.79	72.70	20.79(4/6av.)
8	*trans*-4,5-Me_2	148.92	81.27	32.83	72.26	19.23(4e), 11.95(5e)
9	*cis*-4,5-Me_2 (5 % ae+95 % ea)	149.16	78.09	29.57	72.61	16.99(4e), 9.89(5a) [a]
10	4,4,6-Me_3	149.50	80.89	40.48	72.32	29.89(4e), 26.51(4a), 21.12(6e)
11	*r*-4,*cis*-5,*trans*-6-Me_3 (33 % aae+67 % eea)	149.22	76.37	35.72	78.03	19.86(4e), 11.94(5a), 15.90(6a) [a]
12	*r*-4,*trans*-5,*cis*-6-Me_3	149.24	80.24	39.78	80.24	19.18(4/6e), 12.33(5e)
13	*r*-4,*cis*-5,*cis*-6-Me_3	149.24	79.01	34.24	79.01	19.88(4/6e), 3.78(5a)
14	4,4,5-Me_3 (12 % 5a+88 % 5e)	149.18	84.53	35.28	70.11	27.61(4e), 21.31(4a), 11.31(5e) [a]
15	4,5,5-Me_3	148.79	82.91	31.25	77.77	15.27(4e), 21.18(5e), 16.83(5a)
16	4,4,5,5-Me_4	148.92	86.61	33.72	75.69	23.78(4av.), 20.66(5av.)
17	4,4,6,6-Me_4	150.48	80.57	44.77	80.57	29.69(4/6av.)
18	*cis*-4,5,5,6-Me_4	148.98	83.37	34.44	83.37	14.77(4/6e), 20.89(5e), 11.06(5a)
19	*trans*-4,5,5,6-Me_4	148.92	80.96	33.72	80.96	15.79(4/6av.), 20.34(5av.)
20	*trans*-4,4,5,6-Me_4	149.18	84.14	42.56	77.18	27.74(4e), 21.37(4a), 11.95(5e), 19.62(6e)
21	*cis*-4,4,5,6-Me_4	149.44	83.69	38.07	74.59	28.39(4e), 26.57(4a), 7.15(5a), 18.06(6e)
22	4,4,5,6,6-Me_5	149.96	84.34	45.94	84.34	30.41(4/6e), 22.55(4/6a), 11.89(5e)
23	4,4,5,5,6-Me_5	149.11	86.74	36.58	79.01	24.04(4e), 23.39(4a), 20.40(5e), 14.68(5a), 16.27(6e)
24	4,4,5,5,6,6-Me_6	150.02	87.19	40.41	87.19	26.77(4/6av.), 21.18(5av.)

[a] The more favoured orientation shown in parentheses.

ermore, the possible interdependence of the different substituent effects was carefully checked to minimize their total number.

DISCUSSION

Shift Parameters

α-effects. In the case of C(4/6) both the axial and equatorial α-effects are larger than those of 1,3-dioxanes (5.37 *vs.* 0.95 and 7.83 *vs.* 5.76, respectively). This is indicative for the different chair conformations since the carbonate moiety is planar in 2-oxo-1,3-dioxanes which has both through-frame and through-space influence on their geometry and hence on the magnitude of the shift effects in them.

At C(5) the axial and equatorial α-effects are very close to each other both in 1,3-dioxanes and in their 2-oxo derivatives ($\alpha_e-\alpha_a$=0.26 and –0.13, respectively) although they are about 1 ppm more deshielding in the latter ring system. It is also very interesting to note that the geminal α-effect (G_α) at C(4/6) of (*1*) is zero whereas that of 1,3-dioxanes is –2.60 ppm. This is strong evidence for the importance of the presence or lack of interacting synaxial hydrogen atoms. At C(5) G_α equals –2.35 ppm in *1* and –2.57 ppm in 1,3-dioxanes.

β-effects. $\beta_e-\beta_a$ at C(4/6) is almost the same for *1* and for 1,3-dioxanes [8a] (Table 3) although in the former ring system both β-effects are *ca.* 1 ppm less deshielding. Even in 1,3-oxathianes [11] and 1,3-dithianes [12] $\beta_e-\beta_a$ at C(4/6) has a value which is very close to those in the above compounds.

At C(5) (Table 4) $\beta_e-\beta_a$ is only 1.4 ppm for *1* whereas in the other compounds mentioned

Table 3. Parameters for shifts at C(4/6).

The source of the substituent effects	Parameters/ppm [a]		No. of occurrences
4e	α_e	7.83±0.07	23
4a	α_a	5.37±0.08	12
5e	β_e	5.41±0.09	21
5a	β_a	4.01±0.12	19
6e	γ_e	-0.91±0.08	23
6a	γ_a	-2.29±0.11	12
4e5a	$\alpha_e\beta_a$	-1.89±0.12	12
5a6e	$\beta_a\gamma_e$	1.59±0.11	12
5e6e	$\beta_e\gamma_e$	-0.46±0.11	12
4e6e	$\alpha_e\gamma_e$	0.39±0.09	12
4e6a	$\alpha_e\gamma_a$	-0.79±0.12	8
4e4a5e	$\alpha_e\alpha_a\beta_e$	-1.90±0.11	4
4e5a6a	$\alpha_e\beta_a\gamma_a$	-0.80±0.13	4
4e5e5a	$\alpha_e\beta_e\beta_a$	-0.63±0.10	6
4a5a6e	$\alpha_a\beta_a\gamma_e$	-0.72±0.12	6
4a5e6e	$\alpha_a\beta_e\gamma_e$	0.22±0.12	4
4a5e5a	$\alpha_a\beta_e\beta_a$	0.34±0.14	3
5e6e6a	$\beta_e\gamma_e\gamma_a$	0.21±0.11	4

[a] rms 0.110 ppm, av.diff. ±0.06 ppm, range 21.90 ppm.

Table 4. Parameters for shifts at C(5).

The source of the substituent effects	Parameters/ppm [a]		No. of occurrences
4e	β_e	6.75±0.06	26
4a	β_a	5.39±0.11	11
5e	α_e	4.39±0.08	13
5a	α_a	4.52±0.20	12
4,4	G_β	-1.21±0.12	7
5,5	G_α	-2.32±0.23	6
4e5a	$\alpha_a\beta_e$	-3.32±0.14	14
4a5e	$\alpha_e\beta_a$	-2.19±0.18	7
4e6e	$\beta_e^4\beta_e^6$	1.16±0.13	8
4e5e6e	$\alpha_e\beta_e^4\beta_e^6$	-0.93±0.12	4
4a5e5a	$\alpha_e\alpha_a\beta_a$	-0.33±0.14	4
4e4a5e	$\alpha_e\beta_e\beta_a$	0.76±0.19	4
4e5e5a	$\alpha_e\alpha_a\beta_e$	-0.46±0.15	7
4e5a6a	$\alpha_a\beta_e^4\beta_e^6$	-0.33±0.12	4

[a] rms 0.097 ppm, av.diff. ±0.04 ppm, range 13.60 ppm.

above it varies from 3.0 to 3.5 ppm. This resembles the situation with the α_e–α_a difference discussed above and shows again that the spatial and electronic environment at C(4/6) of *1* differs clearly from that of, *e.g.*, 1,3-dioxane because of the influence of the 2-oxo group. Also the geminal β-effects of *1* and 1,3-dioxane are somewhat different (Tables 3 and 4).[8a]

γ-effects. The effect of the carbonate moiety is clearly seen also in the γ-effects. At C(2) both γ_e and γ_a are small and deshielding whereas in 1,3-dioxanes they are both deshielding and γ_a has

Table 5. Parameters for shifts at C(2).

The source of the substituent effects	Parameters/ppm [a]		No. of occurrences
4e	γ_e	0.46±0.05	26
4a	γ_a	0.36±0.08	11
5a	δ_a	0.20±0.16	12
4,4	G_γ	–0.21±0.07	7
5,5	G_δ	–0.62±0.17	6
4e6e	$\gamma_e^4\gamma_e^6$	–0.17±0.08	8
4e5e	$\gamma_e\delta_e$	–0.11±0.04	14
4e5a	$\gamma_e\delta_a$	–0.17±0.10	14
4e5e5a	$\gamma_e\delta_e\delta_a$	0.33±0.11	7
4a5e6e	$\gamma_a\gamma_e\delta_e$	–0.16±0.07	4

[a] rms 0.077 ppm, av.diff. ±0.05 ppm, range 1.37 ppm.

got a normal value of –7.1 ppm (Table 5). At C(4/6) γ_e is not much different from that in 1,3-dioxanes whereas the γ_a-effect at C(4/6) in *1* is much less shielding than in 1,3-dioxanes (–2.34 *vs.* –4.72 ppm). This is mainly due to the lack of a synaxial hydrogen atom (γ-H) at C(2) and to a lesser extent to the planarity of the carbonate grouping[13] as shown by the ratio of the effects in *1* and 1,3-dioxane (~0.5).

δ-effects. These effects are, in general, small. From the two possible δ-effects in *1* only δ_a at C(2) has a small nonzero value (Table 5).

Vicinal and related polysubstitution effects. In our recent report on 1,3-dioxanes [7] we pointed out that in addition to vicinal effects $\alpha_e\beta_e$,$\alpha_e\beta_a$, $\alpha_a\beta_e$, $\beta_e\gamma_a$, and $\beta_a\gamma_e$ [9] one should take into account their combined influences since, for instance, the real effect of $\alpha_e\beta_a\gamma_e$ substitution can differ significantly from the values of $\alpha_e\beta_a+\beta_a\gamma_e$. This is also the case in 2-oxo-1,3-dioxanes which again lends support to the postulation that vicinal polysubstitution ($n\geq3$) has a further perturbation effect on the basic ring geometry. Comparison of the different $\alpha\beta$ shifts at C(4/6) of 2-oxo-1,3-dioxanes with the corresponding shifts in 1,3-dioxanes[8a] (Tables 3 and 4) shows clearly the influence of the carbonaate moiety. Especially different are $\alpha_a\beta_e$ effects at C(4/6) and $\alpha_e\beta_a$ effects at C(5). As to the polysubstitution effects both their type and magnitude are different in the carbonates and 1,3-dioxanes themselves (Tables 3–5).[7,8a] The fundamental difference in the α_{ae} effects between 2-oxo-1,3-dioxanes and 1,3-dioxanes reflects again the difference in geometries and hence the difference in steric interactions.

Buttressing and syn-*axial effects.* There are three such influence at C(4/6) namely $\alpha_e\gamma_e$, $\alpha_e\gamma_a$, and $\alpha_a\gamma_a$ which all have numerical values very close to those of 1,3-dioxanes. At C(5) the $\beta_e^4\beta_e^6$ effect, 1.16 ppm, is greatly enhanced in comparison with that of 1,3-dioxanes, 0.37 ppm (Table 4).[8a] A fundamental difference iis seen also in the δ-synaxial effects at C(5) since $\beta_a^4\beta_a^6$ is 0.05 ppm (Table 7) in 2-oxo-1,3-dioxanes but –1.02 ppm in 1,3-dioxanes. In general, the similarities and differences in the substituent effects of 2-oxo-1,3-dioxanes and 1,3-dioxanes reflect the magnitudes of their molecular interactions and the basic deviations in their geometries.

Chair–Chair Equilibria and ^{1}H NMR Spectra

4-Methyl-2-oxo-1,3-dioxane (*2*) attains exclusively the equatorial chair form the predominance of which can be concluded from the ^{1}H NMR parameters and is confirmed by the substituent effects on the ^{13}C chemical shifts. We can now use the values of the vicinal coupling constants (Table 6) to evaluate the position of the chair–chair equilibria in *3*, *11* and *14* in order to compare the results with those from the ^{13}C shift data below.

For 5-methyl-2-oxo-1,3-dioxane (*3*) we take the model values from the ^{1}H spectrum of *2*, namely J_{aa} 11.5 and J_{ee} 2.9 Hz. The latter value will be used for the three other compounds, too. The model values for the ea and ae couplings are more difficult to define and hence we do not use

Table 6. ^{1}H NMR parameters of 4-methyl- (*2*), 5-methyl- (*3*) and 4,4,5-trimethyl-2-oxo-1,3-dioxanes (*14*). Solvent $CDCl_3$, internal standard TMS.

Compound	Proton	δ/ppm	J/Hz	
2 [a]	5a	1.93	5a5e	−14.41; 6a6e −11.03
	5e	2.10	5a6a	11.54; 5e6a 3.56
	6a	4.39	5e6e	2.92; 5a6e 5.02
	6e	4.44	4a5e	3.38; 4a5a 10.27
	4a	4.63	4a,CH_3	6.35
	CH_3	1.44		
3 [a,b]	5a	2.38 [2.39] [c]	44	−10.7 [−10.7]; 5a, CH_3 6.8 [6.8]
	4/6a	4.11 [4.09]	45(t)	9.2 [9.2]
	4/6e	4.42 [4.40]	45(c)	4.5 [4.6]
	CH_3	1.05 [1.05]	'4e6e'	1.0 [1.0]; '4a6a' ~0.4
14 [d]	5a	2.19	6e6a	−11.0
	6a	4.11	5a6a	10.85
	6e	4.30	5a6e	5.0
	CH_3(5)	1.00	5a,CH_3	7.0
	CH_3(4a)	1.37		
	CH_3(4e)	1.47		

[a] Jeol FX-200. [b] About 73:27 mixture of the equatorial and axial chair forms (see the text). [c] The values in the brackets obtained on a Jeol PFT-100. [d] About 86:14 mixture of the 5-equatorial and 5-axial chair forms (see the text); Bruker WM 360.

them for our calculations. Consequently, we obtain eqn. (2),

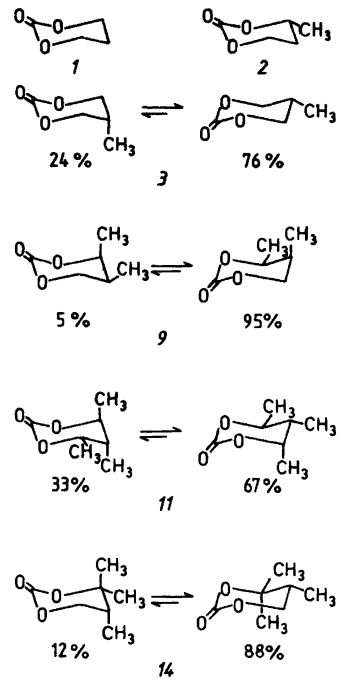
$$9.2 = X_{5e} \cdot 11.5 + (1-X_{5e})2.9 \quad (2)$$

from which $X_{5e}=0.73$. This is in excellent agreement with the result, 0.76, given by the ^{13}C chemical shift correlation taking into account that the error limits are about 0.03 in both calculations. If we assume that the actual value of $J_{4a6a}\sim 0$ as usual we obtain $J_{4e6e}\sim 1.4$ Hz a value which gives 'J_{4a6a}'$=0.27\times 1.4=0.4$ Hz and 'J_{4e6e}'$=0.73\times 1.4=1.0$ Hz in good agreement with the experimental finding (Table 6).

In the case of *r*-4,*cis*-5,*trans*-6-trimethyl derivative (*11*) the weighted average of the J_{aa} and J_{ee} type couplings is 7.8 Hz.[4] In our previous report [4] we did not have a good enough selection of model values. Hence we now reestimate the conformer ratio of *11* using $J_{aa}=10.3$ Hz and $J_{ee}=2.9$ Hz obtained from *2* as models [eqn. (3)].

$$7.8 = X_{aee} \cdot 10.3 + (1-X_{aee})2.9 \quad (3)$$

This leads to $X_{aee}=0.66$ which is not far from the earlier estimate [4] ($X_{aee}=0.58$) and is again



Scheme 1.

practically equal to the result (X_{aee}=0.67) from the ^{13}C chemical shift correlation (Scheme 1).

Finally we calculate the conformer population of *14* (Scheme 1) using J_{aa}=12.1 Hz which has been taken from 4,4,6-trimethyl-2-oxo-1,3-dioxane [4] and J_{ee}=2.9 Hz as above [eqn. (4)].

$$10.85 = X_{5e} \cdot 12.1 + (1 - X_{5e})2.9 \quad (4)$$

Thus the 4,4,5-trimethyl derivative is an 86:14 mixture of the 5e and 5a chair forms, a result which again fits together very well with that obtained from the ^{13}C NMR spectra (Scheme 1).

Chair–Chair Equilibria and the ^{13}C Chemical Shifts

To test the anancomerism of 4-methyl-2-oxo-1,3-dioxane (*2*) we calculated the values of the substituent effects on the ^{13}C chemical shifts using different axial–equatorial conformer ratios. However, we always ended up with the best fit when *2* was taken exclusively equatorial. Since $\Delta_e - \Delta_a$ is relatively small at all of the ring atoms (Tables 3–5) we can only conclude that *2* consists of at least 98 % of the equatorial chair form. The clear predominance of the equatorial conformer is supported also by the observation that the positions of the chair–chair equilibria of *3, 11* and *14* do not deviate significantly from those of the corresponding 1,3-dioxanes [7] (Table 6 and Scheme 1). The conformational energy of an axial 5-methyl group (2.9 kJmol^{-1}, Scheme 1) is somewhat less than that in 1,3-dioxanes and hence we can expect that the ratio of the 4e5a and 4a5e chair forms in *9* is also very close to that in 1,3-dioxanes (97:3).[7] Actually we can easily estimate that the proportion of the 4e5a form should be about 95 %. This estimate fits very well in the multiple linear correlation of the ^{13}C chemical shift data and leads, together with the other data, to the conclusion that the stereochemistry of the C_4–C_5–C_6 moiety of 2-oxo-1,3-dioxanes does not differ fundamentally from that of 1,3-dioxanes.

Finally we will discuss compounds *17, 22* and *24* which include 4a6a, 4a5e6a and 4a5a6a type increments, respectively. The values of these effects have been calculated manually and are shown in Table 7 together with the comparable data for 1,3-dioxanes. The values of the 4a6a effects are fairly close to those of 1,3-dioxanes at all ring atoms although the structural difference is somewhat reflected in the influence at C-5. Also the 4a5e6a effects are very small and almost equal in both sets of compounds whereas the 4a5a6a effects are clearly more deshielding in 1,3-dioxanes. In 2-oxo-1,3-dioxanes the values of the latter effects are quite small in agreement with the chair conformation. *cis*-2,4,4,5,6,6-Hexamethyl-1,3-dioxane has been stated to attain the 1,4-twist form [7] to some extent and this statement finds support both from the magnitude of the 4a5a6a effects and from thermochemical calculations.[8a]

CONCLUSION

A thorough correlation of the ^{13}C NMR chemical shift data with some complementary ^1H NMR results led to a consistent picture of the predominance of the chair conformation and the existence of some chair–chair equilibria in 2-oxo-1,3-dioxanes. Accordingly the present work strongly supports the view [7,8] that ^{13}C chemical shifts, measured at 298 K only, are very useful and sensitive detectors in configurational and conformational analysis. It is obvious that the

Table 7. The values of the substituent effects derived manually from compounds *17, 22* and *24* together with those of 1,3-dioxanes.[8a]

Substituent effect/ppm	Ring carbon atom [a] C-2	C-4/6	C-5
4a6a	+0.77(+0.96)	+2.88(+3.44)	+0.05(−1.02)
4a5e6a	+0.02(−0.21 [b])	+0.29(+0.79 [b])	+0.57(+0.30 [b])
4a5a6a	+0.16(+0.69 [c])	+0.95(+3.18 [c])	+1.15(+3.82 [c])

[a] The values for 1,3-dioxanes in parentheses. [b] Estimated from *trans*-2,4,4,5,6,6-hexamethyl-1,3-dioxane.[7,8a] [c] Estimated from *cis*-2,4,4,5,6,6-hexamethyl-1,3-dioxane.[8a]

more data becomes available the better we can benefit from the application of ^{13}C chemical shifts to solving ring conformations and other structural problems.

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Synthesis and Photocyclization of [2.0.2.0]-, [2.2.2.0]- and [2.2.2.2]Cyclophanediene

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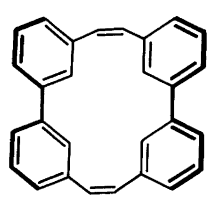
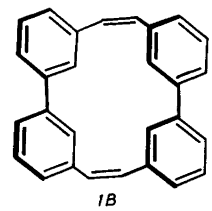
Three cyclophanes, [2.0.2.0]-, [2.2.2.0]- and [2.2.2.2]cyclophanediene, *1*, *5* and *6*, respectively, have been prepared by double Wittig reactions. On irradiation under oxidative conditions, cyclophanes *1* and *6* give bi-4,5-phenanthrylene, *2*, and [2.2]-(3,6)-phenanthreneophane, *7*, respectively. On further oxidation of *2*, dibenzo[*def*,*pqr*]cyclobutano[*jkl*]tetraphenylene, *4*, is formed.

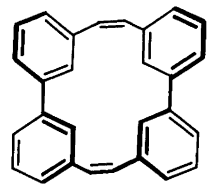
During the last few years we have investigated the application of multiple Wittig reactions to the synthesis of cyclophanes with unsaturated bridges,[1] some of the products being potential precursors for circulenes and other topologically interesting compounds. In this paper, we describe the preparation and attempted photocyclization of three cyclophenadienes. The conformations of the cyclophanes are discussed briefly in relation to the photocyclizations. Part of this work has been reported in a short communication.[2]



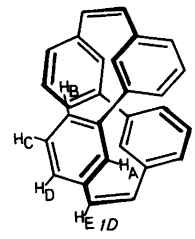
1A



1B



1C



1D

RESULTS AND DISCUSSION

[2.0.2.0]Metacyclophanediene, *1*, can be prepared in 4 % yield from biphenyl-3,3′-dialdehyde and the bis triphenylphosphonium salt from 3,3′-bis(bromomethyl)biphenyl by a double Wittig reaction at –40 °C.[2] An alternative but longer synthetic route *via* the dithiacyclophane and ring contraction rearrangements has been described by Leach and Reiss.[3] The structure of the cyclophane follows from its ^{1}H NMR, UV and MS. The conformation of *1* is of interest when discussing its photocyclization. Four symmetrical conformations, *1A–1D*, can readily be envisaged. Leach and Reiss considered the *syn* conformation *1A* to be the preferred one. Of the four symmetrical conformations, *1A* and *1B* contain relatively planar biphenyl units. In *1A*, the bridging double bonds are almost orthogonal to the π-systems of the biphenyls, while conformer *1B* is sterically rather strained, as apparent by inspection of molecular models (CPK). Conformer *1B*, however, allows for a delocalized π-system. Conformer *1C* is rigid with almost orthogonal π-systems, while conformer *1D* is much less rigid, resembling two *cis*-stilbenes linked together to form a ring, and has the symmetry of a two-bladed propeller (D_2). From the ^{1}H NMR spectrum of *1*

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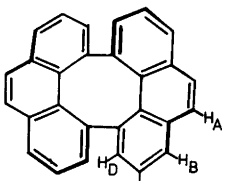
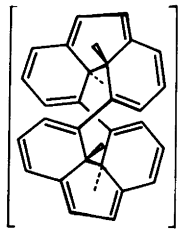
it is not possible to discriminate between the four conformers *1A–1D*. Although the internal aromatic protons appear at unusually low field (δ 7.61) this shift can be attributed either to proximity effects in *1A* or *1C* or to deshielding effects from neighbouring phenyl rings in *1B* or *1D*. However, the UV spectrum of *1* is similar to that of *cis*-stilbene, with almost double the ε-value (see Experimental) which is to be expected only for conformer *1D*.



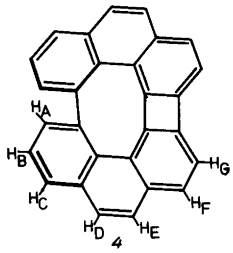
2

Irradiation of cyclophane *1* under oxidative conditions gave bi-4,5-phenanthrylene, *2*, (or dibenzo[*def,pqr*]tetraphenylene) in 65 % yield. The product must be rather strained and the two twisted phenanthrene (or 3 helicene) units could be almost orthogonal giving the molecule a propeller-shaped structure (D_2-symmetry) similar to that of its precursor. This proposal is supported by the UV spectrum of *2* which closely resembles that of phenanthrene. A recent X-ray investigation of *2* shows that the internal deformations of the two phenanthrene subunits are similar to those found in helicenes and that the angle between the two phenanthrene moieties is 69°.[4] The ^{1}H NMR spectrum of *2* is also similar to that of phenanthrene except for the signal from the H_D-protons which is shifted upfield by 0.98 ppm. This shift is probably due to shielding by the opposite phenanthrene unit in the severely twisted structure.

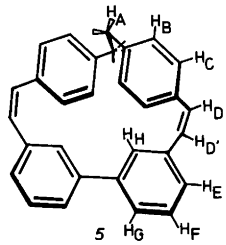
It is conceivable that irradiation of cyclophane *1* under nonoxidative conditions (degassed solution, nitrogen atmosphere) would lead to two successive photocyclizations to give a product with two dihydrophenanthrene units linked together to form a ring. Such an unstable intermediate, ideally with D_2-symmetry, sould be considered as a bridged [24]annulene, *3*. However, we have not been able to detect such an intermediate on irradiation of *1*. Instead, we observed a slow decomposition of the starting material and initially some *cis–trans* isomerization (NMR evidence only).


3

Oxidation of *2* in a melt of sodium chloride and aluminium trichloride [5] gave *4*, dibenzo[*def,pqr*]-cyclobutano[*jkl*]tetraphenylene, the structure of which was determined by ^{1}H NMR and MS (see Experimental). The NMR spectrum of *4* resembles those of the dehydrohelicenes prepared by Wynberg and co-workers.[6] The MS of *4* is very simple, showing M^+ and M^{2+} and almost no fragmentation, which reflects the stability of the compound. Further oxidation of *4* to the fully ring-closed derivative was unsuccessful. In contrast to that of *4* the MS of *1* and *2* showed not only strong molecular ions but the successive loss of six and two mass units, respectively, interpreted as being due to ring closure reactions. The same ring closures could be achieved by irradiation and oxidation. Thus, there seems to be a close parallel between facile ring-closure reactions in the mass spectrometer and chemically-induced reactions in these compounds.



4

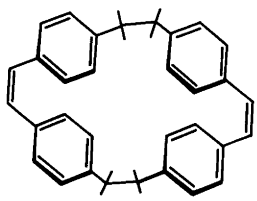


5

In order to increase the flexibility of the cyclophanediene and its ring-closed derivatives, we prepared a new cyclophane in which one of the biphenyl units in *1* had been replaced by a bibenzyl unit. The bis(triphenylphosphonium) salt from 3,3′-bis(bromomethyl)biphenyl was reacted with bibenzyl-4,4′-dialdehyde under the standard conditions to give [2.2.2.0]metaparacyclophanediene, *5*, in 4 % yield after chromatography. To our surprise, irradiation of *5* in various solvents under oxidative conditions gave

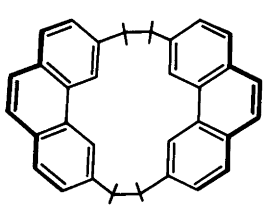
no ring closed product. Instead, slow decomposition of the starting material and, possibly, *cis–trans* isomerization was observed. It is difficult to rationalize the failure of the photocyclization as being due to unfavourable geometry in the ground state. Although no certain conclusions about the ground state geometry can be drawn from available data, there seems to be little steric hindrance in a conformation with two *cis*-stilbene units (from CPK models). Thus, since the UV spectrum of *5* is consistent with such a conformation, we are left with the speculation that unfavourable steric effects in the excited state prevent the photocyclization reaction.[7]

An even more flexible cyclophanediene, *cis,cis*-[2.2.2.2]paracyclophanediene, *6*, can be prepared by a double Wittig reaction from bibenzyl-4,4′-dialdehyde and the bis(triphenylphosphonium) salt from 4,4′-bis(bromomethyl)-bibenzyl. The same cyclophane has also been prepared by preparative electrolysis at constant potential of [2.2.2.2]paracyclophanetetraene [8]

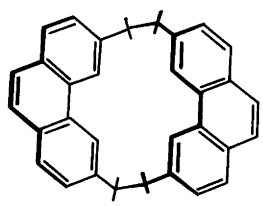


6

and by light-induced isomerization of the *trans,-trans* isomer of [2.2.2.2]paracyclophanediene.[9] We have also reported on the conformations and dynamic processes in the *cis,cis* isomer of [2.2.2.2]paracyclophanediene, *6*, elsewhere.[10] As expected, the cyclophanediene *6* readily undergoes photocyclizations under oxidative conditions to give [2.2](3,6)phenanthrenophane, *7*, which can also be prepared by catalytic hydrogenation of [2.2](3,6)phenanthrenophanediene.[11] The cyclophane *7* is rather rigid and



7A



7B

should adopt a *syn. 7A,* or *anti, 7B,* conformation. The downfield shift of the methylene protons in *7* (δ 3.62) as compared to those observed in a series of similar cyclophanes with ethylene bridges [10] is best explained by assuming the *syn* conformation to be lowest in energy. If so, exchange of the "outer" and "inner" methylene protons in *7* to give a singlet in the NMR spectrum, requires a rapid inversion process *via* the *anti* conformation.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker WH 270 instrument using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were run on an AEI MS 902 spectrometer and UV spectra (in cyclohexane unless otherwise stated) on a Beckman DK-2A instrument. Melting points were determined on a Reichert hot stage apparatus. The photochemical experiments were carried out using a Rayonet reactor with light of maximum intensity at 254 nm and 300 nm.

The bis(triphenylphosphonium) salt from 3,3′-bis(bromomethyl)biphenyl. 3,3′-Dimethylbiphenyl (0.05 mol), recrystallized *N*-bromosuccinimide (0.11 mol) and dibenzoylperoxide, as initiator, were refluxed for 24 h in dry, redistilled, tetrachloromethane. The hot solution was filtered to remove succinimide, and 3,3′-bis(bromomethyl)biphenyl precipitated from the cooled filtrate (38 %, m.p. 114–115 °C, NMR: δ 7.6–7.3 (8H, m), 4.54 (4H, s)). The product was then heated in dry DMF with two equivalents of triphenylphosphine for 10 h. On cooling of the reaction mixture, the white crystalline phosphonium salt precipitated. This was collected, washed with dry ether, and dried *in vacuo* before use.

The bis(triphenylphosphonium) salt from 4,4′-bis(bromomethyl)bibenzyl was prepared from 4,4′-bibenzyldicarbaldehyde. Reduction of the dialdehyde with $LiAlH_4$ gave the hydroxymethyl compound which was converted to the corresponding bromomethyl compound by treatment with HBr in acetic acid. The bis(bromomethyl) compound was then heated with two equivalents of triphenylphosphine in dry DMF for 12 h, and the reaction mixture cooled. The precipitated bisphosphonium salt was collected, washed with ether, and dried *in vacuo* before use.

3,3′-Biphenyldicarbaldehyde. The crude 3,3′-bis(dibromomethyl)biphenyl prepared by fourfold NBS bromination of 3,3′-dimethylbiphenyl was refluxed in a mixture of water–ethanol (1:1) for 24 h. The solution was then stirred overnight

with sodium bisulfite and then extracted with chloroform several times. The aqueous layers were collected and acidified with sulfuric acid. After the gas evolution had ceased, the mixture was extracted with chloroform. The solvent was then distilled off giving 3,3′-biphenyldicarbaldehyde in 60 % yield (m.p. 83–85 °C). NMR: δ 10.2 (2H, s), 8.3–7.5 (8H, m). MS: *m/e* 210 (M^+, 100 %), 209 (93), 182 (11), 181 (23).

The Wittig reactions were performed as previously described and the products were separated and isolated by the standard procedure.[1]

[*2.0.2.0*]*Metacyclophanediene* 1. 3,3′-Biphenyldicarbaldehyde (3 mmol) and the bis(triphenylphosphonium) salt from 3,3′-bis(bromomethyl)biphenyl (3 mmol) were reacted under the standard conditions to give cyclophane *1*, in 4 % yield, (m.p. 114–115 °C). NMR: δ 7.61 (H_A, t, $J_{AB}=J_{AD}=1.5$), 7.31 (H_C, t, $J_{BC}=J_{CD}=7.5$), 7.18 (H_B or H_C, d of t), 7.11 (H_B or H_D, d of t) and 6.60 (H_E, s). MS: *m/e* 356 (M^+, 100 %), 355 (6), 354 (5), 353 (6), 352 (6), 351 (6), 350 (6), 340 (8), 339 (9), 182 (11), 178 (9), 176 (10), 175 (9), 169.5 (12), 168.5 (13). Abs.mass; 356.156±0.003; calc. for $C_{28}H_{20}$ 356.156. UV: λ_{max} 282 nm, ε=19 500.

[*2.2.2.0*]*Metaparacyclophanediene* 5. 4,4′-Bibenzyldicarbaldehyde (3 mmol) and the bis(triphenylphosphonium) salt from 3,3′-bis(bromomethyl)biphenyl were reacted under the standard conditions to give cyclophane, *5*, in 4 % yield (m.p. 192 °C). NMR: δ 7.36 (H_F, t, $J_{EF}=J_{FG}=7.5$ Hz), 7.19 (H_E, H_G, H_H, m), 6.81, 6.68 (H_B and H_C, d, $J_{BC}=8$ Hz), 6.64, 6.47 (H_D and $H_{D'}$, d, $J_{DD'}=12$) and 2.87 (H_A, s). MS: *m/e* 384 (M^+, 100 %), 383 (3), 192 (26), 91 (15). Abs.mass 384.187±0.003; calc. for $C_{30}H_{24}$ 384.187. UV: 262 nm, ε=24 200.

[*2.2.2.2*]*Paracyclophanediene* 6. Bibenzyl-4,4′-dialdehyde (5 mmol) and the bis(triphenylphosphonium) salt from 4,4′-bis(bromomethyl)biphenyl (5 mmol) were reacted under the standard conditions to give cyclophane *6* (156.5 mg, 15.2 %, m.p. 137–139 °C). NMR: δ 7.00 and 6.73 (16 H, d, aromatic protons), 6.53 (4 H, s, olefinic protons), and 2.84 (8 H, s, methylene protons). MS (68 eV): *m/e* 412 (M^+, 100 %), 207 (20, 206 (80), 205 (18), and 191 (14). Abs.mass 412.222±0.005; calc. for $C_{32}H_{28}$ 412.219. UV (ethanol): 265 nm, ε=23 600.

Bi-4,5-phenanthrylene 2. The cyclophanediene *1* was dissolved together with iodine in cyclohexane and irradiated for 24 h. The reaction products were separated by column chromatography on silica gel with tetrachloromethane as eluant, giving *2* in 65 % yield (m.p. 262–263 °C). NMR: δ 7.80 (H_B, d, of d, $J_{BC}=J_{CD}=7.5$), 7.66 (H_A, s), 7.40 (H_C, t), 6.63 (H_D, d of d, $J_{BD}=1.4$). MS: *m/e* 352 (M^+, 67 %),351 (80), 350 (100), 349 (13), 348 (27), 176 (7), 175.5 (13), 175 (47), 174.5 (7), 174 (27). Abs.mass 352.126±0.003; calc. for $C_{28}H_{16}$ 352.125. UV: 253 nm, ε=100 000.

Dibenzo[def,pqr]*cyclobutano*[jkl]*tetraphenylene* 4. To a mixture of *2* (8 mg) and sodium chloride (30 mg) was added powdered aluminium chloride (60 mg) and the compounds were mixed quickly.[5] The reaction vessel was fitted with a drying tube and placed in a preheated oil bath at 140 °C. As soon as the melt was completed (10 min) it was hydrolyzed with water (20 ml). The water phase was extracted with chloroform several times and the combined organic phases evaporated to dryness. The residue was chromatographed on silica gel with chloroform as eluant giving *4* in 40 % yield (m.p. 330 °C decomp.). NMR: δ 9.34 (H_G or H_F, d, $J_{FG}=8.5$), 9.15 (H_A or H_C, d of t, J_{BC} or $J_{AB}=7.5$), 8.52 (H_G or H_F, d), 8.27 (H_A or H_C, d of t), 8.23 (H_D and H_E, AB-quartet, $J_{DE}=8$) and 8.13 (H_B, t). MS: *m/e* 350 (M^+, 37 %), 348 (7), 175 (16), 123 (15), 109 (24). Abs.mass 350.109±0.003; calc. for $C_{28}H_{14}$ 350.109.

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The Kinetics and Mechanisms of the Reactions of Cation Radicals of Phenothiazine Derivatives with Acetate Ion and Water in Acetonitrile

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The kinetics of the reaction of the cation radical of *N*-methylphenothiazine (MPZ) with acetate ion in aqueous acetonitrile were observed to be consistent with mechanism (i)–(iii) and rate law (iv).

$$MPZ^{\cdot+} + AcO^- \overset{K_i}{\rightleftharpoons} MPZ^{\cdot}\text{–}OAc \quad \text{(i)}$$

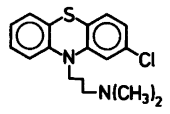
$$MPZ^{\cdot}\text{–}OAc + MPZ^{\cdot+} \underset{k_{-ii}}{\overset{k_{ii}}{\rightleftharpoons}} MPZ^{+}\text{–}OAc + MPZ \quad \text{(ii)}$$

$$MPZ^{+}\text{–}OAc + AcO^- \xrightarrow{k_{iii}} \text{Products} \quad \text{(iii)}$$

$$\text{Rate} = 2k_{iii}K_iK_{ii}[MPZ^{\cdot+}]^2[AcO^-]^2/([MPZ] + k_{iii}/k_{-ii}[AcO^-]) \quad \text{(iv)}$$

The reaction orders in $MPZ^{\cdot+}$, MPZ, and AcO^- were observed to be 2, –0.5 and 1.5, respectively, at $[H_2O]$ equal to 2.78 M. The reaction was observed to be inhibited by both acetic acid and water due to the deactivation of acetate ion by hydrogen bonding. No deuterium kinetic isotope effect was observed when reactions were carried out in the presence of D_2O. Under comparable conditions the reactivity of chlorpromazine cation radical was observed to be about 10^2 times than for $MPZ^{\cdot+}$.

The phenothiazine cation radicals are among the most stable ion radicals known. The ease with which these cation radicals can be studied, as compared to more reactive ones, has led to numerous studies of their properties and reactions.[1–19] We have recently [1] commented on the mechanism of the reactions of chlorpromazine (*1*) cation radical in aqueous buffers. The



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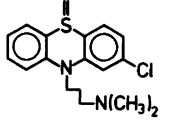
reaction had previously been examined by McCreery and coworkers [2,3,5] and the product was observed to be the oxide (*2*). The sulfurane



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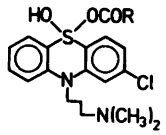
(*3*) was proposed to be a primary intermediate in citrate and phosphate buffers and the mechanism for the reaction was formulated as (1)–(3). We



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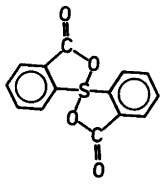
were not able to visualize a reasonable structure for 3^{-} and concluded that the proposed mechanism was not meaningful. Another feature of the mechanism which is unacceptable is the assumption that the homogeneous electron transfer reaction (2) can be treated as an equilibrium with a backward rate constant k_{-2} of a magnitude comparable to k_3, the rate constant for the rate determining step (3). Under the experimental conditions E_o for the redox couple $1/1^{\cdot+}$ was observed to be close to +0.6 V *vs.* SCE. Values of E_o for reduction of sulfuranes like *3* are not available, but may be estimated to be close to the peak potential, –1.9 *vs.* SCE, reported [20] for the related structure *4*. From these values an



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approximate value for K_2 of 10^{42} can be calculated. The analysis presented earlier in connection with the possible disproportionation mechanism for the pyridination of 9,10-diphenylanthracene cation radical [21] then predicts the maximum value of k_{-2} to be of the order of 10^{-32} $M^{-1}s^{-1}$ assuming the forward rate of (2) being diffusion controlled, *i.e.* $k_2 \simeq 10^{10}$ $M^{-1}s^{-1}$. This value of k_{-2} is surely so small that the electron transfer reaction (2) has to be treated kinetically as an irreversible process and not as an equilibrium. Doing this results in a rate law inconsistent with the observed kinetics.

A reexamination of the kinetic data showed that mechanism (4)–(7) gives rise to rate law (8) which is consistent with that observed experimentally. This mechanism, which is similar to that proposed [5] for the reaction in acetate buffer, shows that the kinetics in citrate and phosphate buffers do not require the postulation of any unlikely anion radical intermediate like $3^{\cdot-}$.

$$1^{\cdot+} + RCO_2^- + H_2O \overset{K_1}{\rightleftharpoons} 3^{\cdot-} + H^+ \quad (1)$$

$$3^{\cdot-} + 1^{\cdot+} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} 3 + 1 \quad (2)$$

$$3 \xrightarrow{k_3} 2 + RCO_2H \quad (3)$$

$$1^{\cdot+} + RCO_2^- \overset{K_4}{\rightleftharpoons} 1^{\cdot}\text{–OCOR} \quad (4)$$

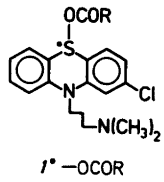
$$1^{\cdot}\text{–OCOR} + 1^{\cdot+} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} 1^{+}\text{–OCOR} + 1 \quad (5)$$

$$1^{+}\text{–OCOR} + H_2O \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} 1(OCOR)(OH) + H^+ \quad (6)$$

$$1(OCOR)(OH) \xrightarrow{k_7} 2 + RCO_2H \quad (7)$$

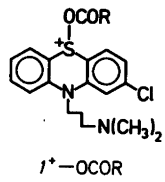
$$\text{Rate} = 2k_7K_4K_5K_6[1^{\cdot+}]^2[RCO_2^-]/[H^+]([1] + k_6/k_{-5}) \quad (8)$$

Mechanism (4)–(7) suggested another possibility. If the reactions were conducted in solvents



$1^{\bullet}$–OCOR

where the nucleophilic activity of RCO_2^- is greater or with more nucleophilic carboxylate ions reactions between 1^{+}–OCOR and RCO_2^- as



1^{+}–OCOR

illustrated by (9) and (10) might become significant.

$$1^{+}\text{–OCOR} + RCO_2^- \begin{cases} \xrightarrow{k_9} 1(OCOR)_2 & (9) \\ \xrightarrow{k_{10}} 2 + (RCO)_2O & (10) \end{cases}$$

This would cause the reaction order in RCO_2^- to approach 2, which would provide convincing evidence for the mechanism.

A mechanism similar to (4)–(7) has recently been ruled out for the hydroxylation of thianthrene cation radical in the presence of trifluoroacetate ion.[22] Significant deuterium kinetic

isotope effects were observed when the kinetics were studied in acetonitrile containing H_2O or D_2O and the reaction was observed to be first order in both water and trifluoroacetate ion. Thus, the mechanism of the hydroxylation of thianthrene cation radical involves water as the nucleophile under the conditions studied, *i.e.* when no other good nucleophiles are present.

The kinetics of the decay of a number of phenothiazine derivative cation radicals were studied in aqueous acetic acid using ESR and visible absorption spectrophotometry.[6] The rate of cation radical decay was observed to be second order in ion radical and disproportionation mechanisms were proposed. A similar study was later carried out in H_2SO_4 on a large number of 10-alkylphenothiazine cation radicals and a disproportionation mechanism was also assumed.[7] In general, the products of the reaction of water with 10-alkylphenothiazine cation radicals are the *S*-oxides analogous to *2*.[17–19]

No fewer than five disproportionation mechanisms were considered by Evans, Lenhard and Blount [9] in their discussion of the kinetics of the decomposition of 10-phenylphenothiazine cation radical in pyridine. They concluded, on the basis of an analysis used earlier to rule out disproportionation during the reaction of thianthrene cation radical with anisole,[23] that disproportionation is not involved. However, the analysis and the entire discussion by Evans, Lenhard and Blount [9] concerning the possibility of disproportionation during the decomposition of 10-phenylphenothiazine (*5*) cation radical is trivial. The equilibrium constant for reaction (11),

$$2\,5^{\cdot+} \underset{}{\overset{K_{11}}{\rightleftharpoons}} 5^{2+} + 5 \qquad (11)$$

was determined using cyclic voltammetry to be equal to 2.2×10^{-12} and apparent second order rate constants as great as 10^3 $M^{-1}s^{-1}$ were determined for the decomposition. The analysis already referred to [21] gives an immediate answer on the feasibility of disproportionation in this case. The assumption that back reaction (11) is diffusion controlled gives the maximum possible value of the rate constant for the forward reaction, k_{11}, to be of the order of 10^{-2} $M^{-1}s^{-1}$. This value is about 10^5 times lower than the observed apparent second order rate constants, a fact which eliminates the necessity to consider disproportionation mechanisms for the decomposition of $5^{\cdot+}$.

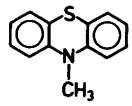
RESULTS

The most simple of the 10-alkylphenothiazines, *6*, was chosen as a model substrate for the

Table 1. Reaction order analysis of the kinetics of the reactions of *N*-methylphenothiazine cation radical in acetonitrile.[a]

C_A^b/mM	C_{AcO^-}/mM	$v_{1/2}$/V s^{-1}	$v_{1/2}/C_{AcO^-}^{1.5}$	$((v_{1/2}/C_{AcO^-}^{1.5})/C_A^{0.5})\times10^{-4}$
0.5	17.4	0.655	285	
0.5	38.1	2.56	344	
0.5	68.8	6.56	364	
			331±41	1.48
1.0	17.4	0.950	414	
1.0	38.1	3.46	465	
1.0	68.8	7.71	427	
			435±27	1.38
2.0	17.4	1.38	601	
2.0	38.1	4.91	660	
2.0	68.8	11.2	621	
			627±30	1.40
				1.42±0.05

[a] Measurements at 19.5 °C. C_{H_2O}=2.78 M. [b] Substrate concentration.



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hydroxylation of phenothiazine derivative cation radicals. The much lower reactivity of $6^{\cdot+}$ as compared to thianthrene cation radical forced us to a much more nucleophilic buffer system than $CF_3CO_2^-LH^+$ (L=2,6-lutidine) which was used in the latter study.[22] Acetate buffers prepared from Bu_4NOAc and AcOH in aqueous acetonitrile provided a sufficiently reactive medium to study the hydroxylation reaction.

Since the derivative cyclic voltammetry kinetic method [24,25] was described in a recent paper [22] discussion of the kinetic procedures is not necessary here.

The data in Table 1 were measured in order to obtain reaction orders in cation radical, substrate and acetate ion. At each substrate concentration, $v_{1/2}$ was obtained over a fourfold range of $[AcO^-]$. Data were obtained at substrate concentrations of 0.50, 1.00 and 2.00 mM. The next to last column in Table 1 gives the values of $v_{1/2}/C_{AcO^-}^{1.5}$. The fact that these values are very nearly constant at all substrate concentrations indicates that the reaction order in acetate ion is very close to 1.5 under the conditions of the measurements. The average values of $v_{1/2}/C_{AcO^-}^{1.5}$ were then used to determine the reaction orders in substrate and cation radical, $R_{A/B}$. The last column in Table 1 shows that $(v_{1/2}/C_{AcO^-}^{1.5})/C_A^z$ is very nearly constant when z is equal to 0.5. It follows that $R_{A/B}$ is very close to 1.5.

The data in Table 2 show that the decomposition of $6^{\cdot+}$ in aqueous acetonitrile acetate buffer is inhibited by acetic acid. However, in both series of experiments, $v_{1/2}$ decreased by only about 25 % as the concentration of added AcOH was changed from 0 to 21.9 mM. The concentration of AcOH in the aqueous acetonitrile solution of AcO^- before addition of the acid was estimated to be less than 10^{-2} mM assuming that the pK_a was the same as in water. This estimation is a maximum value and indicates that [AcOH] is negligibly small before the addition of AcOH.

The data in Tables 3 and 4 indicate an absence of a deuterium kinetic isotope effect in the presence of D_2O when the water concentration was varied in the range of 0.69 to 2.78 M with $[AcO^-]$ ranging from 15.8 to 63.2 mM.

Table 2. The effect of acetic acid on the rate of decomposition of *N*-methylphenothiazine cation radical in acetonitrile.[a]

C_{AcOH}/mM	C_{AcO^-}/mM	$v_{1/2}$/V s^{-1}
0	38.1	4.91
21.9	38.1	3.39
43.8	38.1	2.98
87.6	38.1	1.45
0	63.2	11.0
21.9	63.2	8.22
43.8	63.2	6.54
87.6	63.2	4.08
175.2	63.2	1.82

[a] Measurements at 19.5 °C. C_A=2.0 mM, C_{H_2O}=2.78 M.

Table 3. Search for a deuterium kinetic isotope effect during decomposition of *N*-methylphenothiazine cation radical in acetonitrile.[a]

C_{AcO^-}/mM	Y [b]	$v_{1/2}$/V s^{-1}	k_H/k_D
15.8	H_2O	2.02	1.06
15.8	D_2O	1.91	
31.6	H_2O	4.76	1.03
31.6	D_2O	4.61	
63.2	H_2O	11.7	1.03
63.2	D_2O	11.4	

[a] Measurements at 19.5 °C. C_A=2.0 mM. [b] Y=H_2O or D_2O, C_Y=2.78 M.

Table 4. The effect of H_2O and D_2O concentration on the apparent rate of decomposition of *N*-methylphenothiazine cation radical in acetonitrile.[a]

Y [b]	C_y/M	$v_{1/2}$/V s^{-1}	k_H/k_D
H_2O	0.69	66.4	0.97
D_2O	0.69	68.5	
H_2O	1.39	24.6	1.13
D_2O	1.39	21.8	
H_2O	2.78	3.60	0.99
D_2O	2.78	3.62	

[a] Measurements at 19.5 °C. C_A=2.0 mM, C_{AcO^-}=33.2 mM. [b] Y=H_2O or D_2O.

Table 5. Kinetic data for the reactions of chlorpromazine cation radical in acetonitrile.[a]

C_A^b/mM	C_{H_2O}/M	C_{AcOH}/mM	$v_{1/2}$/V s^{-1}
0.5	1.39	0	225
0.5	2.78	0	62.9
1.0	2.78	0	77.0
2.0	2.78	0	74.0
2.0	2.78	4.38	67.4
2.0	2.78	8.75	60.0
2.0	2.78	17.5	47.1
2.0	2.78	35.0	35.0
2.0	2.78	52.5	20.6
2.0	2.78	70.0	15.0
2.0	2.78	105	9.38

[a] Measurements at 19.5 °C. C_{AcO^-}=8.29 mM. [b] Substrate concentration.

Table 6. The effect of water concentration on the nucleophilic activity of acetate ion in acetonitrile.[a]

C_{H_2O}/mM	$v_{1/2}$/V s^{-1}
0	171.7
69.4	128.0
139	95.9
278	64.4
556	24.9
1112	4.48
2224	0.820

[a] Data for the decomposition of *N*-methylphenothiazine cation radical in solution containing Bu_4NOAc (8.8 mM) and substrate (1.90 mM) at 19.5 °C.

A less extensive set of experiments was also carried out with *1* as substrate under the same conditions. The decomposition of $1^{\cdot+}$ was observed to be significantly more rapid which required that [AcO^-] be limited to lower values. The data in Table 5 were obtained in solutions 8.3 mM in AcO^-. At a water concentration of 2.78 M in the absence of added AcOH, $v_{1/2}$ was observed to be very nearly independent of C_A which indicates that $R_{A/B}$ is very close to 1 under the reaction conditions. The reaction was observed to be inhibited by AcOH in a similar manner to that of $6^{\cdot+}$.

The rate of the decomposition of $6^{\cdot+}$ in acetonitrile in the presence of AcO^- was observed to be strongly dependent on the concentration of water. The data in Table 6 show the effect of increasing [H_2O] successively by factors of 2.

DISCUSSION

The observation of $R_{A/B}$ equal to 1.5 (Table 1) suggests that the reaction order in cation radical is 2 and that in substrate is -0.5 during the decomposition of $6^{\cdot+}$ in aqueous acetonitrile solutions of acetate ion. The observation of a reaction order of 2 in cation radical makes it necessary to consider the possibility of a disproportionation mechanism.[6-9,23,26-32] When possible, the measurement of the reversible electrode potential for the oxidation of the cation radical to the dication [33] provides a reliable estimate of K_{disp}, the equilibrium constant for the disproportionation. It was not possible to measure the reversible oxidation potential of $6^{\cdot+}$ (or for $1^{\cdot+}$) under the conditions used in this study due to the rapid reaction of the cation radicals with acetate ion. When K_{disp} is available, the analysis [21] mentioned in the introduction can be used in the evaluation of the feasibility of disproportionation. However, when K_{disp} is not available as in the present study, it is necessary to rely on the form of the rate law. An effective kinetic analysis to distinguish disproportionation from other mechanisms was presented for the anisylation of thianthrene cation radical.[23] The analysis involves measuring second order rate constants (k_{obs}) for the decomposition of the cation radical in the presence of excess substrate (S). After obtaining several k_{obs} values over a range of [S], a plot of $1/k_{obs}$ will often give a staight line obeying eqn. (12).[23]

$$1/k_{obs} = A[S]+B \qquad (12)$$

This analysis is commonly used for complex reaction schemes where steady state kinetics are assumed.[34] The intercept B can either be a constant independent of the concentration of other reactants, relatable to rate constants, or variable depending on another reactant. In the case of the anisylation of thianthrene cation radical B was observed to be dependent on the anisole concentration and provided a means of eliminating the disproportionation mechanism. This same analysis was later used by McCreery

and coworkers [2] in the analysis of the kinetics of the decomposition of $1^{\cdot+}$ and extensively by Evans, Lenhard and Blount [9] in related reactions.

The observation of a reaction order close to 1.5 for acetate ion provides us with still another tool to analyze for a possible disproportionation mechanism for the decomposition of $6^{\cdot+}$ under the conditions of this study. Disproportionation (13) could be followed by reaction of 6^{2+} with acetate ion to give 6^{+}-OAc which could then react with a second acetate ion to give products.

$$2\,6^{\cdot+} \underset{k_{-13}}{\overset{k_{13}}{\rightleftharpoons}} 6^{2+} + 6 \qquad (13)$$

$$6^{2+} + AcO^{-} \underset{k_{-14}}{\overset{k_{14}}{\rightleftharpoons}} 6^{+}\text{–OAc} \qquad (14)$$

$$6^{+}\text{–OAc} + AcO^{-} \xrightarrow{k_{15}} \text{Products} \qquad (15)$$

In general, dications are at the minimum 10^6 times as reactive towards nucleophiles as cation radicals are.[35] This implies that k_{14} is very large and subsequently (13) cannot be considered as a fast equilibrium prior to (14), but has to be represented by the individual rate constants in the kinetic analysis. Application of the steady state approximation for both 6^{2+} and 6^{+}–OAc leads to rate law (16)

$$\text{Rate} = \frac{2\,k_{15}K_{13}K_{14}[6^{\cdot+}]^2[AcO^-]^2}{[6] + \frac{k_{15}}{k_{-14}}[6][AcO^-] + \frac{k_{15}K_{14}}{k_{-13}}[AcO^-]^2} \qquad (16)$$

for the disproportionation mechanism. Since the dissociation of 6^{+}–OAc is expected to be very slow compared to further reaction with acetate ion *i.e.* $k_{-14} \ll k_{15}[AcO^-]$, rate law (16) reduces to (17),

$$\text{Rate} = \frac{2\,k_{15}K_{13}K_{14}[6^{\cdot+}]^2[AcO^-]}{\frac{k_{15}}{k_{-14}}\left([6] + \frac{k_{14}}{k_{-13}}[AcO^-]\right)} \qquad (17)$$

which predicts a reaction order less than or equal to 1 in acetate ion which is inconsistent with the data. If the assumption is made that K_{13} is the same in the presence of acetate as in acetonitrile with nucleophiles excluded, the disproportionation mechanism is ruled out without resort to eqn. (17).

The most likely mechanism, one which takes into account all of the experimental data, involves reactions (18)–(20) and gives rise to rate law (22).

$$6^{\cdot+} + AcO^{-} \overset{K_{18}}{\rightleftharpoons} 6^{\cdot}\text{–OAc} \qquad (18)$$

$$6^{\cdot}\text{–OAc} + 6^{\cdot+} \underset{k_{-19}}{\overset{k_{19}}{\rightleftharpoons}} 6^{+}\text{–OAc} + 6 \qquad (19)$$

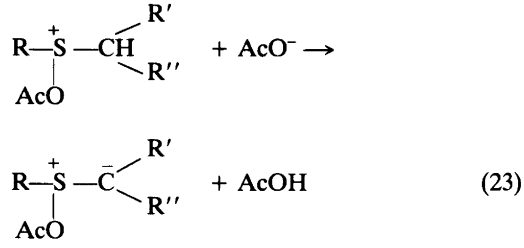
$$6^{+}\text{–OAc} + AcO^{-} \xrightarrow{k_{20}} 6{=}O + Ac_2O \qquad (20)$$

$$6^{+}\text{–OAc} + AcO^{-} \underset{k_{-21}}{\overset{k_{21}}{\rightleftharpoons}} 6(OAc)_2 \qquad (21)$$

$$\text{Rate} = \frac{2\,k_{20}K_{18}K_{19}[6^{\cdot+}]^2[AcO^-]^2}{[6] + \frac{k_{20}}{k_{-19}}[AcO^-]} \qquad (22)$$

The acetic anhydride formed in eqn. (20) suffers hydrolysis in a subsequent slow step which does not influence the kinetics.

A key intermediate in the mechanism is the acetoxysulfonium ion, 6^{+}–OAc. Acetoxysulfonium ions are also believed to play an important role in the Pummerer reaction [36–38] in which a sulfoxide containing an α-hydrogen atom is converted to the corresponding α-acetoxysulfide by treatment with acetic anhydride. The first step of the Pummerer reaction is usually formulated as the reverse of reaction (20), but the step is very slow at room temperature compared to forward (20) which under the conditions of the present study can be treated kinetically as an irreversible step. The second step of the Pummerer reaction involves base attack by acetate ion at the α-position of the acetoxysulfonium ion resulting in the formation of an acetoxysulfonium ylide [eqn. (23)].



(23)

However, this pathway is not possible for 6^{+}–OAc due to the lack of α-hydrogen atoms.

Thus, except for dissociation (back reaction (19)), 6^+–OAc is restricted to suffer nucleophilic attack by acetate (reactions (20) and (21)) or eventually water.

In discussions of the mechanism of the Pummerer reaction [36,37] the possible involvement of sulfuranes as intermediates has been considered. Sulfuranes, which contain a tetracovalent sulfur atom, have been isolated in special cases [39–42] and sulfurane-type intermediates have been proposed in racemization and oxygen exchange reactions of optically active sulfonium ions.[36,43] In the preliminary report on this work [1] a sulfurane, $6(OAc)_2$, was proposed as the primary product which could then undergo transformation to $6{=}0$ (reaction (24)).

$$6(OAc)_2 \xrightarrow{k_{24}} 6{=}0 + Ac_2O \qquad (24)$$

The inclusion of $6(OAc)_2$ in the reaction scheme gives rise to rate law (25) (reaction (21) slow and irreversible) or rate law (26) (reaction (21) reversible, reaction (24) slow and irreversible), which both are of the same form as eqn. (22) and therefore kinetically indistinguishable.

$$\text{Rate} = \frac{2\,k_{21}K_{18}K_{19}[6^{\cdot+}]^2[AcO^-]^2}{[6] + \frac{k_{21}}{k_{-19}}[AcO^-]} \qquad (25)$$

$$\text{Rate} = \frac{2\,k_{21}K_{18}K_{19}[6^{\cdot+}]^2[AcO^-]^2}{\left(1+\frac{k_{-21}}{k_{24}}\right)[6] + \frac{k_{21}}{k_{-19}}[AcO^-]} \qquad (26)$$

It should be pointed out that rate law (25) does not require that $6(OAc)_2$ is long lived, but in lack of further evidence in support of the participation of $6(OAc)_2$ we prefer the simpler scheme which only includes reaction (20).

Nucleophilic attack by acetate ion on an acetoxysulfonium ion intermediate (eqn. (20)) is in agreement with the observation that anodic oxidation of thianthrene in non-aqueous Ac_2O–AcOH–NaOAc gives the *S*-oxide exclusively.[44] Similarly, when *p*-bromothioanisole was subjected to anodic oxidation under the same conditions, a 2:3 mixture of the *S*-oxide and α-acetoxy-*p*-bromothioanisole was obtained.[44]

The cation radical, $1^{\cdot+}$, of chlorpromazine was found to react at a significantly higher rate than did $6^{\cdot+}$. Extrapolation of $v_{1/2}$ for $6^{\cdot+}$ to $[AcO^-] = 8.3\times10^{-3}$ (Table 1) gives an approximate value of 0.2 V s^{-1} which by comparison with the value observed for $1^{\cdot+}$, 62.9 V s^{-1} (Table 5), demonstrates that the latter cation radical is 300 times more reactive under the same conditions. This rate enhancement is most likely due to the presence of the 2-chloro substituent in $1^{\cdot+}$ rather than the difference in the *N*-alkyl groups since both compounds show only small sensitivity to the presence of acetic acid in the solvent. If the increased reactivity of $1^{\cdot+}$ compared to $6^{\cdot+}$ was due to the difference in structure of the *N*-alkyl groups, $1^{\cdot+}$ would be expected to be much more sensitive to the acidity of the solvent because of the terminal *N,N*-dimethylamino group.

Most of the kinetic runs were conducted at a rather high concentration of water, a typical value being 2.78 M. In Table 4 are reported the results of experiments carried out to test for a possible deuterium kinetic isotope effect.[45] Although no such effect could be detected, pointing to the absence of proton transfer reactions before or during the rate determining step, the over-all reaction rate was in fact found to depend significantly on the concentration of water. The value of $v_{1/2}$ increased by a factor of almost 20 when the water concentration was diminished from 2.78 M to 0.69 M. The effect is demonstrated even more dramatically by the data in Table 6 where the concentration of added water was varied between 0 and 2.22 M resulting in a decrease in $v_{1/2}$ from 171.1 to 0.820 V s^{-1} which corresponds to a reduction of the rate by a factor of 200. A similar effect was observed when acetic acid was added to the solvent system. Although this effect cannot be rationalized quantitatively, a qualitative explanation can be found in the influence of water and/or acetic acid on the reactivity of acetate ion in acetonitrile. Numerous studies have been devoted to the question of the interactions between ions and protic or aprotic dipolar solvents.[46–51] The general conclusion drawn from the studies relevant to the present work is that acetate ion in acetonitrile–water mixtures is strongly associated to water, most likely through hydrogen bonding (eqn. (27)),

$$(CH_3CO_2^- \cdot H_2O)_{solv} \overset{K_{27}}{\rightleftharpoons} (CH_3CO_2^-)_{solv} + (H_2O)_{solv} \qquad (27)$$

because of which the observed nucleophilicity is significantly diminished compared to that of

acetate ion in "water-free" acetonitrile. Acetate has been found to be one of the most powerful nucleophiles in acetonitrile being 30 times as reactive as thiocyanate,[48] whereas acetate is generally considered to be a poor nucleophile in protic solvents. The effect of increasing water concentration on equilibria like (27) is not straight-forward to predict,[49] but the data in Tables 4 and 6 are in agreement with the effect intuitively expected at low water concentrations that increasing values of C_{H_2O} will cause the equilibrium to be displaced more and more effectively to the left. It should be mentioned at this point that the inclusion of water in acetonitrile to the extent of 2.78 M does not change the macroscopic properties of the solvent significantly as reflected in the dielectric constant and the viscosity.[52] Thus, the rate reduction observed in aqueous acetonitrile can be satisfactorily explained by deactivation of acetate by water.

At increasing water concentrations the direct reaction of 6^+–OAc with water [eqn. (28)] might be expected to be of importance.

$$6^+\text{–OAc}+H_2O \xrightarrow{k_{28}} 6=0+\text{AcOH}+H^+ \qquad (28)$$

However, the fact that the reaction order in acetate ion even at C_{H_2O}=2.78 M is close to 1.5 demonstrates that reaction (28) is not the major pathway under our experimental conditions. A small contribution from (28) cannot be excluded, but the over-all retardation caused by the presence of water precludes the possibility to detect it kinetically.

EXPERIMENTAL

The instrumentation, electrodes, cells, data handling procedure and solvent and supporting electrolyte purification have recently been described.[53] *N*-methylphenothiazine was prepared by a standard procedure.[54] Chlorpromazine was kindly provided by Dr. J. Jaroszewski. Tetrabutylammonium acetate was FLUKA (*pract.*) and used as received.

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The Significance of α-Sulfone and α-Sulfonate Groups for the Cleavage of β-Aryl Ether Structures in Lignin

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The kinetics of the alkaline cleavage of the β-aryl ether linkage in lignin containing a sulfonate or a sulfone group in the α-position has been studied by the use of model compounds. In the presence of either of these groups the β-ether cleavage reaction is a first order reaction with respect to the cleavage product. The rate constants and their temperature dependence were determined in each case and compared with data for cleavage in normal structures (α-hydroxyl groups). It was found that the activation energy with a sulfone group is 121 kJ/mol; the rate of cleavage is comparably very fast even at 0 °C. In the presence of an α-sulfonate group, the activation energy is somewhat lower, 97 kJ/mol. The reaction rate is much slower than in the presence of a sulfone group but is still faster than cleavage in normal structures. At 110 °C it is about the same as the rate of cleavage in normal structures at 170 °C.

These results support a β-elimination mechanism induced by the electron-withdrawing power of the sulfone and sulfonate groups as has been previously proposed. It is suggested that the un-ionized nature of the sulfone substituent generates a strong inductive effect whereas the ionized nature of the sulfonate groups gives a weaker effect.

In recent papers, the significance of aryl ether cleavage for the kraft delignification of wood is discussed.[1,2] It was concluded that the cleavage of the non-phenolic β-aryl ether linkages in lignin may be of major importance for the rate of lignin dissolution during the bulk phase of kraft or soda pulping. In our view, efforts to promote delignification should, therefore, be concentrated on the search for methods to increase the rate of cleavage of this type of linkage in lignin. One possible method is to modify the lignin structure in the α-position of aryl propane units by substituting the ordinarily present benzyl alcohol group with another group which promotes cleavage of β-aryl ether bonds. It has previously been demonstrated that the presence of an α-carbonyl group increases the rate of β-aryl ether cleavage considerably, especially in non-phenolic lignin models.[3,4] In the presence of hydrogen sulfide ions, this cleavage proceeds at ambient temperature.

The present work shows similar results for the alkaline β-ether cleavage in non-phenolic models containing an α-sulfonate or an α-sulfone group. It has previously been reported [5,6,10–13] that such groups in the α-position of lignin models facilitate the cleavage of the β-aryl ether linkage and it was therefore of interest to extend these qualitative studies in order to acquire quantitative (kinetic) information. The results given here are compared with earlier kinetic data for the cleavage of the original β-aryl ether linkage models containing the α-hydroxyl group *3* and *4* in order to evaluate the effect of the sulfonate and of the sulfone on the rate of the β-aryl ether cleavage. The rate of this cleavage in the presence of these groups was studied using two slightly different non-phenolic β-aryl ethers: 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propanol-1-sulfonate *1* and 1-(3,4-dimethoxyphenyl)-2-(2-methoxy-phenoxy)-ethyl-*p*-tolyl sulfone *2*.

Compound *2* lacks the terminal hydroxymethyl group assumed to be originally present in the side-chains of lignin. This simplified model was chosen because of its facile preparation according to a synthesis reported previously.[6]

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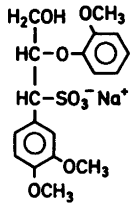
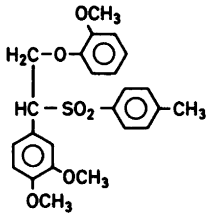
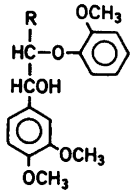


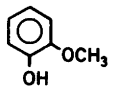
1



2



3 R = CH_2OH
4 R = H



Guaiacol

Aqueous solutions of sodium hydroxide were used as solvents. The aqueous solutions were mixed with methylcellosolve or dioxane to ensure complete solubility of the compounds, the mixtures being carefully made up in the presence of alkali to avoid phase separation (see Experimental).

RESULTS

The kinetic data from the alkaline treatment of compounds *1* and *2* are summarized in Table 1 together with reference data for compounds *3* and *4*.

Figs. 1, 3 and 4 illustrate the course of the ether cleavage reactions at different temperatures and different alkali concentrations.

Cleavage of the β-aryl ether α-sulfonate. Alkaline treatment of the sulfonate *1* affords guaiacol as main product. Fig. 1 shows the liberation of guaiacol at different temperatures as a function of time. A high temperature (above 100 °C) is required if the guaiacol is to be formed in a reasonable time. A logarithmic plot of the yield of guaiacol *versus* time gave straight lines which confirmed that the ether cleavage reaction follows a pseudo first-order behaviour as was expected. The rate constants are given in Table 1 and it is evident that the rate constant for the sulfonate compound *1* at 119 °C is higher than those for the reference compounds *3* and *4* obtained at 170 °C.

The reference compounds *3* and *4* were run in a mixture of organic solvent and water (see Table

Table 1. Alkaline cleavage of the β-aryl ether linkage in compounds *1, 2, 3, 4.* Reaction conditions and rate data. Concentration of substrate: *1*: 3.3×10^{-3} mol l^{-1}, *2*: 0.9×10^{-3} mol l^{-1}. Solvent: H_2O, except: see [b] and [c].

Compound	Conditions		Observed rate constants for guaiacol formation		Final yield of guaiacol
	Temp.[a] °C	$[HO^-]$ mol l^{-1}	$10^3\ k_1$ (min^{-1})	$10^3\ k_2$ (min^{-1} mol^{-1} l)	(mol % of theor.)
1	100	0.53	9.4	17.7	93
	119.0	0.51	41.0	80.4	98
	140.0	0.55	199.0	362	100
2 [b]	0	0.001	–	$30\cdot10^3$	60
	10	0.001	–	$190\cdot10^3$	80
	22	0.001	–	$1500\cdot10^3$	70
3-diacetate [c] (threoform)	172.0	0.30	13.3	44	95
4 [c]	170	0.51	23	45	100

[a] Total error was $<\pm1.6$ °C except in treatment at 0–50 °C where the error was $<\pm0.5$ °C. [b] 60 % dioxane in H_2O, allowance for the solvent effect on rates: see text. [c] Data from literature. Compound *4* was run in 30 % methyl cellosolve [4] and compound *3* in 40 % methyl cellosolve [3] in H_2O.

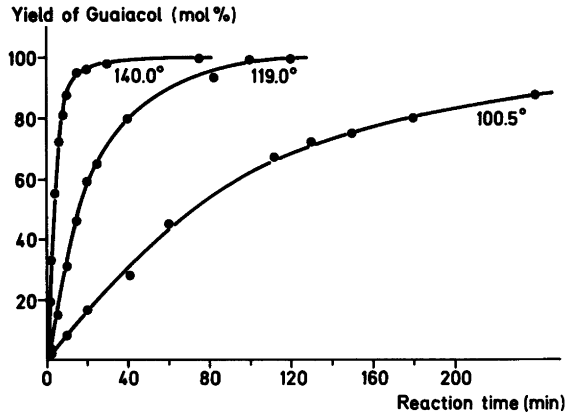


Fig. 1. Formation of guaiacol from cleavage of the β-aryl ether α-sulfonate *1* in 0.5 mol/l sodium hydroxide at different temperatures. Solvent: water.

1). This will in principle give rise to slightly higher rate constants due to increased activity of HO^- ions. The rate constants of the reference compounds in "pure " water would be somewhat lower.*

The fact that the β-aryl ether cleavage in the sulfonate compound *1* proceeds much faster than it does in ordinary β-aryl ether compounds *3* and *4* is further illustrated in Fig. 2 which shows the temperature dependence of the observed (at 0.5 mol/l HO^-) rate constants. The temperature dependence of each compound is of the same order of magnitude as is shown by the similarity of the slopes of the curves. The Arrhenius activation energy of the alkaline ether cleavage reaction in the sulfonated compound *1* is about 97.1 kJ/mol (23.2 kcal/mol) and in the reference compound *4* (or *3*) about 123 kJ/mol (29.6 kcal/mol).[1]

*Cleavage of the β-aryl ether α-(*p*-tolyl) sulfone.* Alkaline treatment of the sulfone *2* had to be performed at very low temperatures due to the great ease of ether cleavage. The formation of guaiacol was found to take place readily at 0 °C and increased with increasing alkali concentration as is depicted in Fig. 3. Since it was difficult to achieve full solubility of the sulfone in the reaction mixture at this temperature, it was necessary to run the reaction with 60 % dioxane in the NaOH water mixture.* Due to difficulties in following the very fast increase of guaiacol at the higher alkaline levels (and temperature), the calculated rate constants given in Table 1 are

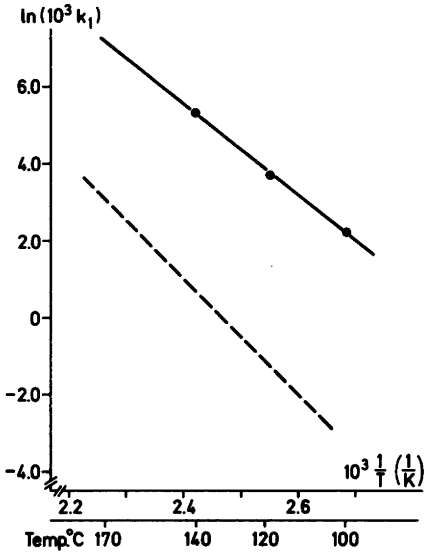


Fig. 2. Arrhenius diagram. Temperature dependence of the observed rate constants (k_1) in 0.5 mol/l sodium hydroxide for the cleavage of the β-aryl ether α-sulfonate *1* (●) and the normal β-aryl ether *4* (*3*) (——) as reference.

* The presence of the organic solvent (as mentioned above) will increase the base strength of HO^--ions and thus raise the rate of cleavage. However, this rate increase is not estimated to be more than one power of ten. This corresponds roughly to only about a 10–20 °C change in temperature and is thus of negligible significance for these rate comparisons.

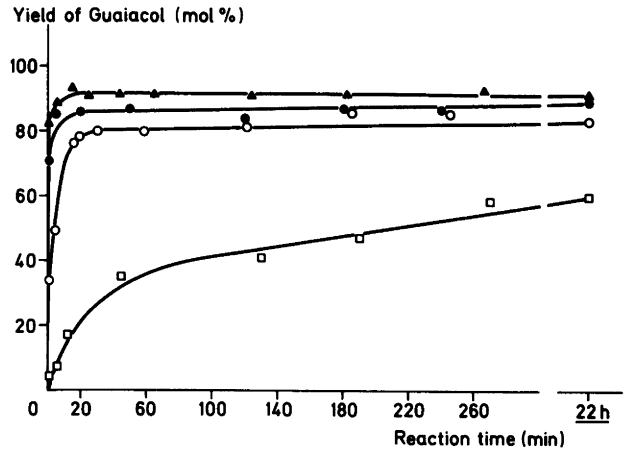


Fig. 3. Formation of guaiacol from cleavage of the β-aryl ether α-sulfone *2* in 0.5 (▲), 0.1 (●), 0.01 (○), 0.001 (□) mol/l sodium hydroxide at 0 °C. Solvent: dioxane/water 3:2.

evaluated from a second-order rate expression using data from runs in 0.001 mol/l sodium hydroxide. Linear plots could be obtained showing the reaction to be of first order with respect to both substrate and alkali (*i.e.* overall second order). In 0.001 mol/l sodium hydroxide, the guaiacol yield reaches a maximum of only about 60–80 %, even after a very long reaction time, 22 h (Figs. 3 and 4). (It could not be decided whether this was real or due to experimental error in determination of the yield, caused by the unusual conditions *e.g.* solvent effects during the extraction.)

As can further be seen in Fig. 4, the rate is dependent on the temperature. The Arrhenius activation energy is 121 kJ/mol (28.8 kcal/mol) (Fig. 5) which is about the same as for the reference compound *4* (or *3*).

Thus, a comparison of the rate constants in Table 1 shows that the cleavage of sulfone *2* is much faster than that of the reference compound *4* (or *3*) even if the solvent effect is considered (see footnote p. 005). Furthermore, a comparison by inserting equal rates in the respective

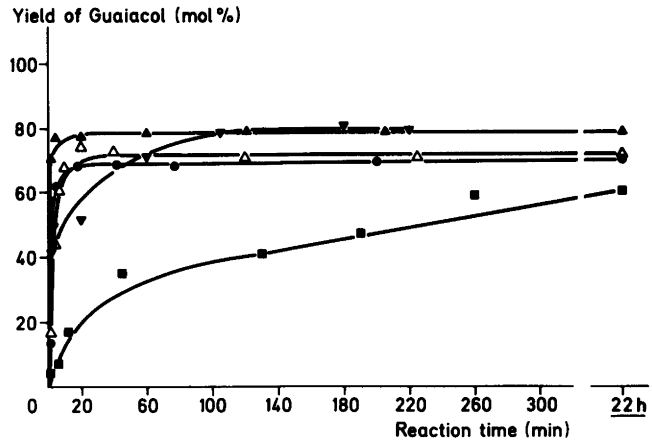


Fig. 4. Formation of guaiacol from cleavage of the β-aryl ether α-sulfone *2* in 0.001 mol/l sodium hydroxide at 0 °C (■), 10 °C (▼), 22 °C (●), 50 °C (△), and in 0.01 mol/l sodium hydroxide at 50 °C (▲). Solvent: dioxane/water 3:2.

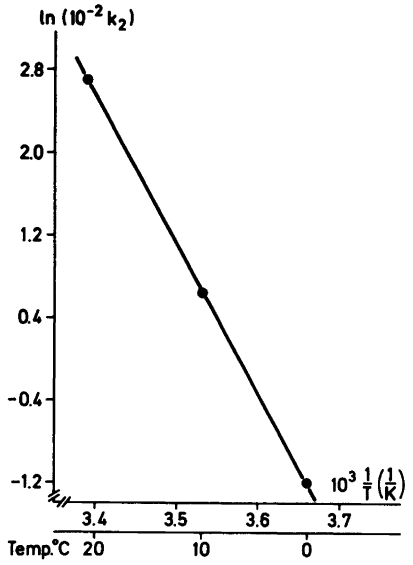
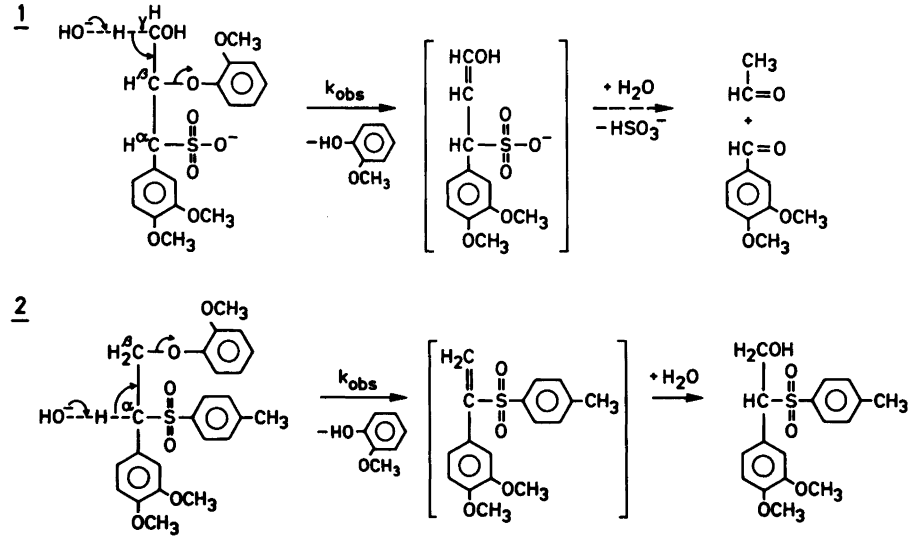


Fig. 5. Arrhenius diagram. Temperature dependence of the observed rate constants (k_2) for the cleavage of the β-aryl ether α-sulfone *2*.

Arrhenius equations shows a temperature difference of about 200 °C for the same rate of cleavage.

DISCUSSION

The great influence of the substituents, the α-sulfonate and the α-sulfone groups, on the alkaline cleavage of the β-aryl ether linkage can be rationalized as an inductive, electron-withdrawing effect on the neighbouring α-carbon-hydrogen bond thus making this hydrogen more acidic. This effect is strongest from the sulfone substituent (because of its un-ionized nature, see below) and brings about a β-elimination of the aryloxy residue with simultaneous formation of an alkene-product of intermediate. The β-elimination mechanism has been studied in the literature and suggested to be the dominating mechanism.[5,7-9] From an alkaline treatment of a non-phenolic *glycol*-β-aryl ether-α-sulfonic acid the corresponding styrene-α-sulfonic acid (*i.e.* lacking the α-hydrogen) has been isolated.[5] Similarily, from the *glycol*-β-aryl ether-σ-sulfone *2* the corresponding β-hydroxy-α-sulfone was isolated by alkaline treatment [6] (Scheme 1, compound *2*). (The β-hydroxy group is likely to be formed by the addition of water to the intermediate styrene α-sulfone.) It could be argued that the β-hydroxy reaction product could also be formed by a direct substitution (*e.g.* an S_N2-reaction, by hydroxide-ions attacking the β-carbon atom) instead of an E2 or E1cB-reaction. However, this



Scheme 1. The β-elimination mechanisms for the alkaline cleavage of the β-aryl ether linkage with electron-withdrawing α-substituents. Corresponding alkene intermediates are formed by hydrogen elimination in the γ- and α-positions. Formation of the main final products according to literature: *cf.* further Refs. 10–13 for compound *1* and Refs. 5–6 for compound *2*.

alternative is ruled out by the extensive studies by Stirling and coworkers [7–9] of the elimination of phenoxide from similar α-substituted ethyl phenyl ethers. In these studies, it was proved that sulfone substituents (among others) activate β-elimination of phenol by a reversible E1cB-mechanism. This was shown by trapping the alkene intermediate with piperidine to give the piperodino adduct.[7] The kinetic data for the formation of phenol (*i.e.* guaiacol) presented here are of the same order of magnitude as the earlier kinetic data although a stronger base was used to induce the β-elimination.[9]

Altogether, it may therefore be concluded that the presence of the α-sulfone group in the *glycol*-β-aryl ether structure (*i.e.* like *4*) activates the β-elimination reaction easily expelling the phenoxy substituent.

Concerning the effect of the α-sulfonate in *glycerol*-β-aryl ether structures, two alternative ways of β-elimination have been discussed in the literature;[10–13] elimination of the α-hydrogen (Saytzeff elimination) or the γ-hydrogen (Hofmann elimination). Product analysis and labelling experiments (of carbon side-chain by ^{14}C) showed that the dominating β-elimination reaction occurs by abstraction of the γ-hydrogen. *i.e.* Hofmann elimination.[11] (See Scheme 1, compound *1*.) This suggested therefore that the expected increase in the acidity of the α-hydrogen is *less* than the increase in the acidity of the γ-hydrogen due to the presence of the α-sulfonate. Thus the lower acidity of the α-hydrogen might partly be due to delocalization of the free electron pair in the ionized sulfonate group towards the α-carbon-hydrogen bond. In addition, the ionized nature of this substituent may exert a shielding effect towards hydroxide ions attacking the α-hydrogen so that these therefore more easily attack the γ-hydrogen.[13] If the latter is the major cause, then the sulfonate group still exerts an inductive electron-withdrawing effect on the γ-hydrogen in spite of the long distance (over two carbon atoms). However, as is also discussed in Ref. 13, steric relationships seem to favour γ-hydrogen elimination in the presence of the sulfonate and this may also contribute to the increase in the reaction rate.

By the use of an un-ionized electron-withdrawing substituent such as the sulfone, electron delocalization and/or charge shielding effect are avoided. This type of substituent would then exert an uncomplicated, stronger electron-withdrawing effect on the nearest carbon–hydrogen bond, *i.e.* the α-hydrogen. Thus, although only the α-hydrogen elimination was studied here with a model containing the sulfone substituent, its ease of elimination supports the mechanism suggested above. Accordingly, in general, electron-withdrawing effects are much stronger from an un-ionized substituent than from an ionized substituent. This explanation accounts for the fact that a much higher temperature is needed to cleave the β-aryl ether linkage by use of sulfonate substituents than by use of sulfone as is shown by the kinetic data in this work.

CONCLUSIONS

Electron-withdrawing substituents such as sulfonate or sulfone groups in the α-position of non-phenolic β-aryl ether structures activate the alkaline fission of the β-aryl ether linkage. In normal β-aryl ether structures (*i.e.* with α-OH) a temperature of about 170 °C is required for the cleavage to occur at a reasonable rate. With the activating sulfonate group in the α-position, the temperature required to attain the same rate is around 110 °C and with the sulfone in the same position the corresponding temperature is less than 0 °C. Earlier work indicates that the sulfone substituent activates the β-elimination reaction (E1cB) from the α-position whereas the sulfonate tends rather to activate a β-elimination from the γ-position in such structures.

The kinetic data obtained suggest that sulfone groups introduced into the α-position of aryl propane units in lignin would exert a very great effect on the rate of the β-aryl ether cleavage during alkaline pulping.

Thus, in order to promote alkaline delignification, sulfone groups seem to be the most interesting substituents to try to introduce into lignin in wood prior to pulping.

EXPERIMENTAL

Materials. Compound *1* [1-(3,4-dimethoxyphenyl)-2-(methoxy-phenoxy)-propanol-1-barium sulfonate] was prepared according to earlier description [14] by heating the compound *3* [15] (2 g) with 80 ml sulfite solution (total SO_2-content 6,2 %, pH 1,5) in a rotating autoclave at 135 °C for 7 h. The work-up procedure in Ref. 14 was in

principle followed: After cooling and extraction with CH_2Cl_2, the residue was passed through a column of Dowex 50W-X8 resin (H^+-form). $BaCO_3$ was added to pH 6 and the mixture was allowed to stand overnight and was finally filtered and evaporated twice. After drying in a desiccator, the barium salt of the sulfonic acid was precipitated by adding ether to a solution in methanol. The mixture was allowed to stand for four days and the solvent was then filtered off and the crystals of the salt collected. Yield 65 % m.p. 178–180 °C.

Methyl ester of compound *1*: The acetate of the sulfonic acid methyl ester [1-(3,4-dimethoxy-phenyl)-3-(2-methoxy-phenoxy)-3-acetoxy-propane-1-sulfonic acid methyl ester] was prepared according to the procedure in Ref. 14. The mass spectra pattern and the ^1H NMR spectra were identical with those described in Ref. 14.

Compound *2* [1-(3,4-dimethoxy-phenyl)-2-(2-methoxy-phenoxy)-ethyl-*p*-tolyl sulfone] was prepared from *4* [15] as described in Ref. 6: Yield 75 % crystalline; m.p. 79–82 °C. (Found C 65.05 H 5.99 O 21.59 S 7.32. $C_{24}H_{26}O_6$ requires: C 65.16 H 5.88 O 21.72 S 7.25.) ^{13}C NMR: δ 151.3–112.4 (16 signals, aromatic) 70.7 (α-C) 68.4 (β-C) 56.2 (OCH_3) 21.5 (CH_3). α-C and β-C was assigned by "off-resonance"-decoupled spectra. ($(CD_3)_2CO$-solvent with TMS as reference.) ^1H NMR (80 MHz): δ 2.38 (s, 3H, ar-CH_3); 3.67, 3.71, 3.83 (3xs, 3x3H, 3x–OCH_3); 4.90–4.52 (m, 3H, $1H_\alpha$ $2H_\beta$); 7.52–6.71 (m, 11H, ar-H). MS: (40 eV) m/e (rel.int %): 442 (M, 0.25), 287 (M-155,127), 259 (0.22), 164 (M-155-123,578.8), 156 (0.13) 150 (6.6), 149 (100), 123 (23.3).

Cooking liquors. Compound 1: NaOH (*p.a.*) was dissolved in 225 ml of distilled water to the desired concentration. Compound *2*: NaOH was dissolved in a mixture (20.0 ml) of 40 % distilled water and 60 % dioxane to the desired concentration. 60 % dioxane was necessary when conducting the experiments at 0 °C in order to achieve full solubility of the sulfone *2*. At a temperature below 0 °C, precipitation occurred (in presence of 0.01 mol/l NaOH). Phase separation occurred at all temperatures if NaOH concentration exceedes 0.5 mol/l.[16,17]

Solutions of substrate. Compound *1*: The model compound (350 mg) was dissolved in H_2O (20 ml) to give an ultimate substrate concentration during the cook of 3.3 mmol/l. Compound *2*: 10 mg were dissolved in 5 ml dioxane to give an ultimate substrate concentration during the run of 0.9 mmol/l.

Cooking apparatus and procedure. Compound *1*: The same apparatus and procedure were used as described in Ref. 4. Compound *2*: 20.0 ml of the alkaline solution was equilibrated at the desired temperature. The run was started by carefully (to avoid precipitation, especially at low temperatures) adding 5.0 ml of the substrate solution to the alkaline solution under vigorous stirring.

Work-up procedure. The same procedure was used for compounds *1* and *2* after the alkaline treatments (cookings) of the model compounds. After a fast cooling of the sample to room-temperature (in the case of cooking) a 3.0 ml aliquot was immediately withdrawn and transferred to a separation funnel diluted with water (5 ml containing 1 mol/l Na_2SO_4), neutralized with 10 % phosphoric acid and extracted with dichloromethane (2.0 ml) containing *p*-cresol as internal standard. The extraction was repeated twice with fresh CH_2Cl_2 (1 ml).

The guaiacol formed from the model compounds was determined directly (GLC) in aliquots from the dried (Na_2SO_4) CH_2Cl_2-extracts. (No other products were identified or isolated in this work.)

GLC-analysis. The analyses of guaiacol and *p*-cresol were performed on a 5 % Castorwax column (steel; 2 m, 3 mm diam) at a temperature of 150 °C (inj. temp. 200 °C; det. temp. 200 °C) isothermically, *cf.* Ref. 3). The GLC-apparatus was F17 or F270 equipped with a flame ionization detector. The areas of the peaks were measured by means of a computing integrator (Minigrator, Spectra Physics) and converted into mg per aliquot using a calibration curve. The amounts of guaiacol are expressed in the figures as mol % of theoretical yield. The starting materials (*1* and *2*) could not be found in the chromatograms (on SE-30 column) probably due to thermal decomposition in the GLC-injector port. It was not therefore certain whether or not any starting material remained after reaction.

Kinetic method. The reactions were in general studied under pseudo first-order conditions using at least a tenfold excess of sodium hydroxide over substrate. Integration of the rate equation gives the expression [18] $\ln a/a-x=k_1t$ where a refers to the maximal and x to the actual concentration of guaiacol. Plots of $\ln (a/a-x)$ *versus* time were linear and the pseudo first-order rate constants (k_1) were calculated from the slopes of the lines. When studying compound *2* under very low [HO^-] (0.001 mol/l), the starting [OH^-] was almost equal to the initial [substrate] and therefore a mathematically equivalent second-order rate expression of one reactant was used.[18] Integration of this rate equation gives the expression [18] $1/(a-x)=kt+1/a$. Plots of $1/(a-x)$ *versus* time were linear with some scattering. From the estimated slopes of the lines, the

second-order rate constants (k_2) were calculated. They are given in Table 1. The Arrhenius activation energies (Ea) for the reactions were caalculated from the integrated expression [18] ln $k=-Ea/RT+A$. Plots of ln k *versus* the inversed absolute temperature $1/T$ are given in Figs. 2 and 5.

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Absolute Configuration of 4-Hydroxy-4-methylglutamic Acids

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The absolute configurations of the naturally occurring (2*S*,4*S*)-4-hydroxy-4-methylglutamic acid and (2*S*,4*R*)-4-hydroxy-4-methylglutamic acid have been determined. L-Amino acid oxidase was used in the determination of the (2*S*)-configuration of the amino acids. The diastereoisomeric (2*S*)-4-hydroxy-4-methylglutamic acids were transformed into (*S*)(+)-citramalic acid and (*R*)(−)-citramalic acid, respectively, by oxidative deamination and decarboxylation using calcium hypochlorite. On the basis of the obtained results and the known absolute configurations of the enantiomeric citramalic acids, it was deduced that the configuration previously assigned the title compounds had to be changed. The structure of (2*S*,4*S*)-4-hydroxyglutamic acid has also been established by use of this method. The L-malic acid liberated was determined by its optical rotation and enzymatically by L-malic acid dehydrogenase.

The present work is a continuation of previous studies on a relatively large group of naturally occurring acidic amino acids which structurally are considered as 3- and/or 4-substituted derivatives of 2-aminopimelic acid, 2-aminoadipic acid and glutamic acid.[1–3] The amino acids in this group have at least two chiral centers, and several diastereoisomers very often co-exist within the same plant.[3–7] Co-occurrence of these diastereoisomers in plant extracts is not a result of the methods used for their isolation.[4,5]

Biogenetic origin of these amino acids in plants has been studied but only little definitive information is available.[8,9] It has been proposed that the reactions are of the aldolase-type in which a 3-carbon unit and a 2-keto acid yield the 2-keto-4-substituted carboxylic acids which finally are transformed into amino acids by transamination.[5–8] Biosynthesis of compounds with both (4*R*)- and (4*S*)-configuration within the same organism is known when aldolase-type reactions are involved.[10] The possibility of both *re*- and *si*-face attacks to a ketimine intermediate is thus a likely explanation of the co-occurrence of diastereoisomeric acidic amino acids within the same plant. Furthermore, it has been shown for all naturally occurring amino acids in this group, where experimental evidences are available, that the configuration at C-2 is (2*S*).[3]

The absolute configuration is established for some of the known 3- and/or 4-substituted acidic amino acids but not for all of them.[3,6,11] Methods widely used for establishing absolute configurations of compounds in this series are based on X-ray crystal structure analysis,[12,13] enzymic methods, NMR-spectroscopy,[3] determination of optical rotation, ORD and CD,[14,15] as well as synthesis of the compounds.[3,16] These methods are not easy to use for all 3- and/or 4-substituted acidic amino acids owing to easy transformation of the compounds into lactones and/or lactams [3] and difficult preparation of crystals suitable for X-ray analysis. Therefore, other methods for structure determination of these amino acids are of interest. Furthermore, recent studies on synthesis and properties of several 3- and/or 4-hydroxylated and alkylated acidic amino acids have revealed that differences in properties of these diastereoisomers are predictable from considerations of their configuration.[3] An exception is the diastereoisomeric 4-hydroxy-4-methylglutamic acids but the configuration assigned to these compounds has previously been questioned.[13]

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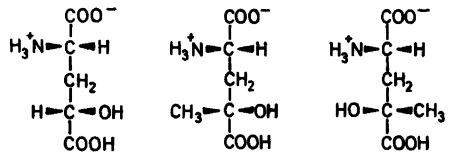

I II III

Fig. 1. Structure of the amino acids isolated from *Phlox decussata* [(2*S*,4*S*)-4-hydroxyglutamic acid, I] and from *Reseda luteola* [(2*S*,4*S*)-4-hydroxy-4-methylglutamic acid, II and (2*S*,4*R*)-4-hydroxy-4-methylglutamic acid, III].

This paper describes a simple method for the structure analysis of 4-substituted acidic amino acids based on oxidative deamination and decarboxylation in alkaline calcium hypochlorite solution. This reaction type in which carbon atom No. 2 in the amino acids is transformed into a carboxylic acid group has been used in the study of other types of compounds.[14,17–20] The carboxylic acids produced in the reaction from different acidic amino acids are easy to identify and the lactone and lactam problems are eliminated. The method is especially sensitive when used to (2*S*,4*S*)-4-hydroxyglutamic acid as the liberated L-malic acid is easy to determine quantitatively by use of malic acid dehydrogenase (EC 1.1.1.37). The method used to the natural occurring diastereoisomeric 4-hydroxy-4-methylglutamic acids appeared to be useful for determination of their absolute configuration.

RESULTS AND DISCUSSION

Methods for isolation and separation of diastereoisomeric 4-substituted glutamic acid derivatives have been described elsewhere.[3] (2*S*,4*S*)-4-Hydroxyglutamic acid was isolated from *Phlox decussata* L.,[7] the two diastereoisomeric (2*S*)-4-hydroxy-4-methylglutamic acids were isolated from *Reseda luteola* L. and their (2*S*)-configuration was determined by use of L-amino acid oxidase.[5] Structures of the *Phlox* amino acid with well-established configuration at both C-2 and C-4 [15] and the *R. luteola* amino acids are shown in Fig. 1.

The amino acids I–III and glutamic acid have been treated with an alkaline solution of calcium hypochlorite. Under the reaction conditions used (see Experimental) the oxidative decarboxylation

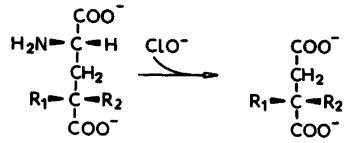

Fig. 2. Oxidation of amino acids in alkaline hypochlorite solution.

	R_1	R_2
Amino acids		
Glutamic acid	H	H
I	H	OH
II	CH_3	OH
III	OH	CH_3
Carboxylic acids		
Succinic acid	H	H
L-Malic acid	H	OH
(*S*)(+)-Citramalic acid	CH_3	OH
(*R*)(−)-Citramalic acid	OH	CH_3

and deamination proceeded in high yield (Fig. 2) and with retention of the configuration at C-4 in the amino acids – *i.e.* C-2 in the carboxylic acids – as shown by transformation of I into (2*S*)- or L-malate. The reactions have also been performed with chloramine-T as described elsewhere,[14] but the reaction modified with respect to use of calcium hypochlorite instead of chloramine-T was preferred owing to an easier isolation procedure for the carboxylic acids.

The carboxylic acids were isolated from the reaction mixtures in a procedure including ion-exchange chromatography (see Experimental). Identity and purity of the isolated carboxylic acids were confirmed by comparison of HVE, PC, TLC and ^{1}H NMR properties of the compounds (Table 1) with corresponding results obtained with authentic reference compounds. Also GLC of trimethylsilyl derivatives of the carboxylic acids were used and the optical rotations were determined for L-malic acid, (*S*)(+)- and (*R*)(−)-citramalic acids. L-Malic acid produced from I was furthermore determined by use of malate dehydrogenase (EC 1.1.37) in a coupled reaction with glutamate-oxaloacetate transaminase (EC 2.6.1.1.) as described for the commercially available test-combination kit.

The results obtained with the two diastereoisomeric (2*S*)-4-hydroxy-4-methylgluta-

Table 1. R_f-values from PC and TLC and ionic mobilities by HVE of some carboxylic acids together with R_t-values by GLC of their per-trimethylsilyl derivatives.

Compound	GLC R_t (min)	R_f on PC in solvent system [a]			HVE mobility (cm) in buffer system [a]		R_f on TLC in solvent [a]	
		(1)	(2)	(3)	pH 3.6	pH 6.5	(4)	(5)
Malonic acid	12.7	0.55	0.18	0.14	34.7	38.7	0.08	0.08
Succinic acid	15.6	0.73	0.24	0.16	7.2	35.8	0.34	0.67
Glutaric acid	17.8	0.72	0.32	0.18	5.5	33.1	0.55	0.75
Malic acid	19.2	0.38	0.12	0.08	19.4	34.4	0.02	0.09
Citramalic acid	18.5	0.42	0.17	0.06	18.2	24.5	0.04	0.14
Aspartic acid [b]	–	0.22	0.16	0.12	9.6	22.5	–	–
Glutamic acid [b]	–	0.28	0.26	0.15	2.6	20.0	–	–

[a] Experimental conditions, see Experimental, the HVE buffer systems were: pH 3.6 (pyridine–HOAc–H_2O) (1:10:200); pH 6.5 (pyridine–HOAc–H_2O) (25:1:500). TLC was performed on Si-gel plates, chromatographic systems were: (1) n-BuOH–HOAc–H_2O (12:3:5); (2) PhOH–H_2O–13 M NH_4OH (120:30:1) (w/v/v): (3) iso-PrOH–H_2O–13 M NH_4OH (8:1:1): (4) $CHCl_3$-*tert*-amyl alcohol–HCOOH–H_2O (136:24:27:83): (5) C_6H_6–HOAc–H_2O (125:73:2). [b] For comparison with properties of 3- and 4-substituted acidic amino acids, see Ref. 3.

mic acids showed that the diastereoisomer with highest pK_{a_2}-value was transformed into (*R*)(−)-citramalic acid (Fig. 2) and (*S*)(+)-citramalic acid was produced from the amino acid with the lowest pK_{a_2}-value. The absolute configuration for the enantiomeric citramalic acids is known,[21–24] and with the established (2*S*)-configuration for both of the diastereoisomeric 4-hydroxy-4-methylglutamic acids, it is deduced that the amino acid III (Fig. 1) has higher pK_{a_2}-value than the isomer II. The absolute configuration now determined for the two chiral atoms in II and III implicates that the previously assigned configuration for carbon atom No. 4 (Ref. 13 and references cited therein) has to be changed. Thereby, the discrepancy no longer exists between the structure and properties of II and III, and there is now full agreement between the stereochemistry of the hitherto examined 3- and/or 4-substituted acidic amino acids and their chemical and spectroscopical properties.[3] CD and optical rotary dispersion curves of several 2-substituted succinic acids previously described [25–27] may also be of value in combination with the method now described for determination of the stereochemistry of 4-substituted glutamic acid derivatives.

EXPERIMENTAL

General methods ^1H NMR spectroscopy, GLC, PC, TLC and HVE were performed as previously described.[5,28,29]

The sodium salts of (*R*)(−)-citramalic acid and (*S*)(+)-citramalic acid as well as L- and D-malic acids were purchased from Sigma Chemical Company.

Isolation and identification of the amino acids I–III are described elsewhere,[3,5,7] as is the use of L-amino acid oxidase [4] to establish their (2*S*)-configuration.

Hypochlorite oxidation of the amino acids. The amino acids (1.35 m mol) were dissolved in 1.5 ml 2 M NaOH and mixed with a solution of $Ca(OCl)_2$ (71.5 mg, 0.5 m mol) in H_2O (1.5 ml). After 2 h at 60 °C the reaction mixture was acidified with HCl (3.5 ml, 1 M) and 10 ml of an H_2O solution containing Br_2 (0.8 ml Br_2 in 50 ml H_2O) were added. This mixture was left at room temperature for 48 h after which excess of Br_2 was destroyed with $Na_2S_2O_3$ (0.1 M in H_2O, *ca.* 8 ml). After concentration almost to dryness, the residue was treated three times with ethyl acetate (5 ml each time) and the extracts were concentrated to dryness. The residue was purified on a strongly acidic ion-exchange resin (Dowex 50 W×8, 200–400 mesh, H^+, 0.7×10 cm). The column was washed with H_2O (30 ml) and the effluent was concentrated to dryness yielding the carboxylic acids: succinic acid from glutamic acid (52 %); (*S*)(+)-citramalic acid from the most acidic of the diastereoisomeric (2*S*)-4-hydroxy-4-

methylglutamic acids from *R. luteola* (48 %); (*R*)(−)-citramalic acid from the *R. luteola* amino acid (2*S*)-4-hydroxy-4-methylglutamic acid with highest pK_{a_2}-value (43 %). Identity of the compounds was established by PC, TLC, and HVE as well as by GLC of the per-trimethylsilylated carboxylic acids [28] (Table 1). ^{1}H NMR spectra of the isolated carboxylic acids were identical with those obtained from authentic carboxylic acids.[23] Optical rotation was determined for: (L)(−)-malic acid, $[\alpha]_D^{23}$ −19.0°, $[\alpha]_{578}^{23}$ −20.8°, $[\alpha]_{546}^{23}$ −21.9°, $[\alpha]_{436}^{23}$ −20.2°, (c 0.35, 0.1 M NaOH); $[\alpha]_D^{23}$ −4.2°, $[\alpha]_{578}^{23}$ −4.6°, $[\alpha]_{546}^{23}$ −5.8°, $[\alpha]_{436}^{23}$ −5.0° (c 0.28, 1 M HCl); (*R*)(−)-citramalic acid, $[\alpha]_D^{23}$ −27.7°, $[\alpha]_{578}^{23}$ −31.0°, $[\alpha]_{546}^{23}$ −34.6°, $[\alpha]_{436}^{23}$ −56.4° (c 0.31, 0.1 M NaOH); $[\alpha]_D^{23}$ −2.6°, $[\alpha]_{578}^{23}$ −9.8°, $[\alpha]_{546}^{23}$ −12.7°, $[\alpha]_{436}^{23}$ −19.2° (c 0.23, 1 M HCl); (*S*)(+)-citramalic acid, $[\alpha]_D^{23}$ +25.5°, $[\alpha]_{578}^{23}$ +27.8°, $[\alpha]_{546}^{23}$ +28.7°, $[\alpha]_{436}^{23}$ +52.0°(c 0.31, 0.1 M NaOH; $[\alpha]_D^{23}$ +10.5°, $[\alpha]_{578}^{23}$ +11.5°, $[\alpha]_{546}^{23}$ +12.4°, $[\alpha]_{436}^{23}$ +16.0° (c 0.23, 1 M HCl). Lit. value [22] $[\alpha]_D^{25}$ +23.6° (c 3.0, H_2O).

Malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) was used in conjunction with glutamate-oxaloacetate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1.) for both qualitative and quantitative determination of L-malate in an assay as described in the information sheet from Boehringer Mannheim GMBH.

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Enantioselective Liquid Chromatographic Retention of a Series of Sulfoxides and *N*-Substituted Sulfoximines on Chiral Stationary Phases

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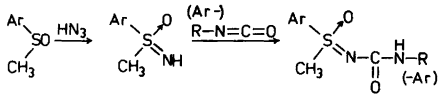
With the use of stationary phases comprised of (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine bound to aminopropyl silica, either covalently *via* an amide bond (CSP 1) or ionically (CSP 2), the liquid chromatographic behaviour of a series of methoxycarbonyl substituted sulfoxides as well as *N*-aryl- and -alkylcarbamyl *S*-methyl *S*-phenyl sulfoximines was studied. Generally, the sulfoximine derivatives were better resolved on columns containing CSP 1, whereas the CSP 2 columns were most suitable for the sulfoxides. The substituted sulfoximines, with one exception, were all shown to be resolvable, giving separation factors $\alpha = 1.10-1.13$; the *R* enantiomer being the first eluted. This contrasts with the behaviour of the parent compound, methyl phenyl sulfoxide as well as other alkyl phenyl sulfoxides where on CSP 2, with no known exception, the *S*-form always is the first eluted enantiomer.

Chiral stationary phases based on (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine have been shown to be highly efficient for the direct liquid chromatographic separation of enantiomers of a wide variety of compounds.[1–5] In this paper the resolution of a type of compounds not earlier investigated, *N*-substituted sulfoximines, is described together with a series of sulfoxides with a methyl or diphenylmethyl ester group.



Scheme 1.

RESULTS AND DISCUSSION

The sulfoximines were synthesized according to the route given below. A series of carboxylic substituted sulfoxides served as precursors for the esters which were obtained *via* reactions with diazomethane or diphenyldiazomethane. The compounds investigated, the types of stationary and mobile phases used and a summary of retention data are shown in Table 1.

A quantitative measure of the degree of chiral recognition exerted by the stationary phase is given by the enantiomeric separation factor, α, which is defined as the ratio of the two capacity factors,[6] k', obtained for the last and first eluted enantiomer, respectively.

The structures of the chiral stationary phases are given in Fig. 1.

It is evident that the methyl aryl sulfoxides investigated, all conform within the chiral recognition model proposed earlier,[5] the *R* enantiomer being the one last eluted (Fig. 2). The relatively high α-value obtained for the non-aromatic methyl 2-ethylsulfinylcyclopentene-1-carboxylate, however, is noteworthy in view of its reduced ability to act as a π-donor. Unfortunate-

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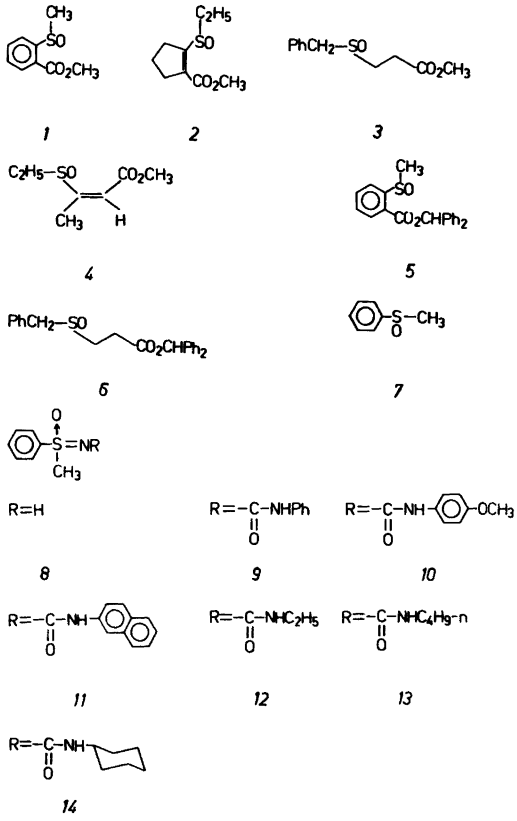
Table 1. Chemical structures and chromatographic retention data of the various compounds investigated.

No.	M.p. °C	*k'* s	*α*	First enantiomer eluted
A. Sulfoxides. Column: CSP 2, mobile phase: 5 % 2-propanol in hexane.				
1	65	15.8, 17.3	1.10	*S*-(−)
2	[a]	8.9, 9.7	1.09	(+)
3	72–75	18.3	(1.0)	–
4	[a]	6.4	(1.0)	–
5	127–9	21.2, 21.9	1.03	*S*-(−)
6	116–9	[b]	(1.0)	–
7	[a]	4.7, 4.9 [c]	1.05	*S*-(−)
B. Sulfoximines. Column: CSP 1, mobile phase: 20 % 2-propanol in hexane.				
8	[a]	5.38	(1.0)	–
9	125–8	12.38, 13.97	1.13	R
10	[b]	24.25, 27.25	1.12	R
11	[b]	27.38	(1.0)	–
12	[b]	8.25, 9.03	1.10	R
13	[b]	4.88, 5.50	1.13	R
14	[b]	4.50, 5.03	1.11	R

[a] Liquid at room temperature. [b] Not determined. [c] 10 % 2-propanol in hexane.



ly, the absolute configurations of the antipodes are not yet known for this compound, so whether the elution order with respect to the configuration at sulfur is the same or not cannot be deduced.

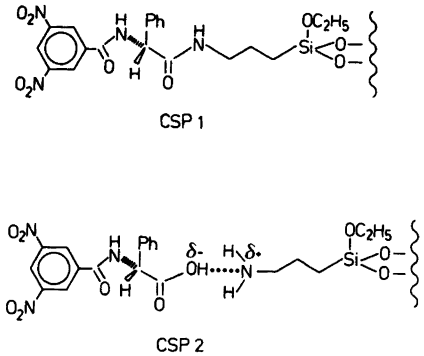


Fig. 1. Structural representation of the chiral stationary phases used; (a) CSP 1, (b) CSP 2.

In the case of the substituted sulfoximines, all compounds that resolved showed the same elution order, *R* before *S*, despite the very large variation in the *k'*-value with the nature of the *N*-substituent. Considering the rules of the Cahn-Ingold-Prelog nomenclature system, however, these results mean that the elution order is reversed when the lone-pair in methyl phenyl sulfoxide is replaced by the RN-group. This, in turn, implies that the sulfoximines adopt another orientation with respect to the stationary phase than the sulfoxides. The limited data obtained so far, however, preclude any suggestion of a chiral recognition model for this case. Fig. 3 shows a chromatogram of one of the compounds in the series of substituted sulfoximines corresponding to an *α*-value of 1.13.

EXPERIMENTAL

Chromatography. The liquid chromatography experiments were performed isocratically with

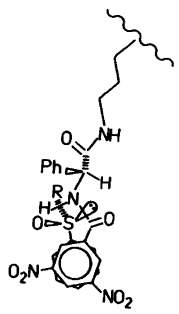


Fig. 2. Chiral recognition model showing the preferred orientation between CSP 1 and the most retained enantiomer (*R*) of an alkyl aryl sulfoxide.

the use of an Altex mod. 100 or 110 A solvent pump, a Beckman 210 injector equipped with a 20-μl loop, and an Altex mod. 152 dual wavelength (254 and 280 nm) or Beckman mod. 165 variable wavelength detector. The analytical columns used (4.6×250 mm) were packed with γ-aminopropylsilica (5 μm) to which (*R*)-*N*-(3,5-dinitrobenzoyl)-phenylglycine is anchored covalently (CSP 1) or ionically (CSP 2). The columns are commercially available from Regis Chem. Co. (CSP 1) and Baker Chem. Co. (CSP 2), respectively. Hexane containing 5–20 % of 2-propanol was used as the mobile phase for all chromatographic runs.

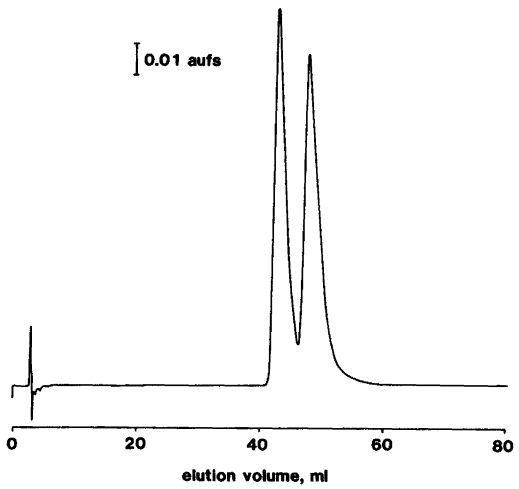


Fig. 3. Illustration of the enantiomeric separation obtained on chromatography of (±)-*N*-phenylcarbamyl *S*-methyl *S*-phenyl sulfoximine (*9*). Conditions: CSP 1-column, hexane −20 % 2-propanol, 2.0 ml/min.

Elution orders were determined by chromatography of samples enriched in one enantiomer of known absolute configuration or sign of optical rotation or by the use of a polarimetric detector. In the latter case the eluate was passed *via* a quartz cell of 200 mm pathlength and the optical rotation registered by means of a Rudolph Auto Pol III polarimeter interfaced with the potentiometric recorder. All compounds were injected as solutions in methylene chloride.

Esterification of the carboxylic substituted sulfoxides. A. Methyl esters. Diazomethane was generated from *N*-nitroso-*N*-methyl-*p*-toluenesulfonamide and distilled.[7] The sulfoxide was dissolved in a small amount of methanol, the solution cooled in ice-water and the diazomethane added until a persistent yellow colour remained. Evaporation of the ether and methanol yielded a product which had the expected structure according to ^{1}H NMR. ^{1}H NMR ($CDCl_3$) of compound Nos. (Table 1): *1*: δ 8.28 (1H,d*, *J* 7.1 Hz), 8.06 (1H,d*, *J* 7.1 Hz), 7.83 (1H,t*, *J* 7.1 Hz), 7.56 (1H,t*, *J* 7.1 Hz), 3.95 (3H,s), 2.85 (3H,s); *2*: δ 3.77 (3H,s), 2.96 (2H,q, *J* 7.5 Hz), 3.1–2.6 (4H,m), 2.08 (2H,m), 1.39 (3H,t, *J* 7.5 Hz); *3*: δ 7.34 (5H,bs), 4.01 (2H,q(AB), *J* −12.4 Hz), 3.70 (3H,s), 2.82(4H,m); *4*: δ 6.22 (1H,m), 3.74 (3H,s), 2.94 (2H,q, *J* 7.5 Hz), 2.21 (3H,m), 1.41 (3H,t, *J* 7.5 Hz). The asterisk denotes further splitting due to m-coupling.

B. Diphenylmethyl esters. Diphenyldiazomethane was prepared from benzophenone hydrazone and mercuric oxide.[8] The red crystals obtained after evaporation of the solvent (light petroleum) were dissolved in ethyl ether. The sulfoxide was dissolved in a small amount of dioxane (purified by filtration through a short column of active alumina). To this solution the diphenyldiazomethane was added in portions and the reaction mixture kept on a water-bath at 70 °C until the red colour obtained after each addition no longer disappeared. Evaporation of the solvent yielded a product which was digerated with ether which in some cases induced crystallization of the product. ^{1}H NMR ($CDCl_3$) of compound Nos. (Table 1): *5*: δ 8.31 (1H,d*, *J* 7.1 Hz), 8.26 (1H,d*, *J* 7.1 Hz), 7.83 (1H,t*, *J* 7.1 Hz), 7.58 (1H,t*, *J* 7.1 Hz), 7.1–7.5 (10H,m), 7.10 (1H,s), 2.72 (3H,s); *6*: δ 7.1–7.5 (15H,m), 6.86(1H,s), 3.96(2H,q(AB), *J* −12.4 Hz), 2.88 (4H,m). The asterisk denotes further splitting due to m-coupling.

Preparation of the N-*substituted sulfoximines.* A. Derivatives of *S*-methyl *S*-phenyl sulfoximine. The unsubstituted sulfoximine was prepared from methyl phenyl sulfoxide and hydrozoic acid according to Johnson *et al.*[9] *N*-Alkyl- or

-arylcarbamyl sulfoximines were obtained *via* reactions with the corresponding alkyl or aryl isocyanate according to the following general procedure: To the sulfoximine (500 μmol), dissolved in ether in a 5 ml screw-cap glass vial containing a small magnetic stir bar, was added an equimolar amount of the isocyanate. The vial was capped and stirred for 0.25–2 h at room temperature or at *ca.* 40 °C, depending upon the isocyanate used. With the aryl isocyanates an immediate reaction occurred with concomitant precipitation of the *N*-arylcarbamyl derivative. After completion of the reaction and cooling, 4 % aqueous sulfuric acid (1 ml) was added to the vial and stirring continued for 1 min. The lower phase (containing extracted unreacted sulfoximine) was removed with a Pasteur-pipette, another ml of acid added and the procedure repeated. The remaining ether solution was dried with a small amount of magnesium sulfate, filtered and evaporated in an air stream. The purity of the product was readily ascertained by HPLC. In the cases where a crystalline material was obtained, this was characterized by its m.p. and ^{1}H NMR spectrum. Compound Nos. (Table 1): *9*: M.p. 125–128 °C (lit.[9] m.p. 129–130 °C). *10–14*: These compounds were only characterized by their retention properties on HPLC. As derivatives of the parent sulfoximine *8*, their structural identities, however, were evident from the resolution pattern obtained from HPLC of these derivatives obtained by synthesis from optically enriched *8*.

Preparative resolutions. The optically active forms of 2-methylsulfinylbenzoic acid were obtained by resolution of the racemic compound with brucine in ethanol as described elsewhere.[10] Esterification of the enantiomers of the acid caused no racemization of the material.

Racemic *S*-methyl *S*-phenyl sulfoximine was resolved partially with (+)-10-camphorsulfonic acid in acetone according to Johnson *et al.*[11]

NMR-spectra. The ^{1}H NMR-spectra were recorded with a JEOL mod. FX-100 Fourier transform NMR-spectrometer.

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Synthesis of Oligosaccharides That Form Parts of the Complex Type of Carbohydrate Moieties of Glycoproteins. Three Glycosides Prepared for Conjugation to Proteins

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Condensation of 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide with *p*-nitrophenyl 3-*O*-benzoyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside, *p*-nitrophenyl 3,6-di-*O*-benzyl-α-D-mannopyranoside and *p*-nitrophenyl 3,4-di-*O*-benzyl-α-D-mannopyranoside gave protected tri- and pentasaccharides. The oligosaccharide glycosides *1*, *2* and *3* were obtained after de-protection of these condensation products. These oligosaccharides will, after suitable conversions, be conjugated to proteins for biological studies.

The complex (*N*-acetyl-D-lactosaminic) type of carbohydrate portions which occur in a multitude of glycoproteins have been suggested to be involved in several biologically important phenomena.[1] Syntheses of different oligosaccharides derived from these carbohydrate portions have been reported.[2–14] In order to study certain biological properties of these oligosaccharides they should be prepared as glycosides suitable for covalent attachment to proteins. We now report the synthesis of three such glycosides, one (*1*) with three and two (*2* and *3*) with five sugar residues.

RESULTS AND DISCUSSION

For the synthesis of trisaccharide glycoside *1* a *p*-nitrophenyl-α-D-mannoside blocked at O-3, O-4 and O-6 and an *N*-acetyl-D-lactosamine precursor were needed. The syntheses of such precursors, namely *p*-nitrophenyl 3-*O*-benzoyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside [15] (*4*) and 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide [2,3] (*5*) have been reported earlier. Compounds *4* and *5* were condensed using silver trifluoromethanesulfonate(silver triflate)–2,4,6-trimethylpyridine as promoter. These conditions are known to give high yields of β-glycosides.[16,17] After work-up and chromatography on silica gel the trisaccharide derivative *6* was obtained in 60 % yield. Compound *6* was converted into *7* (66 %) by successive treatment with sodium methoxide in methanol, hydrazine hydrate in boiling ethanol and acetic anhydride–pyridine.[17] Removal of the *O*-benzylidene and *O*-acetyl groups of *7* was accomplished by treatments with aqueous trifluoroacetic acid and sodium methoxide in methanol, respectively. After purification by gel filtration the trisaccharide glycoside *1* was obtained in 76 % yield as an amorphous powder.

For the synthesis of the pentasaccharide glycoside *2* a mannoside blocked at O-3 and O-6 was needed. *p*-Nitrophenyl α-D-mannopyranoside was treated with bis(tributylstannyl)oxide–benzyl bromide–tetrabutylammonium bromide [18,19] to yield, after chromatography, crystalline *p*-nitrophenyl 3,6-di-*O*-benzyl-α-D-mannopyranoside (*8*) (94 %). Glycosyl bromide *5* and *8* were

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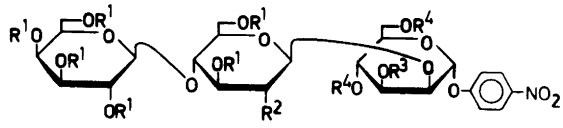


	R^1	R^2	R^3	R^4
6	Ac	NPhth	Bz	*O*-benzylidene
7	Ac	NHAc	Ac	*O*-benzylidene
1	H	NHAc	H	H

condensed as described above and after work-up and chromatography the pentasaccharide derivative *9* was obtained in 41 % yield. Treatment of *9* with hydrazine hydrate followed by acetylation gave *10* (73 %). The nitro group in *10* was reduced by catalytic hydrogenation (Pt catalyst) and the resulting amino group was acylated with trifluoroacetic anhydride to give *11* (71 %). Compound *11* was then de-protected by treatment with sodium methoxide in methanol followed by catalytic hydrogenation (Pd–C catalyst). After gel filtration the pentasaccharide glycoside *2* was obtained in 65 % yield.

For the synthesis of the pentasaccharide glycoside *3* a mannoside blocked at O-3 and O-4 was needed. 2,6-Di-*O*-acetyl-3,4-di-*O*-benzyl-D-mannosyl bromide [14] was condensed with *p*-nitrophenol using silver triflate-2,4,6-trimethylpyridine as promoter. *p*-Nitrophenyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranoside (*12*) was obtained in 37 % yield and this compound was then deacetylated to give *p*-nitrophenyl 3,4-di-*O*-benzyl-α-D-mannopyranoside (*13*) (83 %). Glycosyl bromide *5* and *13* were condensed as described above and the pentasaccharide derivative *14* was obtained in 78 % yield. The 2-deoxy-2-phthalimido groups of *14* were exchanged with 2-acetamido-2-deoxy groups as above to yield crystalline *15* (85 %). Then the *p*-nitrophenyl group *15* was converted into a *p*-*N*-trifluoroacetamidophenyl group as above to give crystalline *16* (51 %) which was finally de-protected to give, after gel filtration, the required pentasaccharide glycoside *3* (65 %).

The structures of *1*, *2* and *3* are evident from their modes of synthesis and from methylation analyses [20] and were also confirmed by ^{1}H and ^{13}C NMR spectroscopy. The spectra of *1*–*3* and their protected derivatives were invariably in agreement with those of similar compounds prepared earlier.

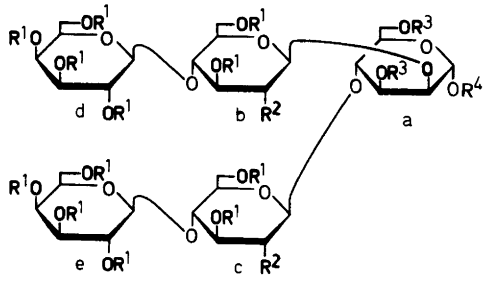
Biological experiments performed with these oligosaccharides will be reported separately.

EXPERIMENTAL

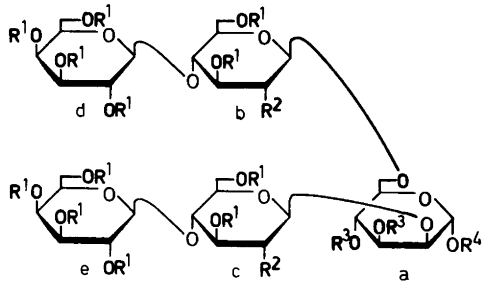
General. Methods and instrumentation for TLC, GLC–MS, NMR and analysis have been described.[3]

Inasmuch as the rational names of compounds *9*–*11* and *14*–*16* are incomprehensible, the reader is referred to the figures for information on the structures of these compounds.

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl-(1→4)*-O-*3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→2)*-O-*3*-O-*benzoyl-4,6-di*-O-*benzylidene-α*-D-*mannopyranoside (6).* A mixture of mannoside *4* [15] (1.3 g), silver triflate (0.79 g), 2,4,6-trimethylpyridine (0.37 g) and molecular sieves (2 g, 3Å) in anhydrous dichloromethane (10 ml) was cooled to −40 °C under nitrogen. Bromide *5* [2,3] (2.4 g) in anhydrous dichloromethane (10 ml) was added



	R^1	R^2	R^3	R^4
9	Ac	NPhth	Bn	*p*-nitrophenyl
10	Ac	NHAc	Bn	*p*-nitrophenyl
11	Ac	NHAc	Bn	*p*-*N*-trifluoroacetamidophenyl
2	H	NHAc	H	*p*-*N*-trifluoroacetamidophenyl



	R^1	R^2	R^3	R^4
14	Ac	NPhth	Bn	*p*-nitrophenyl
15	Ac	NHAc	Bn	*p*-nitrophenyl
16	Ac	NHAc	Bn	*p*-*N*-trifluoroacetamidophenyl
3	H	NHAc	H	*p*-*N*-trifluoroacetamidophenyl

dropwise with stirring. The mixture was allowed to attain room temperature and kept overnight, washed with aqueous hydrogen chloride, water, saturated aqueous sodium hydrogencarbonate and water. After concentration to dryness the product was purified on silica gel with toluene–ethyl acetate (1:1). Compound *6* was obtained as an amorphous power (1.8 g, 60 %), $[\alpha]_D^{21}$ +53° (*c* 1.0, $CHCl_3$); TLC (solvent as above): R_F 0.56; ^{13}C NMR ($CDCl_3$): δ 20.5 (OAc), 54.9 (C'-2), 60.1–78.3 (C-6, C'-6, C''-6, ring C), 95.8, 96.6 (C-1, C'-1), 101.1 (C''-1), 101.8 (*C*HPh), 116.0–160.1 (aromatic C), and 169.4–170.0 (*C*=O).

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl-(1→4)*-O-*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyransyl-(1→2)*-O-*3*-O-*acetyl-4,6-di*-O-*benzylidene*-α-D-*mannopyranoside (7).* A catalytic amount of sodium was added to a solution of compound *6* (910 mg) in anhydrous methanol (50 ml). The mixture was left at room temperature overnight, neutralised with acetic acid and concentrated to dryness. The product was dissolved in 90 % aqueous ethanol (30 ml), hydrazine hydrate (4 ml) was added and the solution was refluxed for 10 h. After cooling the solution was concentrated to dryness, and the product was treated with acetic anhydride–pyridine (1:1, 30 ml) at 100 °C for 30 min. After concentration the product was purified on silica gel with toluene–ethyl acetate (1:4) to yield trisaccharide derivative *7* as an amorphous powder (520 mg, 66 %), $[\alpha]_D^{21}$ +42° (*c* 1.0, $CHCl_3$); TLC (solvent as above): R_F 0.37; ^{13}C NMR ($CDCl_3$); δ 20.6–24.9 (NHAc, OAc), 53.7 (C'-2), 60.9, 62.4, (C'-6, C''-6), 65.2–78.5 (C-6, ring C), 96.7 (C-1), 100.7 (C'-1), 101.0 (C''-1), 102.0 (*C*HPh), 116.3–160.9 (aromatic C), and 169.2, 170.7 (*C*=O).

p-*Nitrophenyl O*-β-D-*galactopyranosyl-(1→4)*-O-*2-acetamido-2-deoxy*-β-D-*glucopyranosyl-(1→2)*-α-D-*mannopyranoside (1).* Compound *7* (325 mg) was treated with 90 % aqueous trifluoroacetic acid (5 ml) for 5 min at room temperature and concentrated to dryness. The product was dissolved in methanol (50 ml) and a catalytic amount of sodium was added. The solution was left at room temperature overnight, neutralised with acetic acid and concentrated to dryness. The product was purified on a Bio-Gel P2 column (5×80 cm) irrigated with water. After freeze-drying trisaccharide *1* was obtained as an amorphous powder (158 mg, 76 %), $[\alpha]_D^{21}$ +38° (*c* 1.0, H_2O); TLC (ethyl acetate–methanol–acetic acid–water, 12:3:3:2): R_F 0.28; ^1H NMR (D_2O, 85 °C): δ 2.03 (3 H, NHAc), 4.45 (d, 1 H, H''-1, $J_{1,2}$ 6.5 Hz), 4.69 (broad d, 1 H, H'-1, $J_{1,2} \simeq 8$ Hz), 5.70 (d, 1 H, H-1, $J_{1,2}$ 1.5 Hz), 7.22–8.28 (4 H, aromatic H); ^{13}C NMR (D_2O): δ 23.5 (NHAc), 56.0 (C'-2), 61.0, 62.1 (C-6, C'-6, C''-6), 69.6–79.6 (ring C), 96.4 (C-1), 100.6 (C'-1), 104.1 (C''-1), 117.6, 127.1, 143.2, 162.1 (aromatic C), and 175.9 (*C*=O). Methylation analysis [20] of *1* gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 3,4,6-tri-*O*-methyl-D-mannose and 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose.

p-*Nitrophenyl 3,6-di*-O-*benzyl*-α-D-*mannopyranoside (8).* *p*-Nitrophenyl α-D-mannopyranoside (3.2 g) and bis(tributylstannyl)oxide (9.0 ml) in toluene (350 ml) was boiled under reflux for 2 h in a Dean-Stark trap. The solution was concentrated to ≃100 ml and benzyl bromide (60 ml) and tetrabutylammonium bromide (1.4 g) were added. The mixture was stirred for 24 h at 80 °C and concentrated to dryness. The product was purified on silica gel with toluene–ethyl acetate (1:1) yielding *8* (4.82 g, 94 %). Crystal-

lisation from ethyl acetate–light petroleum gave prisms, m.p. 112 °C, $[\alpha]_{578}^{22}$ +101° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): 67.0 (C-2), 67.6 (C-4), 69.6 (C-6), 72.1 (C-5), 72.4, 73.5 (CH_2Ph), 79.1 (C-3), 97.9 (C-1), 116.4, 125.7, 127.6, 127.8, 128.0, 128.2, 128.4, 128.6, 137.7, 142.7, 160.8 (aromatic). *Anal.* $C_{26}H_{27}NO_8$: C,H,N.

Pentasaccharide derivative 9. A mixture of mannoside *8* (438 mg), silver triflate (1.18 g), 2,4,6-trimethylpyridine (557 mg) and molecular sieves (4 g, 3Å) in anhydrous dichloromethane (20 ml) was cooled to −40 °C under nitrogen. Bromide *5* [2,3] (3.50 g) in anhydrous dichloromethane (20 ml) was added in two portions after 0 and 1 h reaction time. The mixture was allowed to attain room temperature after the first addition and was then cooled to −25 °C before the final addition. Thereafter the mixture was allowed to attain room temperature (8 h) and worked up as above. The product was purified on silica gel with light petroleum–ethyl acetate (1:2). Compound *9* was obtained as a syrup (712 mg, 41 %), $[\alpha]_{578}^{22}$ +47° (*c* 0.9, $CHCl_3$); TLC (toluene–ethyl acetate, 1:2), R_F 0.55; ^{13}C NMR ($CDCl_3$): 20.4, 20.7 (OAc), 55.2, 55.5 (C-2^b, C-2^c), 61.0–62.4 (4 C, C-6^{b-e}), 66.9–78.6 (C-6^a, ring C, CH_2Ph), 96.4 (C-1^a), 98.0 (2 C, C-1^b, C-1^c), 101.1 (2 C, C-1^d, C-1^e), 116.5, 125.4, 142.6, 161.1 (*p*-nitro-Ph), 123.4–138.6 (aromatic), and 167.8–170.2 (*C*=O).

Pentasaccharide derivative 10. Compound *9* (680 mg) was treated with sodium methoxide in methanol, hydrazine hydrate (2.0 ml) in ethanol (30 ml), and acetic anhydride–pyridine (1:1, 15 ml) as described for compound *7*. The product was purified on silica gel with chloroform–acetone (3:2) yielding *10* as a syrup (457 mg, 73 %), $[\alpha]_{578}^{22}$ +19° (*c* 1.3, $CHCl_3$); TLC (solvent as above): R_F 0.42: ^{13}C NMR ($CDCl_3$): 20.5, 20.8 (OAc), 23.1 (NHAc), 54.3 (2 C, C-2^b, C-2^c), 61.0–62.4 (4 C, C-6^{b-e}), 66.9–78.6 (C-6^a, ring C, CH_2Ph), 96.8 (C-1^a), 99.9 (C-1^b), 100.9, 101.2 (2 C, 1 C, (C-1^b, C-1^d, C-1^e), 116.8, 125.7, 142.8, 161.3 (*p*-nitro-Ph), 127.6–138.6 (aromatic), and 169.1–170.5 (*C*=O).

Pentasaccharide derivative 11. Compound *10* (397 mg) was dissolved in ethyl acetate (10 ml), platinum oxide (80 mg) was added and the mixture was treated with hydrogen at atmospheric pressure. When the hydrogen consumption had ceased the mixture was filtered and concentrated to dryness. The product was dissolved in anhydrous pyridine (5.0 ml), cooled to −20 °C under nitrogen and trifluoroacetic anhydride (0.15 ml) was added. The solution was allowed to attain room temperature and kept overnight. The mixture was distributed between dichloromethane and water and the organic phase was washed with water, dilute sulfuric acid, water, saturated aqueous sodium hydrogencarbonate and water. The product was purified on silica gel with chloroform–acetone (3:2) yielding *11* as a syrup (291 mg, 71 %), $[\alpha]_{578}^{22}$ +17° (*c* 1.0, CH_2Cl_2); TLC (solvent as above): R_F 0.46; ^{13}C NMR ($CDCl_3$): 20.6, 20.8 (OAc), 23.1 (2 C, NHAc), 54.3 (2 C, C-1^b, C-1^c), 61.0–62.5 (4 C, C-6^{b-e}), 67.0–78.5 (C-6^a, ring C, CH_2Ph), 96.8 (C-1^a), 99.9 (C-1^b), 101.0, 101.3 (2 C, 1 C, C-1^c, C-1^d, C-1^e), 117.4, 122.5, 130.8, 154.2 (*p*-trifluoroacetamido Ph), 127.6–138.7 (aromatic), and 169.3–170.8 (*C*=O).

p-*Trifluoroacetamidophenyl 2,4-di*-O-[β-D-*galactopyranosyl-(1→4)*-O-*2-acetamido-2-deoxy-β*-D-*glucopyranosyl-α*-D-*mannopyranoside (2)*. Sodium (10 mg) was added to a solution of *11* (260 mg) in anhydrous methanol (20 ml). The mixture was kept at room temperature for 3 h, neutralised with acetic acid and concentrated to dryness. The product was dissolved in 90 % aqueous acetic acid (20 ml) and hydrogenated at 400 kPa over 10 % palladium–charcoal (500 mg). After filtration and concentration the product was purified by gel-filtration on a Sephadex G-15 column (2.5×90 cm) irrigated with water. After freeze-drying *2* was obtained as an amorphous powder (103 mg, 65 %), $[\alpha]_{578}^{22}$ +10° (*c* 0.4, H_2O); TLC (ethyl acetate–methanol–acetic acid–water, 4:3:3:2): R_F 0.79; ^1H NMR (D_2O, 85 °C): 2.00 (s, 2×3 H, NHAc), 4.47 (d, 2 H, $J_{1,2}$ 6.4 Hz, H-1^d, H-1^e), 4.64 (m, 2 H, H-1^b, H-1^c), 5.50 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1^a), and 7.13–7.53 (4 H, aromatic); ^{13}C NMR (D_2O): 23.3, 23.6 (2 C, NHAc), 56.3 (2 C, C-2^b, C-2^c), 61.3–62.2 (5 C, C-6^{a-e}), 72.2–79.7 (ring C), 96.6 (C-1^a), 101.0 (C-1^b), 102.7 (C-1^c), 104.1 (2 C, C-1^d, C-1^e), 111.4, 122.8 ($COCF_3$), 118.7, 125.1, 130.8, 155.0 (aromatic), 157.3, 158.8 ($COCF_3$), and 175.5, 175.9 (*C*=O). Methylation analysis [20] of *2* gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, and 3,6-di-*O*-methyl-D-mannose.

p-*Nitrophenyl 2,6-di*-O-*acetyl-3,4-di*-O-*benzyl-α*-D-*mannopyranoside (12)*. A mixture of *p*-nitrophenyl (3.23 g), molecular sieves (7 g, 3Å), silver triflate (5.97 g), and 2,4,6-trimethylpyridine (1.31 g) in anhydrous dichloromethane (30 ml) was cooled to −40 °C under nitrogen. A solution of 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-D-mannosyl bromide [14] (7.85 g) in dichloromethane (10 ml) was added with stirring over 45 min. The mixture was allowed to attain room temperature, pyridine (1.5 ml) was added and the mixture was worked up as above. The product was purified on silica gel with toluene–ethyl acetate (6:1). Compound *12* was obtained as a syrup (3.22 g, 37 %), $[\alpha]_{578}^{22}$ +81° (*c* 2.3, $CHCl_3$); TLC (toluene–ethyl

acetate, 4:1): R_F 0.57; ^{13}C NMR ($CDCl_3$): δ 20.6, 20.8 (OAc), 62.7 (C-6), 67.9 (CH_2Ph), 71.1 (C-2),72.0 (CH_2Ph), 73.5 (C-5), 75.2 (C-4), 77.6 (C-3), 96.0 (C-1), 116.4, 125.6, 142.8, 160.3 (*p*-nitro-Ph), 128.0–137.8 (aromatic), and 169.9, 170.3 (*C*=O).

p-*Nitrophenyl 3,4-di-O-benzyl-α-*D-*mannopyranoside (13).* Compound *12* was deacetylated as described for *2* and the product was purified on silica gel with toluene–ethyl acetate (3:1). Compound *13* was obtained as an amorphous powder (2.12 g, 83 %), $[\alpha]_{578}^{22}$ +111° (*c* 1.0, $CHCl_3$); TLC (toluene–ethyl acetate, 1:2): R_F 0.45; ^{13}C NMR ($CDCl_3$): δ 61.3 (C-6), 68.1 (CH_2Ph), 72.5, 73.2, 73.3 (3 C, C-2, C-5, CH_2Ph), 75.3 (C-4), 79.4 (C-3), 97.6 (C-1, $^1J_{C,H}$ 173 Hz),[21] 116.3, 125.8, 142.6, 160.7 (*p*-nitro-Ph), and 127.8–138.1 (aromatic).

Pentasaccharide derivative 14. Compound *14* was prepared from *13* (423 mg) analogously to *6* by using bromide *5* [2,3] (2.43 g), molecular sieves (2 g, 3Å) and silver triflate (1.15 g)–2,4,6-trimethylpyridine (0.54 g). The product was purified on silica gel with light petroleum–chloroform–acetone (2:2:1). Compound *14* was obtained as an amorphous powder (1.30 g, 78 %), $[\alpha]_{578}^{22}$ +58° (*c* 1.0, $CHCl_3$); TLC (toluene–ethyl acetate, 1:2): R_F 0.60; ^{13}C NMR ($CDCl_3$): δ 20.5 (OAc), 55.0 (2 C, C-2^b, C-2^c), 60.8–62.3 (4 C, C-6^{b-e}), 66.9–78.5 (C-6^a, ring C, CH_2Ph), 95.9 (C-1^a), 97.3, 98.3 (2 C, C-1^b, C-1^c), 101.0, 101.1 (C-1^d, C-1^e), 116.6, 125.4, 142.6, 161.1 (*p*-nitro-Ph), 123.4–138.0 (aromatic), and 167.4–170.1 (*C*=O).

Pentasaccharide derivative 15. Compound *14* (365 mg) was treated with sodium methoxide in methanol, hydrazine hydrate (1.6 ml) in ethanol (30 ml), and acetic anhydride–pyridine (1:1, 20 ml) as described for compound *7*. The product was purified on silica gel with toluene–ethyl acetate (1:4) yielding *15* (280 mg, 85 %). Crystallisation from ethanol gave small crystals, m.p. 233–235 °C, $[\alpha]_{578}^{22}$ +14° (*c* 0.7, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 20.5, 20.7 (OAc), 23.4, 24.7 (2 C, NHAc), 54.2, 56.5 (2 C, C-2^b, C-2^c), 61.0–62.7 (4 C, C-6^{b-e}), 67.0–78.6 (C-6^a, ring C, CH_2Ph), 95.5 (C-1^a), 97.8 (C-1^c), 101.0, 101.6, 101.8 (C-1^b, C-1^d, C-1^e), 116.4, 125.8, 142.8, 161.0 (*p*-nitro-Ph), 127.7–139.0 (aromatic), and 169.0–171.6 (*C*=O). *Anal.* $C_{78}H_{98}N_3O_{40}$: C,H,N.

Pentasaccharide derivative 16. Compound *15* (800 mg) was subjected to catalytic hydrogenation over platinum oxide catalyst (200 mg) and treatment with trifluoroacetic anhydride (0.2 ml) in pyridine (5.0 ml) as described for *11*. The product was purified on silica gel with chloroform–acetone (2:1) yielding *16* (420 mg, 51 %). Crystallisation from ethanol–water gave small crystals, m.p. 145–148 °C, $[\alpha]_{578}^{22}$ +0.5° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 20.6 (OAc), 23.3 (2 C, NHAc), 54.0 (2 C, C-2^b, C-2^c), 60.8–62.8 (4 C, C-6^{b-e}), 66.8–78.5 (C-6^a, ring C, CH_2Ph), 95.0 (C-1^a), 98.3, 101.0 (1 C, 3 C, C-1^{b-e}), 116.5, 122.7, 130.3, 153.6 (*p*-trifluoroacetamido Ph), 127.7–138.7 (aromatic), and 169.1–170.8 (*C*=O). Found: C 53.12l H 5.39; N 2.29. Calc. for $C_{80}H_{98}N_3O_{39}F_3$: C 53.90; H 5.50; N 2.36.

p-*Trifluoroacetamidophenyl 2,6-di-*O-[*β-*D-*galactopyranosyl-(1→4)-*O-*2-acetamido-2-deoxy-β-*D-*glucopyranosyl]α-*D-*mannopyranoside (3).* Compound *16* (385 mg) was *O*-deacetylated and debenzylated as described for *2*. The product was desalted on a Sephadex G-15 column (2.5×90 cm) irrigated with water. After freeze-drying, *3* was obtained as an amorphous powder (154 mg, 65 %), $[\alpha]_{578}^{22}$ +2° (*c* 0.8, H_2O); TLC (ethyl acetate–methanol–acetic acid–water, 4:3:3:2): R_F 0.76; ^1H NMR (D_2O, 85 °C): δ 1.92, 2.04 (2 s, 2×3 H, NHAc), 4.40, 4.45 (2 d, 2 H, $J_{1,2}$ 7.2 Hz, H-1^d, H-1^e), 4.50 (d, 1 H, $J_{1,2}$ ~7 Hz, H-1^b), 4.68 (d, 1 H, $J_{1,2}$ ~7 Hz, H-1^c), 5.50 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^a), and 7.11–7.53 (4 H, aromatic); ^{13}C NMR (D_2O): δ 23.4, 23.7 (2 C, NHAc), 56.1 (2 C, C-2^b, C-2^c), 61.3–62.2 (4 C, C-6^{b-e}), 68.7–80.0 (C-6^a, ring C), 97.0 (C-1^a), 100.9 (C-1^c), 102.1 (C-1^b), 104.2 (2 C, C-1^d, C-1^e), 111.4, 122.9 ($COCF_3$), 157.2, 158.7 ($COCF_3$), 118.8, 125.1, 130.9, 155.3 (aromatic), and 175.2, 175.7 (*C*=O). Methylation analysis [20] of *3* gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose and 3,4-di-*O*-methyl-D-mannose.

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Synthesis and NMR Studies of *Z*- and *E*-Isomers of 10-Oxo and 10-Hydroxy Derivatives of Amitriptyline and Nortriptyline

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The *N,N*-dimethyl and *N*-methyl derivatives of (*Z*)- and (*E*)-(10,11-dihydro-10-hydroxy-5*H*-dibenzo[*a,d*]cycloheptene)-$\Delta^{5,\gamma}$-propylamine have been synthesized from the 10-oxo isomers of the *N,N*-dimethyl derivatives with conservation of the geometrical configuration. Structural assignments were based upon chemical shift displacement of the aromatic protons by addition of tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium. The presence of solvent dependent equilibria of intramolecular hydrogen bonded and non-hydrogen bonded conformations of the *Z*-hydroxy isomers were demonstrated by ^{1}H and ^{13}C NMR spectra.

The 10-hydroxy derivatives of the antidepressant drugs amitriptyline and nortriptyline have considerable interest as metabolites of these drugs with a pharmacological activity of their own.[1–4] The synthesis of these compounds and the corresponding 10-oxo compounds, the separation of *Z*- and *E*-isomers, the assignments of the geometrical configuration to the isomers and some NMR data have been reported by Remy *et al.*,[5] and some additional data are given in Refs. 6–8, but the *Z*-10-oxo and the *Z*-10-hydroxy derivatives have not been properly described in these reports. The reason is that their method of synthesis results in mixtures of isomers with a low content of *Z*-isomers.

We describe here another synthetic approach which gives the pure isomers in high yield. The structure of the isomers are assigned by analysis of their ^{1}H and ^{13}C NMR spectra and their IR spectra.

Chemistry. The route we have used for the synthesis is shown in Scheme 1.

Compound *1* is obtained as a 1:1 mixture of isomers and this proportion is conserved in *2* and *3*.[6] Compound *3* is separated into isomeric fractions giving a total yield of 40 % *3E* and 35 % *3Z* isolated as pure isomers. The following steps are performed with the pure isomers under conditions that give very little isomerization. In this way we get two series of compounds from *3* and *7*, each series consisting of compounds with the same configuration. The assignments of structure by spectral analysis are in accordance with this, so that the *E*-isomers are found in one of the series and the *Z*-isomers in the other.

The route used by Remy *et al.*[5] is shown in Scheme 2.

Two of the steps, from *8* to *3* and from *9* to *6* require prolonged heating in strong acid solution and give rearrangement to mixtures of isomers predominantly containing *E*-isomers. The isomers of the oxo compounds are separated and reduced to the hydroxy compounds with $NaBH_4$. The reported melting points and NMR spectra of *E*-isomers are in accordance with our results, while both the physical data and the NMR spectra reported for the *Z*-isomers are inconsistent with our data.

Surprisingly the acid hydrolysis of the enamine *8* to *3* is slow whereas the corresponding hydrolysis of *2* and *3* (Scheme 1) is more rapid. The reason for this is probably that *8* first is converted to the epoxy compound *8a* and that *3* is formed very slowly from this *endo*-bridged compound.

We have isolated *8a* as a dihydrochloride after

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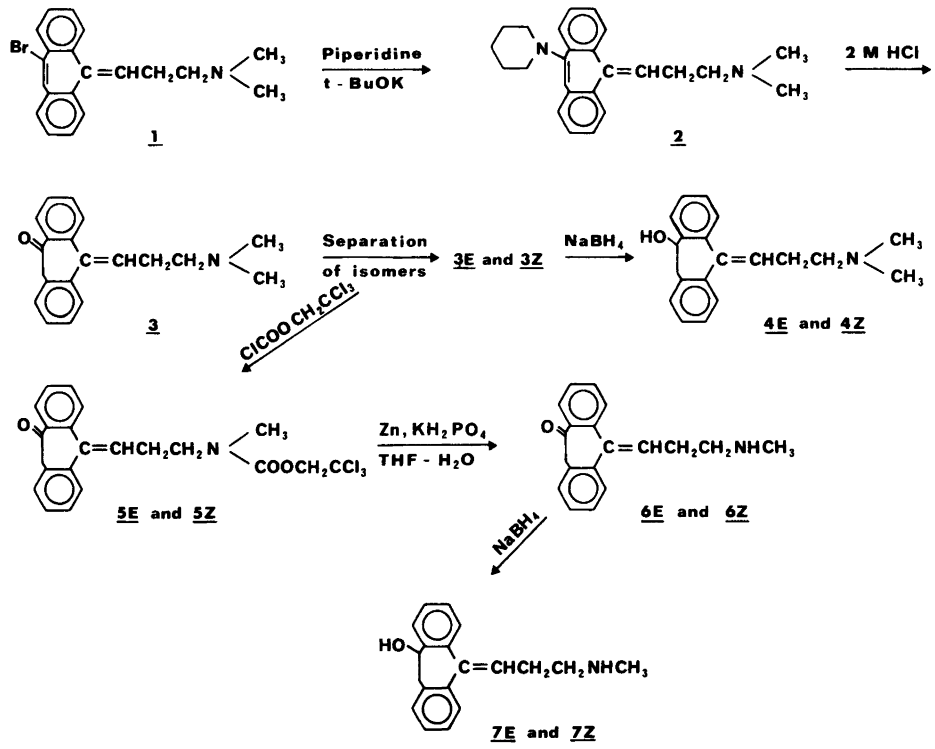
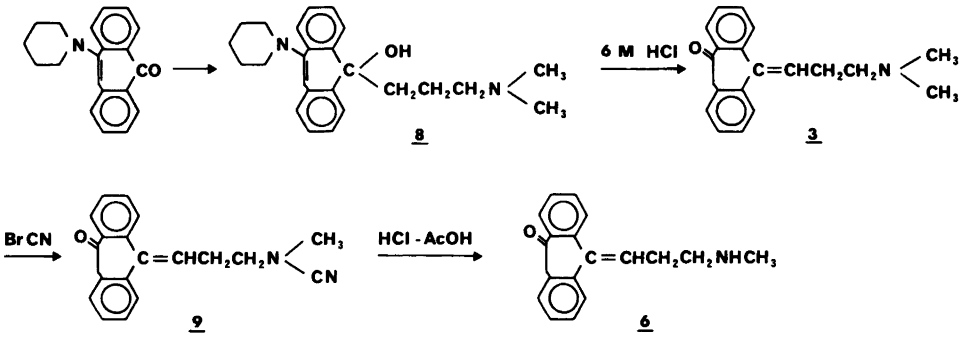


Scheme 1.

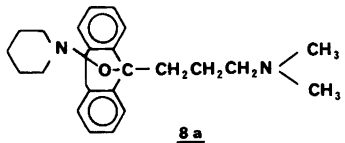
short-term treatment of *8* with acid. The structure was confirmed by the NMR spectrum of the free base.

NMR studies. The use of ^{1}H NMR to the assignment of *E*- or *Z*-structure to tricyclic aromatics containing an amine function in a double bonded side chain, by addition of chemical shift reagents, has been demonstrated in several cases.[5,8,10] It has been found that only the chemical shifts of protons in the aromatic nucleus on the same side of the double bond as the complexing amine function are affected by the shift reagent.

We have found that tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium in relatively low concentration is a very useful reagent for elucidation of the structure of the isomers of the monomethyl keto derivative *6*.



Scheme 2.



8a

The signals from the four protons in the ring out of conjugation to the carbonyl group appear in the aromatic envelope within a rather narrow range (δ ~7.15 ppm, marked with an arrow in Fig. 1). By addition of 40 mg of shift reagent to 50 mg of sample in $CDCl_3$ solution these signals are split and the absorptions are displaced downfield for the *6E* compound, while under the same experimental conditions the corresponding signals from the *6Z* compound remain almost uneffected (Fig. 1). Remy *et al.* reported the fluorinated shift reagent to be unsatisfactory for the assignment as they suggest a strong complexing capability to the keto group.[5]

We did not observe this type of complex formation to any significant extent as the proton ortho to the carbonyl group was only slightly displaced downfield ($\Delta\delta_{ortho}$ ~0.15 ppm), which is similar to the displacement found with the tris(dipivalo-methanato)europium reagent.[5] However, with the dimethylamino compounds *3* the complexing ability of the amine function under the same experimental conditions was found to be much less and complex formation with the keto group was recognized by significant downfield chemical shift displacements of the protons ortho to the keto groups ($\Delta\delta_{ortho}$ (*3E*): 0.69 ppm; $\Delta\delta_{ortho}$ (*3Z*): 0.60 ppm). Unfortunately, Remy *et al.* did not report the effect of chemical shifts reagent upon their claimed *Z*-isomer (*6Z*).[5] However, in our synthesis the *3E* and *3Z* isomers are uniquely related to the *6E* and *6Z* isomers as the intermediate synthetic steps are performed with conservation of the isomer configuration.

The absorption of the vinylic protons from the *E*- and *Z*-isomers are distinctly different in our spectra ($\delta_{CH=}$(*3Z* and *6Z*) ~5.9 ppm (t) J=7 Hz, $\delta_{CH=}$(*3E* and *6E*) ~6.2 ppm (t) J=7 Hz), while Remy *et al.* reported vinylic shifts ~6.2 ppm for both *E* and *Z* compounds.

In a previous patent [7] it was suggested that the hydroxy group and the amine side chain were linked together by an intramolecular hydrogen bond in compound *4Z*, while the corresponding *E*-isomer (*4E*) was observed to be non-hydrogen bonded. In the *E*-isomers (*4E* and *7E*) intramolecular hydrogen bonding was confirmed to be absent as we observed sharp singlet signals from the methyl groups with chemical shifts independent of solvent ($CDCl_3$ or DMSO-d_6) (Table 1).

However, when the spectra of *Z*-isomers (*4Z* and *7Z*) were obtained in $CDCl_3$, two relatively broad peaks appeared from the methyl groups

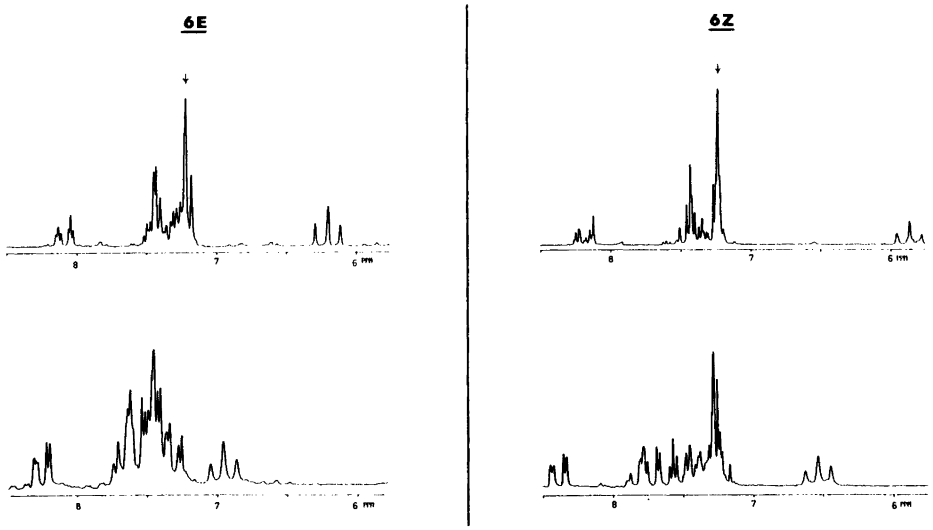


Fig. 1. Aromatic part of ^1H NMR spectra of compounds *6E* and *6Z* before (upper spectra) and after (lower spectra) addition of tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium.

Table 1. ^{1}H NMR chemical shifts of *N*-methyl groups at 30 °C.

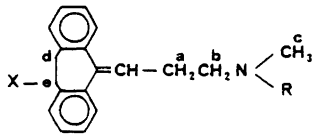
Compound	Solvent	δ (CH_3)/ppm
4E	$CDCl_3$	2.05 (6H)
	DMSO-d_6	2.00 (6H)
7E	$CDCl_3$	2.24 (3H)
	DMSO-d_6	2.18 (3H)
4Z	$CDCl_3$	1.73 (3.8H); 2.08 (2.2H)
	DMSO-d_6	2.03 (6H)
7Z	$CDCl_3$	2.05 (~1.5H); 2.30 (~1.5H) [a]
	DMSO-d_6	2.18 (3H)

[a] At 45 °C these signals collpase to a broad singlet at δ=2.20 ppm.

(Table 1). On heating, these peaks collapse to a broad singlet signal. These findings suggest the *Z*-hydroxy compounds exist in an equilibrium of intramolecular hydrogen bonded and a non-hydrogen bonded conformation. In the hydrogen bonded conformation the amine side chain must be folded with the hydroxy group in a pseudo-axial position, while the distance in the conformation with the hydroxy group in a pseudo-equatorial position obviously is too large for an internal hydrogen bond. In a polar aprotic solvent (DMSO-d_6) the methyl signals appear as sharp singlets (Table 1) indicating that the intramolecular hydrogen bonds are broken and stronger hydrogen bonds established to the solvent. IR spectra recorded in $CHCl_3$ solution also indicate a higher proportion of free OH in *4E* and *7E* than in the corresponding *4Z* and *7Z* isomers as measured by higher peak intensities at ν_{OH} ~3560 cm^{-1}. The OH stretching bond for hydrogen bonded OH groups at ν_{OH} ~3300 cm^{-1} was strong and broad for all four hydroxy compounds.

^{13}C NMR spectra were run of both the ketones *3* and *6* and the hydroxy compounds *4* and *7* to confirm the structural assignments and to study the conformational equilibria of the hydroxy compounds (Table 2). The assignments of saturated carbons were based upon off-resonance

Table 2. ^{13}C NMR spectra [δ (ppm) (intensity)].[a]



Compound	X	R	Temp. (°C)	Solvent	a	b	c	d	e
3E	=O	CH_3	45	$CDCl_3$	27.8 (1.2)	59.0 (1.0)	45.0 (2.3)	50.2 (1.1)	193.6 (0.4)
6E	=O	H	45	$CDCl_3$	29.6 (0.9)	51.2 (1.0)	35.7 (0.7)	50.3 (1.0)	193.7 (0.2)
3Z	=O	CH_3	45	$CDCl_3$	27.9 (1.0)	59.2 (1.0)	45.1 (2.2)	50.4 (1.0)	194.2 (0.2)
6Z	=O	H	45	$CDCl_3$	29.8 (1.0)	51.5 (1.0)	35.9 (0.8)	50.5 (0.9)	194.1 (0.3)
4E	−OH	CH_3	45	$CDCl_3$	27.6 (1.0)	59.2 (1.0)	44.9 (2.2)	40.0 (0.09) 42.0 (0.04)	68.5 (0.08) 72.0 (0.04)
			58		27.6 (1.0)	59.2 (1.0)	44.9 (2.5)	41.0 (0.23)	70.5 (0.17)
7E	−OH	H	45	$CDCl_3$	29.3 (1.0)	51.1 (1.0)	35.4 (1.1)	40.0 (0.14) 42.0 (0.05)	68.5 (0.15) 72.0 (0.05)
4Z	−OH	CH_3	45	$CDCl_3$	26.9 (0.1)	59.3 (1.0)	44.9 (1.2)	41.9 (0.2)	68.0 (0.06) 73.0 (0.06)
			58		26.9 (0.3)	59.3 (1.0)	44.9 (1.7)	41.9 (0.5)	70.0 (0.05)
			45	DMSO-d_6	27.2 (0.9)	58.7 (1.0)	44.7 (2.5)	42.7 (0.3)	67.5 (0.2)
7Z	−OH	H	45	$CDCl_3$	29.1 (0.1)	51.4 (1.0)	35.7 (0.3)	42.0 (0.4)	68.0 (0.11) 75.1 (0.07)

[a] Measured as relative peak heights (peak b chosen as unity).

decoupling and selective ^{1}H-heterodecoupling experiments. Both *E*- and *Z*-isomers of the hydroxy compounds clearly exist in two conformations in $CDCl_3$ solution at 45 °C as the C_e carbon and, to some extent, the C_d carbon atoms are split into two broad (low intensity) signals (Table 2). These signals gradually collapse to a single peak of higher intensity as a function of increasing temperatures. This conformational equilibrium is probably due to ring inversion with a relatively high energy barrier. In the *Z*-isomers (*4Z* and *7Z*) the intramolecular hydrogen bonding results in a broadening of the peaks from the carbon atoms in the side chain, especially peaks from C_a and C_c carbon atoms. By heating the samples further the lines sharpen. In DMSO-d_6 the intramolecular hydrogen bonds are broken and sharp peaks of approximately unit intensity are obtained.

EXPERIMENTAL

Melting points were determined on a Büchi SMP-20 and are corrected. IR spectra were recorded in $CHCl_3$ solution (10 % W/V) on a Perkin Elmer 377 instrument. ^{1}H NMR spectra were recorded at 80 MHz and ^{13}C NMR spectra were recorded at 20 MHz on a Bruker WP 80 DS spectrometer. TMS was used as internal reference standard. The samples were dissolved in $CDCl_3$ or DMSO-d_6 (10 % W/V in 5 mm tubes for ^{1}H NMR spectra and 20 % W/V in 10 mm tubes for ^{13}C NMR spectra).

N,N-*Dimethyl-3-(10,11-dihydro-10-oxo-5*H*-dibenzo[*a,d*]cycloheptene)-*$\Delta^{5,\gamma}$*-propylamine (3).* 10-Bromo-5-(3-dimethylaminopropyl)-5*H*-dibenzo[*a,d*]cycloheptene-5-ol [11] (447 g, 1.2 mol) is dehydrated by refluxing for 1.5 h with a mixture of 2.4 l AcOH and 1.2 l conc. HCl, followed by distillation of 1.5 l of the mixture. The dehydrated base (*1*) is isolated and dried by evaporating with toluene. It is dissolved in a mixture of 1.5 l dry toluene and 0.5 l dry piperidine, and to the mixture is added *t*-BuOK (225 g, 2 mol) during 10 min (cooling, temp. <65 °C). The mixture is stirred for 2 h at 85 °C, cooled and washed with water. The toluene phase containing *2* is extracted with 3 M HCl (2 l), and the acid solution is heated for 2 h at 80 °C. The isomer mixture of *3* is converted to the free base, dissolved in light petroleum (0.7 l) and cooled. One of the isomers of *3* crystallizes from the solution and the other is obtained from the mother liquor after evaporation and crystallization as hydrochloride from EtOH. The mother liquor from the hydrochloride is converted to the free base, and the crystallization procedure is repeated giving a further crop of the two isomers. 140 g (40 %) of the crystalline base m.p. 65–67 °C is obtained. This is sufficiently pure for the following steps. Recrystallization raises the m.p. to 75–76 °C (lit.[5] 71–73 °C). Anal. ($C_{20}H_{21}NO$) C,H,N. The *E*-configuration is assigned to this isomer on the basis of its conversion without isomerization to *4E, 6E* and *7E*.

^{1}H NMR ($CDCl_3$): δ 2.11 (s, 6H), 2.0–2.5 (m, 4H), 3.74 (d, 1H, J=13 Hz), 4.45 (d, 1H, J=13 Hz), 6.19 (broad t, 1H, J=7 Hz), 7.1–7.5 (m, 7H), 8.0–8.2 (m, 1H).

Of the other isomer of *3*, isolated as hydrochloride, 138 g (35 %) m.p. 250–255 °C is obtained, which is used without purification in the following steps. Recrystallization from EtOH raises the m.p. 262–265 °C (lit.[6] 251–253 °C). Anal. ($C_{20}H_{21}$ NO·HCl) C,H,N. The chemical conversion of this isomer to *4Z, 6Z* and *7Z* has established that it has the *Z*-configuration. ^{1}H NMR ($CDCl_3$); δ 2.18 (s, 6H), 2.1–2.7 (m, 4H), ~3.8 (broad d, 1H, J~14 Hz), ~4.4 (broad d, 1H, J~14 Hz), 5.85 (broad t, 1H, J=7 Hz), 7.1–7.5 (m, 7H), 8.1–8.3 (m, 1H).

*(E)-N-Methyl-3-(10,11-dihydro-10-oxo-5*H*-dibenzo[*a,d*]cycloheptene)-*$\Delta^{5,\gamma}$*-propylamine (*6E*).* To a solution of *3E* (115 g, 0.395 mol) in 1 l dry toluene is added $ClCOOCH_2CCl_3$ (90 g, 0.425 mol) and the mixture is heated at 80–90 °C for 3 h. The mixture is cooled, washed with 0.5 M HCl, and the toluene phase is evaporated leaving 190 g of *5E* as a yellow oil. According to the procedure described by Just *et al.*[12] this is dissolved in 1.2 l THF and stirred with 1 M KH_2PO_4 (0.24 l) and Zn-powder (240 g) for 2 h. The temperature is kept below 30 °C. The mixture is filtrated and the THF is evaporated. The residue is dissolved in 10 % AcOH and the acid solution is washed with ether. The free base of *6E* is isolated and the hydrochloride is crystallized from 1-propanol yielding 75 g (60 %), m.p. 217–220 °C. Recrystallization from EtOH raises the m.p. to 226–228 °C (lit.[5] 219–224 °C). Anal. ($C_{19}H_{19}NO$·HCl) C,H,N. ^{1}H NMR of the free base ($CDCl_3$): δ 2.38 (s, 3H), 2.2–2.8 (m, 4H), 1.48 (broad s, 1H), 3.78 (d, 1H, J=13 Hz), 4.41 (d, 1H, J=13 Hz), 6.20 (t, 1H, J=7 Hz), 7.1–7.5 (m, 7H), 8.0–8.2 (m, 1H). The *E*-configuration was assigned to this isomer on the basis of the ^{1}H NMR spectrum after addition of a chemical shift reagent (see text).

*(Z)-N-Methyl-3-(10,11-dihydro-10-oxo-5*H*-dibenzo[*a,d*]cycloheptene)-*$\Delta^{5,\gamma}$*-propylamine (*6Z*).* This is prepared as above from *3Z*. The free base of *6Z* is isolated as an oil (yield 85 %), which is

used without purification in the last step. A hydrochloride can be crystallized from EtOH, m.p. after recrystallization 210–212 °C. Anal. ($C_{19}H_{19}NO$·HCl) C,H,N. ^{1}H NMR of the free base ($CDCl_3$): δ 1.93 (broad s, 1H), 2.38 (s, 3H), 2.3–2.9 (m, 4H), ~3.8 (broad d, 1H, J~14 Hz), ~4.4 (broad d, 1H, J~14 Hz), 5.86 (t, 1H, J=7 Hz), 7.1–7.6 (m, 7H), 8.1–8.3 (m, 1H).

The oxo compounds *3E, 3Z, 6E* and *6Z* are reduced to hydroxy compounds with $NaBH_4$ in MeOH. Typically, the oxo compound as free base (50 g, 0.18 mol) is refluxed for 1.5 h with $NaBH_4$ (25 g, 0.66 mol) in 1.5 l MeOH. All the hydroxy compounds are crystallized as free bases from diethyl ether or isopropyl ether. The yields of recrystallized bases are 50–70 %.

(E)-N,N-Dimethyl-3-(10,11-dihydro-10-hydroxy-5H-dibenzo[a,d]cycloheptene)-$\Delta^{5,\gamma}$-propylamine (4E). M.p. 102–104 °C (lit.[7] 96 °C). Anal. ($C_{20}H_{23}NO$) C,H,N. IR ($CHCl_3$): 3560 (m, free OH), 3340 (s,broad, hydrogen bonded OH) cm^{-1}. ^{1}H NMR ($CDCl_3$); δ 2.05 (s, 6H), 2.2–2.4 (m, 4H), 2.8–3.8 (m, 3H), 4.6–5.2 (m, 1H), 5.88 (broad t, 1H, J=7), 7.1–7.5 (m, 8H).

(Z)-N,N-Dimethyl-3-(10,11-dihydro-10-hydroxy-5H-dibenzo[a,d]cycloheptene)-$\Delta^{5,\gamma}$-propylamine (4Z). M.p. 133–135 °C (lit.[7] 135–136 °C). Anal. ($C_{20}H_{23}NO$) C,H,N. IR ($CHCl_3$): 3550 (w, free OH), ~3300 (s, broad, hydrogen bonded OH) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.73 (broad s, 3.8H), 2.08 (broad s, 2.2H), 1.9–2.7 (m, 5H), 2.9–3.8 (m, 2H), 4.9–5.5 (m, 1H), 5.89 (broad t, 1H, J=7 Hz), 7.0–7.6 (m, 8H).

(E)-N-Methyl-3-(10,11-dihydro-10-hydroxy-5H-dibenzo[a,d]cycloheptene)-$\Delta^{5,\gamma}$-propylamine (7E). M.p. 113–114 °C. Anal. ($C_{19}H_{21}NO$) C,H,N. IR ($CHCl_3$): 3560 (m, free OH), 3310 (s, broad, hydrogen bonded OH) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.85 (broad s, 1H), 2.24 (s, 3H), 2.1–2.7 (m, 4H), 2.9–3.7 (m, 3H), 4.6–5.1 (m, 1H), 5.88 (t, 1H, J=7 Hz), 7.1–7.5 (m, 8H).

(Z)-N-Methyl-3-(10,11-dihydro-10-hydroxy-5H-dibenzo[a,d]cycloheptene)-$\Delta^{5,\gamma}$-propylamine (7Z). M.p. 108–110 °C. Anal. ($C_{19}H_{21}NO$) C,H,N. IR ($CHCl_3$): 3560 (w, free OH), 3310 (s, broad, hydrogen bonded OH) cm^{-1}. ^{1}H NMR ($CHCl_3$): δ 2.05 (broad s, 1.5 H), 2.30 (broad s, 1.5 H), 2.0–2.8 (m, 5H), 2.9–3.6 (m, 3H), 4.9–5.4 (m, 1H), 5.85 (t, 1H, J=7 Hz), 7.0–7.6 (m, 8H).

1-(10,11-Dihydro-5-(3-dimethylaminopropyl)-5,10-epoxy-5H-dibenzo[a,d]cyclohepten-10-yl)-piperidine (8a). 5-(3-Dimethylaminopropyl)-10-piperidino-5*H*-dibenzo[*a,d*]cyclohepten-5-ol (*8*) [7] is heated at 80–90 °C with 2 M HCl for 2 h, converted to the free base and the dihydrochloride is crystallized from Me_2CO as a monohydrate. The same product is obtained from *8* by short-term heating in $CHCl_3$ with excess of HCl, evaporating and crystallizing from Me_2CO. M.p. 215–216 °C. Anal. ($C_{25}H_{32}N_2O \cdot 2HCl \cdot H_2O$) C,H,N. ^{1}H NMR ($CDCl_3$) (of the free base): δ 1.2–3.2 (m, 17H), 2.24 (s, 6H), 3.87 (d, 1H, J=17 Hz), 6.9–7.3 (m, 8H).

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Synthesis of *S*- and *R*-4-Amino-3-hydroxybutyric Acid (GABOB) and *S*- and *R*-Carnitine from Arabinose or Ascorbic Acid

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Through a simple series of reactions D- and L-arabinose have been converted into the optically pure forms of *S*- and *R*-4-amino-3-hydroxybutyric acid, (GABOB), respectively, and of *S*- and *R*-carnitine. *R*-GABOB and *R*-carnitine have also been prepared from L-ascorbic acid.

Reaction of hexono- or pentono-lactones with hydrogen bromide in acetic acid (HBA) has been shown to give bromodeoxy- or dibromodideoxy-lactones, which have subsequently been converted into deoxylactones, deoxysugars, or into simpler, optically active compounds.[1,2,3] In the present work the reaction of salts of erythronic and threonic acid with HBA has been studied and the resulting dibromoderivatives have been converted into optically pure *R*- and *S*-4-amino-3-hydroxybutyric acid (GABOB) and into *R*- and *S*-carnitine. *R*-GABOB and *R*-carnitine have been prepared from L-glutamic acid *via* an enzymatic decarboxylation [4] and, recently, *R*-GABOB has been synthesized from L-ascorbic acid.[5] The latter synthesis did not, however, produce an optically pure product.

Reaction of potassium D-erythronate (*1*) with HBA for 24 h followed by treatment with methanol gave the crystalline methyl ester of 2,4-dibromo-2,4-dideoxy-D-threonic acid (*2*) in 76 % yield. Selective hydrogenolysis of *2* subsequently yielded the 2,4-dideoxy-4-bromo ester (*3a*) in ~85 % yield. Reaction of *3a* with sodium azide in dimethylformamide gave the azide (*3b*) which was hydrogenated in acidic solution in the presence of platinum to give the aminoester (*3c*). Neither *3b* nor *3c* were isolated in a pure state, but were only characterized through their ^{13}C NMR spectra. Hydrolysis of *3c* and purification on an ion exchange resin gave *S*-(+)-GABOB (*4c*) in 58 % yield, calculated from the dibromoester (*2*). By the same procedure, *R*-(−)-GABOB (*8c*) was prepared from potassium L-erythronate (*5*) *via* the dibromoester (*6*), the 2,4-dideoxy-4-bromoester (*7a*), the azide (*7b*) and the aminoester (*7c*). The optical rotations of *4c* and *8c* were in close agreement with those reported previously on products obtained by resolution of derivatives of racemic GABOB.[6]

Treatment of the bromocompound (*3a*) with trimethylamine in methanol gave the trimethylammonium derivative (*3d*) as the hydrobromide. Subsequent hydrolysis and purification on an ion exchange resin yielded *S*-(+)-carnitine (*4d*), isolated as the hydrochloride. Similarly, *R*-(−)-carnitine (*8d*) was prepared from (*7a*). Comparison of the optical rotations of the two products with those reported [4,7] indicates that they are optically pure.

The potassium salts of D- and L-erythonic acid (*1* and *5*) are readily prepared from D- and L-arabinose, respectively.[8] Oxidation of L-ascorbic acid with hydrogen peroxide gives L-threonic acid which can be easily isolated as its crystalline calcium salt (*9*).[9] Treatment of *9* with HBA followed by reaction with methanol gave the methyl ester of 2,4-dibromo-2,4-dideoxy-L-erythronic acid (*10*). Selective hydrogenolysis of *10* gave *7a*, identical with the product obtained from *6*. Since, however, *10* could not be induced to crystallize it was found most convenient to prepare *7a* from the crystalline *6*.

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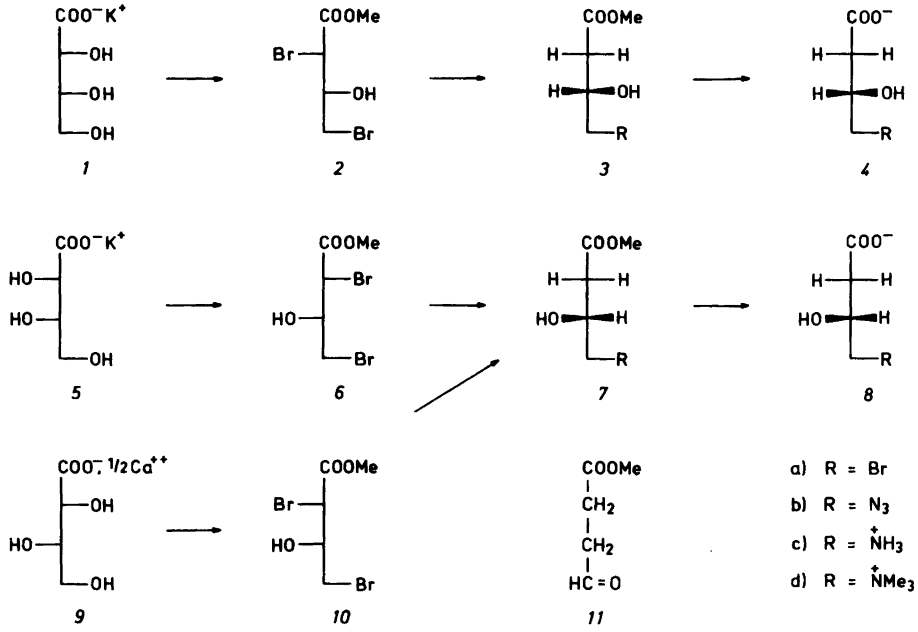

The configuration at C–2 of *2*, *6*, and *10* was not proved, but was assumed to be as indicated, since reactions of aldonic acids [1,2,3] and of diethyl tartrate [10] with hydrogen bromide in acetic acid in all cases give 2-bromo-derivatives with inversion of the configuration at C–2.

The selective removal of the 2-bromine from *2*, *6* or *10* was performed by hydrogenolysis using palladium on carbon as catalyst and sodium acetate–acetic acid as the acid acceptor, whereas in previous work on dibromolactones [2,3] triethylamine was used as the acid acceptor. Attempts to used triethylamine in the hydrogenolysis of *2* or *6* resulted in formation of *ca.* 10 % of methyl 4-oxo-butanoate (*11*) in addition to *3a* or *7a*. With the weaker base, sodium acetate–acetic acid, only traces of *11* were observed.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. NMR spectra were obtained on Bruker WH-90 and HX-90 instruments. For ^{1}H and ^{13}C NMR spectra measured in $CDCl_3$ solution TMS was used as internal reference whereas 1,4-dioxane (67.4 ppm) was used for ^{13}C NMR spectra measured in D_2O solution. Microanalyses were performed by NOVO microanalytical laboratory.

Potassium D- and L-erythronate (*1* and *5*) were prepared by treatment of D- and L-arabinose, respectively, with oxygen in potassium hydroxide solution at 40 °C.[8] The products were recrystallized from methanol–water. Yields in a series of preparations varied from 50–60 %.

Calcium L-threonate, monohydrate (*9*) was obtained in 79 % yield by oxidation of L-ascorbic acid with hydrogen peroxide.[9]

Methyl 2,4-dibromo-2,4-dideoxy-D-threonate (2). Potassium D-erythronate (*1*) (20 g) was stirred with 140 ml of a 32 % solution of hydrogen bromide in acetic acid for 24 h at room temperature. Methanol (500 ml) was then added and the mixture was kept overnight. The potassium bromide was filtered off and the solution was evaporated; the residue in methanol (200 ml) was boiled for 2 h and the methanol was evaporated. The residue thus obtained was dissolved in ethyl acetate (100 ml) and washed twice with water, the solution was dried and evaporated leaving 28.5 g (90 %) of crude, crystalline *2*, m.p. 68–71 °C. Recrystallization from ether–pentane gave 24.0 g (76 %) of a product with m.p. 75–77 °C. Further recrystallization gave an analytical sample, m.p. 76–77 °C, $[\alpha]_D^{20}$ −44.4° (c 3.4, $CHCl_3$). Anal. $C_5H_8Br_2O_3$:C, H, Br. ^{13}C NMR ($CDCl_3$): 169.0 ppm (C–1); 70.5 (C–3); 53.4 (OMe); 48.5 (C–2); 32.9 (C–4).

Methyl 2,4-dibromo-2,4-dideoxy-L-threonate

(6) was prepared in the same manner from potassium L-erythronate (5), m.p. 75–76.5 °C, $[\alpha]_D^{20}$ +43.8° (c 3.5, $CHCl_3$). Anal. $C_5H_8Br_2O_3$: C, H, Br. ^1H and ^{13}C NMR spectra were identical with those of *2*.

Methyl 2,4-dibromo-2,4-dideoxy-L-erythronate (10). Calcium L-threonate, monohydrate (*9*) (10 g) was stirred with HBA (70 ml) for 24 h followed by treatment with methanol and subsequent work-up as described above. This gave *10* as a crude syrup (15.0 g, 89 %) which could not be induced to crystallize. The product was virtually pure as seen from a ^{13}C NMR spectrum. Distillation in vacuum gave 11.0 g (65 %), b.p. 115–116 °C (2 mmHg), $[\alpha]_D^{20}$ −26.0° (c 17, $CHCl_3$). Anal. $C_5H_8Br_2O_3$: C, H, Br. ^{13}C NMR ($CDCl_3$): 168.7 ppm (C–1); 70.4 (C–3); 52.6 (OMe); 44.5 (C–2); 35.8 (C–4).

Methyl S-4-bromo-3-hydroxybutanoate (3a). The dibromoester (*2*) (8.0 g) in ethyl acetate (80 ml) and acetic acid (8 ml) was treated with hydrogen at 1 atm. pressure in the presence of anhydrous sodium acetate (8.0 g) and 5 % palladium on carbon (500 mg). After *ca.* 1 h 1 molar equivalent of hydrogen was consumed and the hydrogen uptake became much slower. The mixture was then filtered, the solution was washed with aqueous sodium hydrogencarbonate, dried and evaporated leaving 5.1 g (89 %) of *3a* as a colourless liquid which was almost pure as seen from a ^1H NMR spectrum. Distillation gave 4.67 g (82 %) of product, b.p. 79–80 °C (1 mmHg), $[\alpha]_D^{20}$ −16.2° (c 8, $CHCl_3$). Anal. $C_5H_9BrO_3$; C, H, Br. ^{13}C NMR ($CDCl_3$): 171.7 ppm (C–1); 67.2 (C–3); 51.7 (OMe); 39.0 (C–2); 37.1 (C–4).

Methyl R-4-bromo-3-hydroxybutanoate (7a) was prepared analogously from methyl 2,4-dibromo-2,4-dideoxy-L-threonate (*6*) in 86 % yield, b.p. 80–81 °C (1 mmHg), $[\alpha]_D^{20}$ +16.1° (c 9, $CHCl_3$). Anal. $C_5H_9BrO_3$: C, H, Br. A ^{13}C NMR spectrum was identical with that of the *S*-enantiomer.

Alternatively, methyl 2,4-dibromo-2,4-dideoxy-L-erythronate (*10*) was hydrogenolyzed as described above. This gave 70 % of *7a*, b.p. 79–80 °C (1 mmHg), $[\alpha]_D^{20}$ +16.0° (c 5, $CHCl_3$).

S-4-Amino-3-hydroxy-butanoic acid (4d). The dibromoester (*2*) (8.0 g) was hydrogenolyzed as described above to give 5.1 g (89 %) of crude 2-deoxy-ester (*3a*). This product was stirred with sodium azide (10 g) in DMF (30 ml) for 5 h at 65 °C. The DMF was then evaporated and the residue in water (25 ml) was extracted 4 times with ethyl acetate. The combined extract was washed once with water, dried and evaporated leaving 4.1 g (89 %) of crude, syrupy methyl *S*-4-azido-3-hydroxy-butanoate (*3b*). ^{13}C NMR ($CDCl_3$): 171.5 ppm (C–1); 66.6 (C–3); 55.1 (C–4); 51.1 (OMe); 38.1 (C–2).

The crude azide (*3b*) was dissolved in methanol and conc. hydrochloric acid (3 ml) and platinum oxide (100 mg) was added. The mixture was shaken with hydrogen for 5 h at 3 atm. pressure. Filtration and evaporation gave the crude, syrupy hydrochloride of methyl *S*-4-amino-3-hydroxy-butanoate (*3c*). ^{13}C NMR (D_2O): 173.6 ppm (C–1); 65.0 (C–3); 53.2 (OMe); 44.7 (C–4); 39.9 (C–2).

The product (*3c*) in water (20 ml) was boiled for 4 h and the solution was then poured on a column of ion exchange resin (Amberlite IR-120, H^+). The column was washed with water until the eluate was neutral. Subsequent eluation with 10 % aqueous ammonia (500 ml) and evaporation gave 2.9 g of crude product which was recrystallized from water–ethanol to give 2.0 g (58 %) of *S*-GABOB (*4c*), m.p. 213–214 °C (dec.), $[\alpha]_D^{20}$ +20.1° (c 2.3, H_2O), (reported [6] m.p. 210–212 °C, $[\alpha]_D$ +20.4°). ^{13}C NMR (D_2O): 179.3 ppm (C–1); 66.4 (C–3); 45.0 (C–4); 43.2 (C–3).

R-4-Amino-3-hydroxy-butanoic acid (*8c*) was prepared in the same way from the L-dibromoester (*6*) in a yield of 55 %, m.p. 213–214 °C; $[\alpha]_D^{20}$ −20.2° (c 4.0, H_2O), (reported [6] m.p. 210–212 °C, $[\alpha]_D$ −21.4°). Its ^{13}C NMR spectrum was identical with that of the *S*-form described above.

S-(+)-1-Propanaminium-3-carboxy-2-hydroxy-N,N,N-trimethyl chloride, [*S-(+)-carnitine, hydrochloride*], (4d). The bromoester (*3a*) (9.6 g) in methanol (40 ml) containing trimethylamine (6 g) was heated to 60 °C for 20 h in a closed container. The yellow solution was then evaporated and the crystalline residue was stirred with ether and filtered off yielding 11.0 g of crude methyl ester of *S*-carnitine (*3d*) as the hydrobromide. ^{13}C NMR (D_2O): 175.5 ppm (C=O); 70.5 (CHOH); 63.7 ($CH_2\overset{+}{N}\equiv$); 55.2 ($\overset{+}{N}Me_3$); 53.5 (OMe); 41.1 (CH_2). A small signal at 45.9 ppm showed that the product contained some trimethylammonium bromide.

The product was boiled for 3 h in 50 ml 2 M hydrochloric acid and the solution was evaporated. The residue in water was put on a column of ion exchange resin (Amberlite IR-120, H^+); the column was then eluted with water until the eluate was neutral then with 10 % aqueous ammonia (500 ml). The latter eluate was evaporated and coevaporated with water. The residue in water was acidified with hydrochloric acid and evaporated leaving a product which crystallized when stirred with acetone. Filtration and drying gave 6.2 g (65 %) of *S*-carnitine, hydrochloride (*4d*), m.p. 133–136 °C, $[\alpha]_D^{20}$ +22.9° (c 3.3,

H_2O). Recrystallization from 2-propanol gave 5.5 g (57 %) of product with m.p. 135–137 °C, $[\alpha]_D^{20}$ +23.2° (c 2.3, H_2O). These values were not changed on further recrystallization, (reported [7] m.p. 142 °C, $[\alpha]_D$ +23.7°). ^{13}C NMR (D_2O): 174.8 ppm (C=O); 70.4 (CHOH); 63.6 ($CH_2\overset{+}{N}\equiv$); 55.1 ($\overset{+}{N}Me$); 40.8 (CH_2).

R-*(−)-1-Propanaminium-3-carboxy-2-hydroxy*-N,N,N-*trimethyl chloride, (*R-*(−)-carnitine, hydrochloride), (8d)* was synthesized in the same manner from the bromoester (*7a*). The crude product was obtained in 64 % yield, m.p. 132–134 °C $[\alpha]_D^{20}$ −22.7° (c 3.1, H_2O). Recrystallization from 2-propanol gave 57 %, m.p. 135–137 °C, $[\alpha]_D^{20}$ −23.2° (c 1.9, H_2O), (reported [7] m.p. 142 °C, $[\alpha]_D$ −23.7°). Its ^{13}C NMR spectrum was identical with that of the *S*-enantiomer.

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α-Haloalkyl Ethers in Alkylations of 2-Pyrimidinones

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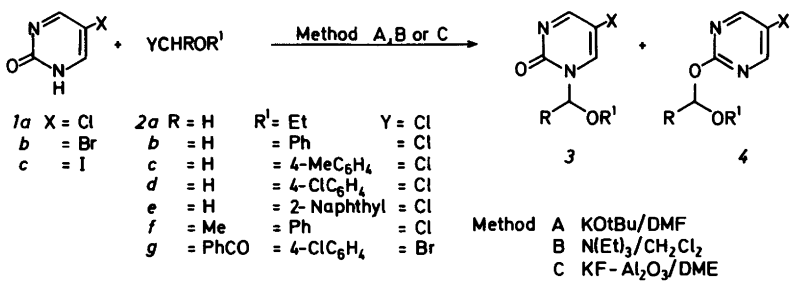
The ambident 5-halopyrimidin-2-one anion with potassium as counterion in DMF is preferentially *O*-alkylated using the relatively hard α-chloroalkyl ether electrophiles. As ammonium salts in dichloromethane or by the use of KF-alumina in DME the preference is for *N*-alkylation. TMS ethers of the pyrimidinones are exclusively *N*-alkylated.

Anions of 5-halopyrimidin-2-ones are ambident and can thus in principle be either *O*- or *N*-alkylated. The preferential site of reaction seems to depend on factors such as the structure of the alkylating agent, the solvent used in the reaction, the nature of the counterion, the nature of the leaving group and on the temperature used.[1] In our previous report on alkylation reactions of this system, simple halides like methyl iodide, allyl bromide and benzyl bromide were studied in which case the *N*-substituted pyrimidines were obtained almost exclusively.[2] These reagents have a rather soft carbon where the substitution occurs, and hence the softer part of the ambident system (*N*) is preferentially alkylated. An increasing amount of *O*-alkylation of the ambident system is to be expected when the reacting carbon of the alkylating agent is made harder. This would appear to be the case when the alkylating agent is an α-halo ether. We herein report such studies.

The *N*-β-oxa-alkyl products *3* coming out of these reactions are also of special interest since we have found that *N*-substituted 5-halopyrimidin-2-ones affect the cell cycle during mitosis by possessing reversible metaphase arresting properties.[3]

The 5-halopyrimidin-2-ones were available by literature methods.[4] There was, however, the need for an improved synthesis of the 5-iodo analogue *1c*. It has been found that the latter can conveniently be prepared from 2-pyrimidinone using iodine monochloride under acidic conditions.

Various methods were studied for the alkylation with α-halo ethers (Scheme 1). In *N,N*-dimethylformamide (DMF), potassium was used as the counterion of the pyrimidinone. (Method A); the major product from the reaction was the *O*-alkylated isomer (Table 1). Presumably the hard potassium ion is well solvated by DMF so that the harder part of the ambident pyrimidine system (*O*) can compete successfully with the



Scheme 1.

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Table 1. Alkylations of 5-halopyrimidin-2-ones using α-chloro ethers.

	X	R	R'	Method [a]	mmol *1* and *2*	Yield (%) [b]	*3*:*4* [c]
3a/4a	Cl	H	Et	B [d]	10	74	2:1
3b/4b	Cl	H	Ph	B	5	85	3:1
3b/4b	Cl	H	Ph	C	– [e]	48	3:1
3c/4c	Cl	H	4-MeC_6H_4	A [d]	2	80	2:3
3c/4c	Cl	H	4-MeC_6H_4	B [d]	10	76	3:1
3c/4c	Cl	H	4-MeC_6H_4	C	– [e]	66	7:2
3d/4d	Cl	H	4-ClC_6H_4	A [d]	20 [f]	70 [g]	2:3
3d/4d	Cl	H	4-ClC_6H_4	B	6	91	4:1
3e/4e	Br	H	4-ClC_6H_4	A	5	70	2:3
3e/4e	Br	H	4-ClC_6H_4	C	– [e]	53	3:1
3f/4f	I	H	4-ClC_6H_4	A [h]	5	87	3:5
3f/4f	I	H	4-ClC_6H_4	B	10	56	4:1
3g/4g	Cl	H	2-naphthyl	B [d]	5	86	4:1
3h/4h	Cl	Me	Ph	B [d]	4.5	76	3:2
3i/4i	Cl	PhCO	4-ClC_6H_4	B [d,i]	5	77	8:1

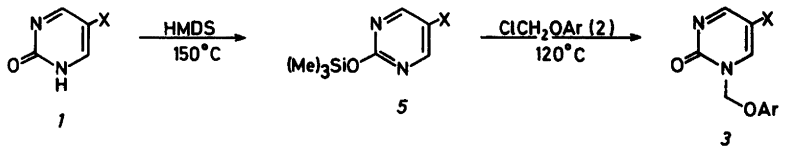
[a] *Method A:* KOtBu/DMF (*3c/4c*: RT, 3 h; *3d/4d*: RT, 1/2 H and 60 °C, 1/2 h; *3e/4e*: 60 °C, 4 h: *3f/4f*: RT, 3 1/2 h). *Method B:* $N(Et)_3/CH_2Cl_2$ (*3a/4a*: RT, 1 h; *3b/4b–3d/4d*: RT, 24 h; *3f/4f*: reflux 3 h and RT, 24 h; *3g/4g–3i/4i*: RT, 24 h). *Method C:* KF/Al_2O_3 (RT, 4 d); [b] crude product; [c] estimated from ^1H NMR spectrum of the crude product; [d] the hydrochloride of *1* was used with 2 equivalents of base; [e] 2 mmol of *1* and 2.5 mmol of *2*. [f] 20 mmol of *1* and 23 mmol of *2*; [g] 74 % yield when the free base of *1* was used; [h] the potassium salt of *1* was premade; [i] the α-bromo ether was used as alkylating agent.

softer part (*N*) for the alkylating agent. Preferential *O*-alkylation also agrees with the empirical rule which states that the more electronegative atom is preferentially alkylated when the anion is "free".[1a] When triethylamine was used as the base for the pyrimidinone in DMF, the *O*:*N*-alkylated isomer was *ca.* 1:1.

The α-haloalkyl ethers are reactive alkylating agents. Less polar solvents can therefore be used in the reaction provided the reactants can be solubilized. The pyrimidinones *1* constitute a solubility problem. Quaternary alkylammonium salts are popular for solvation of substances of low solubility in solvents with low polarity.[5] We find that addition of triethylamine to 5-chloropyrimidin-2-one in dichloromethane results in full dissolution. The solubility of the ion pair [6] between triethylamine and the 5-chloro-, the 5-bromo- and the 5-iodo-2-pyrimidinone decreased with increase in the size of the halogen but the solubility is sufficient in all cases for the alkylation to be carried out (Method B). The more basic secondary alkylamines are known to react slowly with dichloromethane and even tertiary amines of low steric hindrance such as in trimethylamine are attacked.[7,8]

The main product from the reaction is the *N*-alkylated isomer (Table 1); the ammonium nitrogen is apparently closer associated with the *O*-atom of the ambident system in dichloromethane than in DMF thereby favouring *N*-alkylation. It should also be pointed out, however, that anionic oxygen nucleophiles in dichloromethane have been reported to possess low nucleophilicity.[9] When *3* or *4* was stirred together with α-haloalkyl ether in dichloromethane at room temperature for 2 days simulating the reaction condition for the formation of the former, no transalkylation was observed showing that the product isomer ratios result from kinetic control.

Organic synthesis using reagents absorbed on insoluble inorganic supports is gaining importance;[10] thus alumina has been used to promote the hydrogen-bond assisted reactions of potassium fluoride,[11,12] and it has been claimed that this system is an effective reagent in selective *N*-alkylations of carboxamides, lactams and other *N*-heterocycles.[12] This reagent in 1,2-dimethoxyethane (DME) (Method C) applied to the pyrimidinones *1*, however, resulted in partial *O*-alkylation, the *N*:*O*-alkyl isomer ratio being similar to



1 5 3

Scheme 2.

that of Method B but the reaction was slow and the yields were inferior.

The silyl variant of the Hilbert-Johnson method, as used in the synthesis of pyrimidine nucleosides,[13] has been applied to simple alkylations of uracils.[14] Adapted for the pyrimidinones *1* the silyl ethers *5* were readily prepared using hexamethyldisilazane (HMDS). The alkylation in refluxing dichloromethane was slow resulting in low yield; better yields were obtained when the reactants were heated together at 120 °C in the absence of a solvent (Scheme 2). Only the *N*-alkylated isomers were obtained. The low reactivity is attributed to low nucleophilicity of the nuclear nitrogens. Nucleophilicity and basicity are interrelated, and it has been shown that the reactivity of heteroaromatic *O*-trimethylsilyl lactims decreases with decreasing pK_a values of the corresponding methoxy lactims.[15] The pK_a value −0.77 for 5-bromo-2-methoxypyrimidine [16] therefore explains why the silyl ethers *5* react sluggishly. On the other hand, Table 2 shows that the yield in the alkylation increases with increase in the size of the 5-halogen which we attribute to a gradual decrease in the electronegativity which results in an increase in the pK_a of the molecule.

For the preparative synthesis of *N*-alkylated pyrimidinones Method B is favoured because of good yields; the *N*- and *O*-alkyl isomers are readily separated since the *O*-alkyl isomer is less polar than the *N*-isomer which allows for selective solvent extraction, or separation by fractional crystallization or chromatography.

EXPERIMENTAL

The mass spectra are reported as MS [70 eV; (% rel. int.)].

5-Iodopyrimidin-2-one 1c *as potassium salt.* An aqueous solution of iodine monochloride stabilized with sodium chloride (3.67 M, 8.30 ml, 30 mmol) was added to 2-pyrimidinone hydrochloride (3.30 g, 25 mmol) in water (20 ml). The mixture was heated at 60 °C for 1 h, cooled and kept in the refrigerator overnight. The solid material was collected, washed with water, acetone and ether before dissolving in warm 5 M potassium hydroxide (15 ml). The title compound precipitated on cooling and was washed with a little cold ethanol and ether; yield 2.30 g (35 %). Anal. $C_4H_2IN_2KO$: C, H.

α-Halo ethers 2b–2f were prepared as recently described.[17]

2-Bromo-2-(4-chlorophenoxy)-1-phenylethanone 2g. A mixture of 2-(4-chlorophenoxy)-1-phenylethanone [18] (4.2 g, 17 mmol) and *N*-bromosuccinimide (4.0 g, 22 mmol) with a catalytic amount of α,α′-azoisobutyronitrile in tetrachloromethane (50 ml) were heated under reflux for 3 h. The mixture was filtered and the solid washed with tetrachloromethane and ether. The filtrate was evaporated and the residue recrystallized from light petroleum; yield 2.6 g (47 %), m.p. 120 °C. ^{1}H NMR ($CDCl_3$): δ 6.9–7.7 (m, 8 H) 8.0–8.3 (m, 2 H).

Formation of 3 *and* 4 *by alkylation of* 1. *Method A.* Potassium *t*-butoxide (2.0 mmol) in dry DMF (5 ml) was added to a solution of 5-halopyrimidin-2-one (2.0 mmol) in dry DMF (20 ml). The mixture was stirred at room temperature for 10 min before the α-halo ether (2.0

Table 2. Alkylations of 5-halo-2-trimethylsilyloxypyrimidines using α-chloroalkyl ethers.

	X	Ar	mmol *5*	mmol *2*	Time (min)	Yield (%)
3c	Cl	4-MeC_6H_4	3.0	2.5	15	48
3d	Cl	4-ClC_6H_4	4.2	3.8	60	27
3e	Br	4-ClC_6H_4	6.7	6.0	30	37
3f	I	4-ClC_6H_4	2.3	2.3	15	60

mmol) was added. The resultant mixture was stirred at room temperature or at 60 °C (time given in Table 1) before the solvent was removed at reduced pressure. The residue was triturated with water, extracted into chloroform, washed with water (5 x), dried ($MgSO_4$) and evaporated. The residual product was a mixture of the *N*- and *O*-alkylated isomer. For analysis the isomers were separated by their different solubility in ether, the *O*-isomer being the more soluble. The *N*-isomer was recrystallized and the *O*-isomer purified on a silica column (chloroform).

Method B. Triethylamine (10 mmol) was added to a suspension of the 5-halopyrimidin-2-one (10 mmol) in dry dichloromethane (50 ml). The mixture was stirred for 10 min at room temperature before the α-halo ether (10 mmol) in dry dichloromethane (15 ml) was added dropwise over 10 min. The mixture was stirred at room temperature for 24 h before the solvent was distilled off. The residue was washed with water, extracted into chloroform and worked up as above.

Method C. A mixture of the 5-halopyrimidin-2-one (2.0 mmol), the α-halo ether (2.5 mmol) and $KF–Al_2O_2$ (Type B) [11] (6.0 mmol) was stirred together in dry 1,2-dimethoxyethane (20 ml) for 4 days before chloroform (60 ml) was added and the mixture filtered, washed with water and worked up as above.

Formation of 3 *by alkylation of* 5. The 5-halo-2-trimethylsilyloxypyrimidine *5* and the α-chloro ether *2* were heated together at 120 °C (time given in Table 2). Chloroform was added and the mixture heated under reflux for 30 min. The cooled solution was filtered and evaporated. The residue was extracted into chloroform, filtered, evaporated and recrystallized.

Physical data for the compounds *3* and *4* are as follow:

5-Chloro-1-(ethoxy)methylpyrimidin-2-one 3a. M.p. 89 °C (ether). Anal. $C_9H_7ClN_2O_2$: C, H. ^1H NMR ($CDCl_3$); δ 1.15 and 3.67 (Et), 5.30 ($–CH_2O–$), 8.22 and 8.53 (H-4 and H-6, respectively, *J* 3 Hz). IR (KBr): 1680 cm^{-1} (CO). MS: 188 (1, M), 146 (15), 144 (45), 131 (13), 130 (11), 116 (12), 102 (19), 59 (100).

5-Chloro-2-(ethoxy)methoxypyrimidin-2-one 4a. Oil. ^1H NMR ($CDCl_3$): δ 1.22 and 3.80 (Et), 5.55 ($–CH_2O–$), 8.42 (H-4 and H-6).

5-Chloro-1-(phenoxy)methylpyrimidin-2-one 3b. M.p. 146 °C. (Chrom.) Anal. $C_{11}H_9ClN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 5.92 (CH_2), 6.8–7.4 (Ph), 7.80 and 8.43 (H-4 and H-6, respectively, *J* 3 Hz). IR (KBr): 1660 cm^{-1} (CO). MS: 236 (7, M), 145 (32), 143 (100), 116 (31), 94 (6), 77 (18).

5-Chloro-1-(4-tolyloxy)methylpyrimidin-2-one 3c. M.p. 186 °C (acetone). Anal. $C_{12}H_{11}ClN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 2.25 (Me), 5.74 (CH_2), 6.8–7.3 (Ph), 7.80 and 8.50 (H-4 and H-6, respectively, *J* 3 Hz). IR (KBr): 1680 cm^{-1} (CO). MS: 250 (7, M), 145 (31), 143 (100) 116 (28).

5-Chloro-2-(4-tolyloxy)methoxypyrimidine 4c. M.p. 72 °C (MeOH). Anal. $C_{12}H_{11}ClN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 2.25 (Me), 6.00 (CH_2), 6.8–7.3 (Ph), 8.45 (H-4 and H-6). IR (KBr): 1560 cm^{-1} (pyrimidine). MS: 250 (15, M), 145 (31), 143 (100), 121 (15), 116 (23).

5-Chloro-1-(4-chlorophenoxy)methylpyrimidin-2-one 3d. M.p. 163 °C ($CHCl_3$/light petroleum). Anal. $C_{11}H_8Cl_2N_2O_2$: C, H. ^1H NMR (DMSO-d_6): δ 5.78 (CH_2), 7.0–7.4 (Ph), 8.64 and 8.71 (H-4 and H-6, respectively, *J* 4 Hz). IR (KBr): 1670 cm^{-1} (CO). MS: 274/272/270 (1/6/9, M), 145 (62), 113 (100).

5-Chloro-2-(4-chlorophenoxy)methoxypyrimidine 4d. M.p. 91 °C (MeOH). Anal. $C_{11}H_8Cl_2N_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 6.00 (CH_2), 6.9–7.3 (Ph), 8.45 (H-4 and H-6). IR (KBr): 1560 cm^{-1} (pyrimidine). MS: 274/272/270 (1/6/10, M), 145 (36), 143 (100).

5-Bromo-1-(4-chlorophenoxy)methylpyrimidin-2-one 3e. M.p. 200 °C (acetone). Anal. $C_{11}H_8BrClN_2O_2$: C, H. ^1H NMR (DMSO-d_6, $CDCl_3$): δ 5.81 (CH_2), 7.0–7.3 (Ph), 8.49 and 8.60 (H-4 and H-6, respectively, *J* 3 Hz). IR (KBr): 1660 cm^{-1} (CO). MS: 316/314 (6/5, M), 189 (96), 187 (100), 162 (16), 160 (17).

5-Bromo-2-(4-chlorophenoxy)methoxypyrimidine 4e. M.p. 102 °C (MeOH). Anal. $C_{11}H_8BrClN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 6.00 (CH_2), 6.9–7.4 (Ph), 8.52 (H-4 and H-6). IR (KBr): 1570 and 1560 cm^{-1} (pyrimidine). MS: 316/314 (14/13, M), 189 (90), 187 (100), 162 (16), 160 (20).

1-(4-Chlorophenoxy)methyl-5-iodopyrimidin-2-one 3f. M.p. 216 °C (EtOAc). Anal. $C_{11}H_8ClIN_2O_2$: C, H. ^1H NMR (DMSO-d_6/$CDCl_3$): δ 5.85 (CH_2), 6.9–7.5 (Ph), 8.68 and 8.82 (H-4 and H-6, respectively, *J* 3 Hz). IR (KBr): 1650 cm^{-1} (CO). MS: 362 (37, M), 235 (100), 222 (6), 108 (22).

2-(4-Chlorophenoxy)methoxy-5-iodopyrimidine 4f. M.p. 106 °C (MeOH). Anal. $C_{11}H_8ClIN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 6.00 (CH_2), 6.9–7.3 (Ph), 8.70 (H-4 and H-6). MS: 362 (16, M), 235 (100), 205 (20), 108 (70).

5-Chloro-1-(2-naphthyloxy)methylpyrimidin-2-one 3g. M.p. 168 °C (acetone). Anal. $C_{15}H_{11}ClN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 5.82 (CH_2), 7.0–7.9 (naphthyl and H-6), 8.45 (H-4, *J* 3 Hz). IR (KBr): 1660 cm^{-1} (CO). MS: 288/286 (5/15, M), 145 (32), 143 (100), 116 (22), 115 (16).

5-Chloro-2-(2-naphthyloxy)methoxypyrimidine 4g. M.p. 81 °C (MeOH). Anal. $C_{15}H_{11}ClN_2O_2$:

C, H. ^{1}H NMR ($CDCl_3$): δ 6.11 (CH_2), 7.0–7.7 (naphtyl), 8.40 (H-4 and H-6). MS: 288/286 (13/40, M), 157 (15), 156 (22), 145 (32), 144 (13), 143 (100), 127 (46).

5-Chloro-1-(1-phenoxy)ethylpyrimidin-2-one 3h. M.p. 92 °C (CH_2Cl_2/light petroleum). Anal. $C_{12}H_{11}ClN_2O_2$: C, H. ^{1}H NMR ($CDCl_3$): δ 1.72 (CH_3, J 5 Hz), 6.4–7.3 (Ph and CH), 7.79 and 8.40, (H-4 and H-6, respectively, J 3 Hz). IR (KBr): 1665 cm^{-1} (CO). MS: 250 (1, M), 159 (30), 157 (100), 121 (23), 77 (30), 65 (17).

5-Chloro-2-(1-phenoxy)ethoxypyrimidine 4h. Oil. ^{1}H NMR ($CDCl_3$): δ 1.72 (CH_3, J 5 Hz), 6.5–7.4 (Ph and CH); 8.41 (H-4 and H-6). MS: 250 (4, M), 159 (33), 157 (100), 131 (63), 121 (65), 94 (72), 77 (90).

1-[Benzoyl(4-chlorophenoxy)]methyl-5-chloropyrimidin-2-one 3i. M.p. 144 °C (CCl_4). Anal. $C_{18}H_{12}Cl_2O_3N_2$: C, H. ^{1}H NMR ($CDCl_3$): 6.8–8.0 (11 H), 8.51 (H-6, J 3 Hz). IR (KBr): 1710 and 1665 cm^{-1} (CO). MS: 374 (14, M), 269 (15), 247 (22), 201 (22), 199 (70), 105 (100).

2-[Benzoyl(4-chlorophenoxy)]methoxy-5-chloropyrimidine 4i. M.p. 116 °C. Anal. $C_{18}H_{12}Cl_2O_3N_2$: C, H. ^{1}H NMR ($CDCl_3$): 6.9–8.2 (10 H), 8.37 (H-4 and H-6). MS: 374 (2, M), 271 (65), 269 (100), 199 (15), 116 (30), 105 (53), 77 (47).

5-Halo-2-trimethylsilyloxypyrimidines 5 were made using hexamethyldisilazane.[13b] 5-Chloro-2-trimethylsilyloxypyrimidine; yield 83 %, b.p. 90 °C/8–9 mmHg. 5-Bromo-2-trimethylsilyloxypyrimidine; yield 61 %, b.p. 62 °C/0.01 mmHg. 5-Iodo-2-trimethylsilyloxypyrimidine; yield 77 %, b.p. 65–69 °C/0.01 mmHg.

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Chemical Feasibility Studies Concerning Potential Prodrugs of Acetylsalicylic Acid

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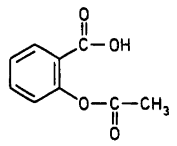
A rationale is developed for aspirin prodrugs based on non-acidic latentiated derivatives. Knowledge of the gastro-intestinal liabilities and pharmacological profile is required for this approach whereby aspirin is built into a common ortho ester function of the type 2-substituted 2-methyl-4*H*-1,3-benzodioxin-4-one with latentiated carboxyl and acetoxy groups. Twelve compounds of this type, ten substituted with various alkoxy and aryloxy groups and two with arylthio groups, have been isolated and characterized. A new synthetic route, comprising the reaction of 2-acetoxybenzoyl chloride with TMS derivatives of the corresponding alcohols and phenols, has been devised for the preparation of some of the compounds while others were prepared according to known methods. Subsequently, the prodrug candidates have been subjected to non-enzymatic hydrolysis for a first rapid screening *in vitro*. Only 2-*tert*-butoxy-2-methyl-4*H*-1,3-benzodioxin-4-one is observed to act as a true proaspirin, releasing aspirin, under these conditions, but analogous compounds with *tertiary* substituents may display the same behavior, and this chemical approach to aspirin modification may offer a viable rationale for aspirin prodrugs with reduced gastric irritancy or for making "superaspirins".

Aspirin (*O*-acetylsalicylic acid) *1* is widely used for its analgesic, antiinflammatory and antipyretic properties, while its clinical value in the prevention of platelet aggregation is less well-established. Among its disadvantages are the relatively narrow therapeutic margin, *1*'s irritancy towards the gastric mucosa (especially in the crystalline state), and occasionally hypersensitivity towards *1*.



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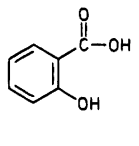

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The mode of action of *1* is reasonably well understood though important unresolved problems remain. Administered orally, *1* has a short plasma half-life of 15–20 min., primarily due to its enzymatic hydrolysis (first-pass effect) to salicylic acid *2* whose plasma half-life is longer by about an order of magnitude. The pharmacological profile of *1* is thus determined by the two half-lives mentioned, as well as by the similar, though not identical, pharmacological properties possessed by *1* and *2*. Often in pharmacological discussions the distinction between *1* and *2* (and their anions) is dropped altogether and the all-encompassing term "salicylates" is used. Among clinical practitioners there is a strong consensus that the oral administration of *1* is a valid therapy and could not be substituted by the administration of *2* or precursors of *2* without loss of efficacy.[1-3]

We felt that it might well be worthwhile to develop non-acidic prodrugs of *1* which probably could eliminate the gastro-intestinal liabilities of *1* while at the same time a substantially improved therapeutic index could be hoped for.[4] A useful prodrug of *1* could thus either constitute a "superaspirin" or at least reduce the long-term xenobiotic burden of chronic *1* users. Probably a

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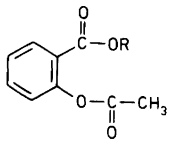
well-designed prodrug of *1* would be most beneficial in inflammation therapy while a different series of experimental candidate prodrugs might lead to another prodrug of *1* most useful as an analgesic. Ironically, *1* itself was developed as a prodrug of *2* half a century before the prodrug concept was explicitly presented in its rational, presently accepted context.[5]

It is interesting to note that most of the considerable number of "aspirin prodrugs" which so far have been described in the literature [6-9] are basically salicylic acid prodrugs since they release *2* and not *1* upon hydrolysis. However, a few proaspirins of the type *3* have also been described [10-12] (*vide infra*).



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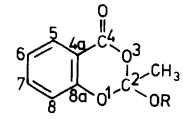
Off-hand, one can envisage two levels of sophistication in the latentiation of the *1* molecule. If the acetoxy group is left intact and only the carboxyl group latentiated, the rate of hydrolysis of the acetoxy group will set the lowest limit for the rate at which the prodrug can release *1* unless, of course, the less likely event occurs in which the latentiated carboxyl group anchimerically retards the hydrolysis of the acetoxy group relative to the rate of hydrolysis of *1*'s acetoxy group. This means that a prodrug of this type will have an even shorter half-life than *1* itself or constitute a prodrug of *2*. Thus, even methyl 2-acetoxybenzoate *3* ($R = CH_3$), the simplest latentiated *1* imaginable, is a prosalicylic acid and not a proaspirin.[13] A number of compounds of type *3* with extremely labile R groups are indeed proaspirins, but only by virtue of a correspondingly short half-life [10,11] (*vide supra*).

This strongly suggests that a useful prodrug of *1* would need to be comprised of both a latentiated carboxyl and acetoxy group, the most obvious possibility being the incorporation of both latent groups into a common ortho ester function. A straightforward choice would be system *4* where the substituent R ideally should



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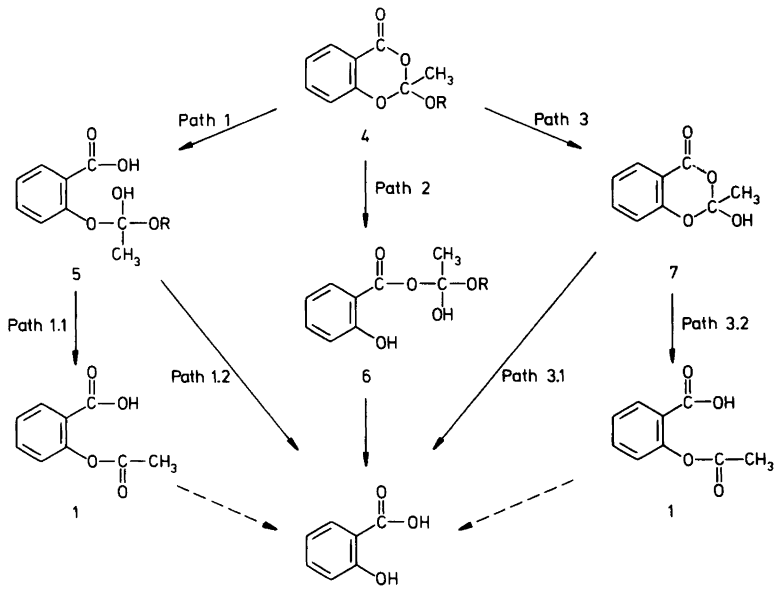
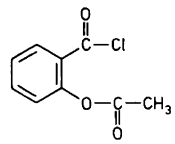


Fig. 1. Model pathways for hydrolytic breakdown of *4*(*13*) to *1* and *2* (ortho ester hydrolysis).

be chosen so as to ensure a proper balance of hydrophilic/lipophilic properties and an appropriate rate of hydrolysis of *4*. However, since the ortho ester carbon atom is linked to three non-equivalent oxygen atoms the hydrolysis of *4* can, in principle, involve the cleavage of any of these three carbon–oxygen bonds in its first step (Fig. 1).

Thus, only path 1 followed by path 1.1 and path 3 followed by path 3.2 are productive for the hydrolytic formation of *1* from *4* while the remaining pathways yield *2* only. Unless a serendipitous choice of R can be made, useful prodrug candidates *4* should be tailored with a built-in stereoelectronic control of the hydrolytic pathways [14] in order to make them true *1* precursors. The theory of ortho ester hydrolysis is a well-developed subject,[15] so that the desired stereoelectronic control ought to be achievable, but on the other hand it might be inherently difficult to optimize the choice of R with regard to three, probably unrelated, properties of *4*. In order to mimic the *in vivo* fate of *4*, separate hydrolysis experiments in gastric juice and in plasma would be required. Interestingly, the literature does not appear to contain any data concerning the hydrolysis of ortho esters in the presence of hydrolytic enzymes. For the sake of simplicity we chose to screen our candidate compounds in the first instance by *in vitro* non-enzymatic hydrolysis.

2-Alkoxy(aryloxy)-2-methyl-4*H*-1,3-benzodioxin-4-ones *4* have, in fact, been prepared by a number of authors for different purposes. Rüchardt and Rochlitz [16] prepared a number of *4* from 2-acetoxybenzoyl chloride *8* and alcohols or phenols, but did not investigate their hydrolysis. Paris *et al.* obtained, *inter alia,* the prodrug candidates *9* [9b] and *10* [17] and characterized them in whole animal studies as roughly equipotent (on a molar basis) with *1* while being essentially devoid of *1*'s ulcerogenic properties. However, Paris *et al.* did not characterize *9* and *10* as



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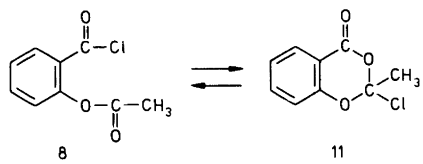
proaspirins or prosalicylic acids by studying their hydrolysis. Similarly, an analogous compound (*4*, R = 2-methoxyphenyl) with claimed antiinflammatory and antipyretic activity similar to that of *1*, but without gastric side-effects was patented recently.[19]

RESULTS AND DISCUSSION

Synthesis. The formation of *3* and/or *4* according to eqn. (1) depends in a complicated manner upon the presence or absence of base, the choice of solvent, and other reaction conditions.[16–18] The known syntheses of *4* must be said to be based on trial and error, and our own experience has been similar. While there is no direct evidence of an equilibrium (2) in neat 2-acetoxybenzoyl chloride (according to a ^{13}C NMR study [20] no *11* is present), Rüchardt and Brinkmann [21] found *8* to form *12* according to eqn. (3):

$$8 + R{-}OH \rightarrow 3 + 4 + HCl \quad (1)$$



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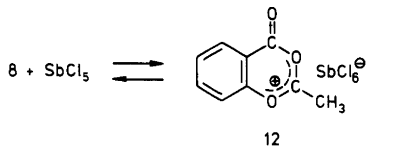

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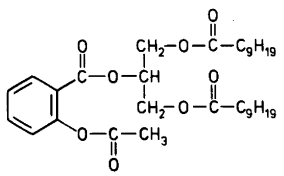

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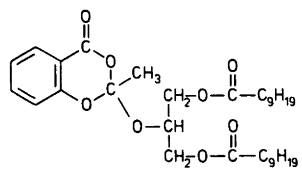


10

So far, however, *12* has not successfully been used as a starting material for the synthesis of *4*.[22]

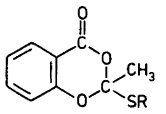
We did devise a new synthetic pathway leading to *4*, *i.e.* eqn. (4):

$$8 + R{-}O{-}Si(CH_3)_3 \rightarrow 3 + 4 + (CH_3)_3SiCl \quad (4)$$

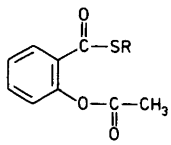
While the preparation of esters from acid chlorides and alkoxysilanes according to eqn. (5) is a known procedure,[23] we are not aware of any previous ortho ester syntheses *via* the TMS route. In spite of the mild conditions of this synthetic approach, it failed to bring about a dramatic improvement in selectivity or predictability with regard to the *4*:*3* ratio.

$$R^1C(=O){-}Cl + R^2O{-}SiR^3_3 \rightarrow R^1C(=O){-}OR^2 + Cl{-}SiR^3_3 \quad (5)$$

The ortho thioesters *13*, related to the thioesters *14* as *4* are to *3*, are also obvious candidates as potential proaspirins. Examples of *13* and *14*



13



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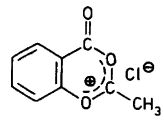
are known from the literature [16] and we prepared *13* according to standard procedures [16] and also according to (6), patterned after (4).

$$8 + R{-}S{-}Si(CH_3)_3 \rightarrow 13 + 14 + (CH_3)_3SiCl \quad (6)$$

The potential hydrolytic pathways that can be envisaged for *13* are parallel to what is shown for *4* in Fig. 1 and will not be elaborated further.

The formation of 2-alkoxy(aryloxy)-2-methyl-4*H*-1,3-benzodioxin-4-ones *4*, cyclic isomers of the alkyl (aryl) 2-acetoxybenzoates *3*, by reaction of *8* with alcohols and phenols, respectively, was first reported by Rüchardt and Rochlitz [16] as well as by Fried [24] and shown to follow (1). The cyclic isomers *4* are assumed to form in a kinetically controlled reaction proceeding *via* the intermediate 2-methyl-4-oxo-4*H*-1,3-benzodioxin-2-ylium salt *11'*, *3* being the thermodynamically more stable group of products. Analogous ambident reactivity caused by anchimeric assistance of an



11'

acetoxy group has also been observed with α-acetoxy acid chlorides.[25]

Table 1. Preparation of *4*/*3*.

4/*3*	Substituent R	Synthetic method I [a]	Synthetic method II [a]	Total yield/% I [b]	Total yield/% II [b]	*4*:*3* ratio I [c]	*4*:*3* ratio II [c]
a	*tert*-butyl	+	−	72	−	6:1	−
b	*neo*-pentyl	−	+	−	90	−	9:1
c	4-chlorophenyl	+	+	78	95	4:1	5:1
d	2-nitrophenyl	+	−	<5	−	*n.d.*	−
e	phenyl	+	+	85	95	6:1	4:1
f	benzyl	−	+	−	90	−	8:1
g	2-methyloxycarbonylphenyl	+	−	<5	−	*n.d.*	−
h	2-[(3-pyridinylcarbonyl)amino]ethyl	+	−	78	−	9:1	−
i	(±)-1-phenylethyl	−	+	−	95	−	1:1
j	2-naphthyl	+	−	62	−	6:1	−
k	(−)-bornyl	−	+	−	90	−	3:1
l	(±)-menthyl	−	+	−	90	−	3:1

[a] See Experimental for details. [b] Based on crude product. [c] Determined from ^{1}H NMR spectra of the crude product. *n.d.* not determined.

Table 2. Preparation of *13/14*.

13/14	Substituent R	Synthetic method I [a]	Synthetic method II [a]	Total yield/% I [b]	Total yield/% II [b]	*13:14* ratio I [c]	*13:14* ratio II [c]
a	*tert*-butyl	+	–	90	–	*14a* only	
b	4-chlorophenyl	+	–	73	–	3:1	–
c	phenyl	–	+	–	95		*14c* only
d	3-methylphenyl	+	–	54	–	1:1	–

[a] See Experimental for details. [b] Based on crude product. [c] Determined from ^{1}H NMR spectra of the crude product.

Table 3. Physical properties of 2-alkoxy(aryloxy)-2-methyl-4*H*-1,3-benzodioxin-4-ones *4*.

4	Formula	B.p./°C [e] (m.p./°C)	^{1}H NMR [a] δ_{CH_3}/ppm	^{13}C NMR [a] $\delta_{C(2)}$/ppm	IR $\nu_{C=O}$/cm^{-1}	UV [d] λ(log ε)/nm	Analysis (Reference)
a	$C_{10}H_{16}O_4$	100–110	1.80	112.7	1750 [b]	301(3.40)	(16)
b	$C_{11}H_{18}O_4$	80– 90	1.85	113.4	1745 [b]	298(3.74)	C, H
c	$C_{15}H_{11}ClO_4$	170–180	1.87	113.2	1755 [b]	300(3.58)	(16)
e	$C_{15}H_{12}O_4$	150–160	1.86	113.3	1750 [b]	300(3.26)	(16)
f	$C_{16}H_{14}O_4$	110–120 (49–50)	1.91	113.0	1735 [c]	300(3.43)	(16)
h	$C_{17}H_{16}N_2O_5$	(98–99)	1.82	–	1745 [c]	300(3.38)	Found: C 60.23; H 5.01; N 8.45 Calc.: C 62.19; H 5.01; N 8.53
j	$C_{19}H_{14}O_4$	(88.5–89.5)	1.97	113.6	1745 [c]	300(3.72)	(16)
k	$C_{19}H_{24}O_4$	130–140	1.83	113.8	–	–	Found: C 70.28; H 7.81 Calc.: C 72.13; H 7.65
l	$C_{19}H_{26}O_4$	130–140	1.80	112.7	1740 [b]	304(3.57)	Found: C 71.58; H 8.85 Calc.: C 71.67; H 8.23

[a] In $CDCl_3$. [b] As film. [c] In KBr. [d] In 6:1 (v:v) aqueous phosphate buffer–dioxane solution at 37.0 °C and pH 7.40. [e] At 0.01 torr.

With the exacting demands on the substituent R of a true proaspirin *4* or *13* in mind, our present synthetic study summarized in Tables 1–4 was devised as a somewhat crude pilot study and not as an optimization program. In order to obtain as much mechanistic information as possible, we did not restrict ourselves to latentiated entities releasing pharmaceutically acceptable alcohols, phenols or thiols. However, our series does include pharmaceutically acceptable examples such as *4h*, *4k* and *4l*. Another important consideration is the molecular weight of *4* or *13*. If an ortho ester *4* or *13* contains so elaborate a group R that its maximum release of *1* amounts to only a small fraction of the molecular weight of *4* or *13*, any therapeutic advantage achieved by the latentiation might be more than offset by the increase in equivalent weight (based on *1* released) of *4* or *13*. For this reason we expressly restricted ourselves to low-to-moderate molecular-weight groups R.

When the cyclic aspirin derivatives *4* and *13*

Table 4. Physical properties of 2-(4-chlorophenylthio)-2-methyl-4*H*-1,3-benzodioxin-4-one *13b*.

13	Formula	M.p./°C	^{1}H NMR [a]	^{13}C NMR [a]	IR [b]	UV [c]	Analysis
			δ_{CH_3}/ppm	$\delta_{C(2)}$/ppm	$\nu_{C=O}$/cm^{-1}	λ(log ε)/nm	
b	$C_{15}H_{11}ClO_3S$	60–61	2.00	112.4	1745	304(3.30)	C, H, Cl, S

[a] In $CDCl_3$. [b] In KBr. [c] In 6:1 (v:v) aqueous phosphate buffer–dioxane solution at 37.0 °C and pH 7.40.

were first described,[16] ^{1}H NMR and IR data were used to distinguish them from their acyclic isomers *3* and *14*, respectively. In our experimental series we could confirm and extend these assignments with additional support from ^{13}C NMR and UV data. For some of the ^{13}C NMR signals an upfield shift similar to what has recently been reported for the analogous compounds *9* and *10* [17] is observed. The UV absorption of *4* and *13* resembles that of *2* [26] and is found at 300–305 nm, while the esters *3* and *14* with a weak absorption at 275–280 nm resemble *1*.[26] No UV data of the ortho esters *4* and *13* have previously been reported in the literature.

Unfortunately, the MS fragmentation patterns of *3*(*14*) and *4*(*13*) are too similar to allow safe structural conclusions to be drawn. In order to rule out the remote possibility that the compounds assigned the structures *4* and *13* are in fact *3* and *14*, respectively, and *vice versa*, an X-ray structure investigation of *4j* was initiated which confirmed the spectroscopic structure assignment.[27] Thus, an absolute degree of reliability has now been achieved for the spectroscopic identification of *3*/*14* and *4*/*13*.

Hydrolysis. The hydrolytic breakdown of the isolated cyclic aspirin derivatives *4*(*13*) in water–dioxane mixtures (37.0 °C, pH 7.40) was studied spectrophotometrically by monitoring the decrease in ultraviolet absorbance *versus* time. With *4a* this was best done at 300 nm and with the other compounds at 240–250 nm, because the products, *1* or *2*, respectively, (Fig. 1), absorb only weakly at these wavelengths [26] (Fig. 2).

Table 5 gives the obtained pseudo-first-order rate constants and in Fig. 2 typical spectra showing different stages of the breakdown to *1* (*4a*) and *2* (*4f*) can be seen. By comparing the product spectra following completion (10–12 half-lives) with reference spectra of *1* and *2* recorded under identical conditions, the salicylate formed was identified. In principle, the hydrolytic breakdown of *4*(*13*) may result in productive formation of either *1* or *2*, or both (Fig. 1). With simultaneous formation of both salicylates the minor component may go undetected by this method due to overlapping spectra. However, the presence of *2* cannot arise from the hydrolysis of preformed *1* as the rate constant for this reaction is slower by more than an order of

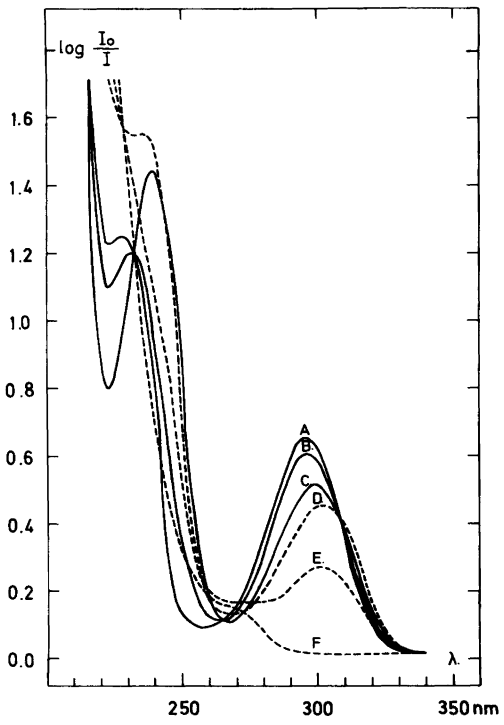


Fig. 2. Ultraviolet spectra of different stages of the hydrolysis of *4a* in aqueous phosphate buffer–dioxane solution (1:1 (v:v)) and of *4f* in 6:1 (v:v) solution at pH 7.40 (μ = 0.26) and 37.0 °C. *4a*: *D*, spectrum after 1 min; *E*, after 4 min; *F*, after 43 min (*1*). *4f*: *C*, spectrum after 1 min; *B*, after 121 min; *A*, after 721 min (*2*).

Table 5. In vitro hydrolysis of 2-substituted 2-methyl-4*H*-1,3-benzodioxin-4-ones. Salicylate product, pseudo first-order rate constants and relative rates in dioxane–aqueous phosphate buffer (pH 7.40 and $\mu = 0.26$) at 37.0 °C.

Compound	Salicylate product	k_{obs}/min^{-1} [a,d]	Relative rate
1	*2*	$1.1 \cdot 10^{-3}$ [b]	0.15
4a	*1*	$1.7(1) \cdot 10^{-1}$ [c]	22
4b	*2*	$3.4(2) \cdot 10^{-2}$	4.4
4c	*2*	$1.9(1) \cdot 10^{-2}$	2.4
4e	*2*	$5.0(3) \cdot 10^{-3}$	0.6
4f	*2*	$1.01(2) \cdot 10^{-2}$	1.3
4h	*2*	$7.8(1) \cdot 10^{-3}$	1.0
4j	*2*	$7.7 \cdot 10^{-3}$	1.0
4k	*2*	$1.4(1) \cdot 10^{-2}$	1.8
4l	*2*	$1.08(4) \cdot 10^{-2}$	1.4
13b	*2*(?)	$1.7(2) \cdot 10^{-3}$	0.2

[a] Averages of three or more runs. [b] *Cf.* with values is Refs. 13 and 26b. [c] In 1:1 (v:v) dioxane–aqueous phosphate buffer solution. [d] In 1:6 (v:v) dioxane–aqueous phosphate buffer solution.

magnitude compared with the rate constants for formation of *2* from most *4*. Only in the case of *13b* does this remain speculative because the rate constant obtained here is of the same magnitude as for the hydrolysis of *1* (Table 5).

Similarly, to obtain a more detailed mechanistic picture of the hydrolytic breakdown of these compounds, the hydrolysis experiment would have to be complemented with more careful product detection and identification (*e.g.* HPLC, GLC-MS, *etc.*) and kinetics measurements (pH rate profiles and isotope effects). However, for a first rapid screening of the prodrug candidates for subsequent and more detailed enzymatic studies these experiments are considered sufficient.

Of the compounds studied, only *4a* is observed to form *1* in detectable amounts (*vide supra*) upon hydrolysis while all the other compounds form *2*. With *2* formation from different *4* the rate constants obtained are of the same magnitude while the rate constant for the hydrolysis of *4a* is higher by more than an order of magnitude, even in a 1:1 (v:v) water–dioxane solution. In a 6:1 solution the reaction is too rapid to be followed by conventional ultraviolet spectrophotometry.

Considering the three-stage mechanism now generally accepted for the hydrolysis of ortho esters [15] and the possible hydrolytic pathways so derived for *4*(*13*) (Fig. 1), the shift in mechanism observed with *4a* may simply be explained by the initial cleavage of a different C–O bond. According to recent studies on hydrolysis of ortho esters and related substrates, several possible routes for hydrolytic breakdown exist. Cyclic ortho esters have been observed to hydrolyze with initial cleavage of the exocyclic C–O bond under stereoelectronic control,[15,28] whereas in acylals [29] and acyl ortho esters [30] the acyloxy group is split off in a first rate-determining step. Besides, the structurally related 3-methoxyphthalides are observed to hydrolyze as acetals at low pH values and as esters at higher (neutral-basic) values.[31]

The hydrolysis experiments performed here do not allow detailed mechanistic conclusions to be drawn. Interestingly, however, *4a* is the only compound observed definitely to release *1* just as it is the only compound containing a *tertiary* alkoxy group as substituent. Hence, the *tert*-butyl group may by virtue of its steric or electronic effects be responsible for the shift in mechanism, and even though its relatively short half-life (4 min) probably makes it of no practical value in prodrug formulations, *4a* and analogous cyclic aspirin derivatives *4*(*13*) containing *tertiary* exocyclic substituents should be considered seriously as potential aspirin prodrugs.

EXPERIMENTAL

Kinetics. For measuring the kinetics of the hydrolytic breakdown of the cyclic aspirin derivatives *4* and *13b*, a Varian Cary 219 UV-VIS double beam spectrophotometer was used. The apparatus was equipped with a jacketed multiple cell block through which water was circulated from a constant-temperature bath operating at 37.0±0.1 °C and an automatic sample-changing and recording facility. In a typical run 3.0 ml aliquots of prethermostatted buffer solution were placed in 1-cm quarz cells. Reactions were initiated by adding a stock solution (0.5 ml) of the substrate in dioxane (spectral grade), shaking a few seconds, and replacing the cell in the cell block. The stock solutions of substrates were prepared so as to give a final concentration of $1-2\times10^{-4}$ M which resulted in maximum absorbances about 2. Reactions were followed to completion and pseudo first-order rate constants were calculated by least-squares analysis as gradients by $\ln|A-A_t| = k_{obs}t+b$. Experiments with correlation coefficients lower than 0.999 were rejected. For very slow reactions the modified Guggenheim expression

$$\ln|A_i'-A_i|+k_{obs}t = constant$$

was used.[32]

The buffer solution was purchased from Struers A/S, Copenhagen, Denmark, and adjusted to pH 7.40 at 37.0 °C with $[H_2PO_4^-/HPO_4^{2-}] = 0.2857$ and ionic strength (μ) = 0.26.

Synthesis. Starting mateerials were all prepared by known methods as follows: *8*,[16] *N*-(2-hydroxyethyl)-nicotinamide,[33] *neo*-pentoxytrimethylsilane,[34], benzyloxytrimethylsilane,[35] (±)-1-phenylethoxytrimethylsilane,[36] (−)-bornyloxytrimethylsilane,[37] (±)-menthyloxytrimethylsilane,[35] phenoxytrimethylsilane [38] and 4-chlorophenoxytrimethylsilane.[38] *1* was kindly placed at our disposal by Alfred Benzon A/S, Copenhagen, Denmark.

All solvents used were analytical grades. Elemental analyses were carried out by Elemental Micro-Analysis Ltd., 33 Cambridge Road, Kingston upon Thames, Surrey KT1 3NQ, England.

Method I. Reaction of 8 *with alcohols, phenols, and thiols. General procedure.* To a solution of *8* in an anhydrous solvent (tetrahydrofuran–acetonitrile) an equimolar solution of alcohol (phenol, thiol) was added over a period of 30 min. The mixture was then stirred under a stream of nitrogen until the evolution of hydrochloric acid had stopped and the starting materials could no longer be detected (TLC, silica gel, dichloromethane). The solvent was then evaporated and the crude product was dissolved in chloroform, washed successively with 0.5 N sodium hydroxide and brine and dried over sodium sulfate. After evaporation of solvent the product was purified by *kugelrohr* distillation in the case of oils or by recrystallization in the case of solids.

Method II. Reaction of 8 *with trimethylsilylated alcohols, phenols, and thiols. General procedure.* A solution of trimethylsilylated alcohol (phenol, thiol) in anhydrous tetrahydrofuran (acetonitrile) was added to an equimolar solution of *8* over a period of 30 min at ambient temperatures. A catalytic amount of triethylamine was added and the mixture refluxed under nitrogen allowing the trimethylchlorosilane to distill off as formed. When starting materials were no longer detectable (TLC) the reaction was stopped and the solvent evaporated. Products were purified by *kugelrohr* distillation.

Detailed descriptions of the methods of preparation for each individual compound are available on request.

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Kinetics and Mechanisms of Electrochemical Dimerizations

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9-Cyanoanthracene is a particularly attractive model molecule for mechanistic studies on reductive electrochemical dimerization reactions in aprotic media. It does not give rise to side-reactions in very dry media and the dimerization reaction is reversible allowing the determination of the forward and backward rate constants as well as of the equilibrium constant. This was done as a function of temperature using double-potential step chronoamperometry. The effect of water was investigated using the same technique. The dimerization involves the radical–radical coupling of two anion radicals in dry media. Steric factors play an important role in the coupling reaction as revealed by temperature dependency investigations. These conclusions contrast those of recent studies implying that radical–radical dimerization of anion radicals, if even possible, is very uncommon. The causes of this misinterpretation of the experimental data are discussed. Addition of water accelerates the dimerization reaction. It is shown that this does not only occur through an "anion radical–proton donor complex mechanism" but also involves, at high water concentration, the coupling of two water-complexed anion radicals. Radical–radical coupling also occurs in the reductive dimerization of other molecules such as activated olefins although some evidence exists that a radical-substrate mechanism tends to prevail in very dry media. The role of water is again best rationalized in terms of specific solvation by water rather than in terms of an "anion radical–proton donor complex mechanism" which prevails only under particular conditions. The investigation of the mechanism of electrodimerization reactions raises important methodological problems. It is shown that the use of "reaction order, without calculation" approaches in cases where the reaction is not controlled by a single rate determining step may lead to erroneous mechanistic conclusions. Besides the reduction of 9-substituted anthracenes, the oxidative dimerization of 4-methoxybiphenyl is a typical illustration of the shortcomings of such oversimplified approaches. Important consequences regarding the chemistry of cation radicals ensue.

Being the base of a famous commercial electroorganic process, the reductive dimerization of activated olefins has been the object, among other organic molecules, of particularly active mechanistic investigation.[1–5]. Most studies in this field have been carried out in solvents of low proton availability, such as acetonitrile, DMF and DMSO, in an effort to decrease the reactivity of the initially formed anion-radicals and hence to reach a better control of the kinetics of the follow-up reactions. It has thus been shown that: (i) for a number of activated olefins, the apparent number of electrons at the first wave, where dimerization occurs, is less than one per molecule on mercury, implying the occurrence of other reactions besides dimerization; enough water was then added to avoid these reactions with the aim of investigating the dimerization itself;[2a] (ii) *under these conditions,* the investigated compounds undergo a radical–radical dimerization accelerated by further addition of water;[2a–2b] (iii) the latter effect derives from *specific solvation of the anion radicals by water* and not from protonation in the investigated water concentration range;[2b] (iv) other activated olefins undergo an electrodimerization reaction which appears as insensitive to the presence of water in a given water concentration range;[2a–2b] (v) for these two classes of activated olefins the electrodimerization is of the radical–radical type.[2a,2b] The latter conclusion was thus regarded to hold in wet

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medium as far as the first class of activated olefins is concerned. In other words, the mechanism was *not* considered as being the same in anhydrous and wet solutions as erronecously stated in recent literature.[6a] Where aprotic solvents containing low water concentrations are concerned, it was emphasized that the interference of radical-substrate coupling is likely with this type of activated olefins.[2g] Recent work [6a–6b] reached the same conclusion and appears therefore as bringing about a confirmation rather than a revelation. Note that although the interference of radical–substrate coupling under dry conditions can be viewed as likely,[2g] an unambiguous proof is still awaited since the experimental data do not exactly fit the predicted kinetics [6a] and the possible kinetic interference of the side-reactions has not been taken into account so far. Some more precision about how the specific solvation by water works has been recently brought up in the case of diethyl fumarate. The dimerization reaction appears as first order in water between 0.139 and 0.556 M,[6c] implying the coupling of two anion radicals one of which is complexed by one water molecule. This is still a mechanism involving the coupling of two anion radicals since the complex with water is regarded as not undergoing internal proton transfer and is therefore still an anion and still a radical. It should however be emphasized that this particular nature of the rate-determining step may well not be general when passing to other substrates and/or higher water concentrations. As will be shown on the example of other molecules, specific solvation by water [2b] thus appears as a safer and more general description of the role of water.

ARE ANION RADICALS UNABLE TO UNDERGO RADICAL–RADICAL DIMERIZATION?

In connection with the case of activated olefins, the reduction of a structurally related molecule, 9-cyanoanthracene (ANCN) is of particular interest. There is no complication involving side-reactions by dry aprotic media. The dimerization reaction is reversible allowing the determination of the equilibrium constant and of both the forward and backward rate constants and of their dependence upon water and countercations. The kinetic and thermodynamic data obtained by double step chronoamperometry in dry DMSO containing Bu_4N^+ as supporting cations will be described and discussed under their energetic and entropic aspects. In this connection, the conclusion of a recent investigation of the electrodimerization of anthracenes

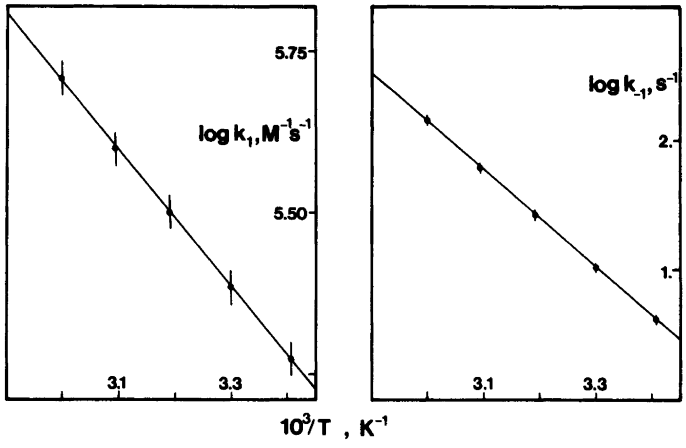

Fig. 1. Double potential step chronoamperometric analysis of the electrodimerization of 9-cyanoanthracene in DMSO containing Bu_4NBF_4 (0.1 M) in the presence of activated neutral alumina. ——: working curve, ●: experimental data. C_{ANCN}=1 mM. The value of the dimerization equilibrium constant, K, is indicated on each curve. On the horizontal axis: θ is the potential inversion time and k_1 the forward dimerization rate constant. R is the normalized ratio of the anodic current at 2θ over the cathodic current at θ: $R(\theta)=[i(2\theta)/i(\theta)]/[2^{-1/2}-1]$. Temperature: (a): 20.5 °C, (b): 40 °C, (c): 50 °C.

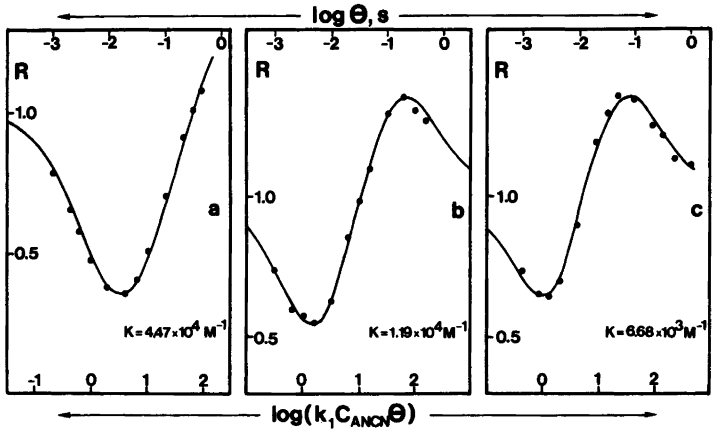

Fig. 2. Electrodimerization of 9-cyanoanthracene in DMSO containing Bu_4NBF_4 (0.1 M) in the presence of activated neutral alumina. Arrhenius plots between 20.5 and 60 °C. k_1, k_{-1}: forward and backward dimerization rate constants.

substituted with electron-withdrawing substituents, including ANCN, in DMF, according to which the simple radical–radical dimerization of the corresponding anion radicals surely does not take place,[7] will be discussed. This is indeed a rather striking statement which, if true, would cast doubts on the reality of radical–radical coupling for any kind of anion radicals, in particular for those deriving from activated olefins in wet medium since the mechanism is then still viewed as involving the coupling of two anion radicals.[2b,6c]

In dry DMSO containing 0.1 M Bu_4BF_4, we observed that the variations of the cyclic voltammetric cathodic peak potential of ANCN with the sweep rate and the substrate concentration are compatible with the occurrence of a radical–radical coupling and not with a radical–substrate coupling. The results obtained using double step chronoamperometry (typical examples of these are shown in Fig. 1) are also compatible with the radical–radical coupling pathway provided the reversibility of the dimerization is duly taken into account. The working curves were generated from the numerical resolution of the pertinent partial derivative equation systems as a function of two dimensionless parameters: $\kappa=KC_{ANCN}$ and $\lambda=k_1C_{ANCN}\theta$ (K: dimerization equilibrium constant, k_1: forward dimerization rate constant, θ: potential inversion time). Fitting of the experimental data with the working curves thus leads to the values of k_1 and K and thus of k_{-1}, the backward dimerization rate constant. This was done at several temperatures ranging between 20 and 60 °C. The ensuing Arrhenius plots are linear (Fig. 2) leading to $E^a_1=4.6$, $E^a_{-1}=17.4$ K cal/mol for the forward and backward activation energies. Regarding the forward dimerization reaction, the Arrhenius pre-exponential factor is thus equal to $7.9 10^8$ M^{-1} s^{-1}, *i.e.*, much smaller than the collision frequency, $\sim 2\times10^{11}$ M^{-1} s^{-1}, that can be estimated for ANCN.[8] This is actually not very surprising for a molecule such as $ANCN^{\cdot-}$ which is obviously sterically hindered toward dimerization. There is indeed ample theoretical background substantiating the role of steric factors for a number of reactions [9a–9c] particularly for dimerization reactions.[9d] A typical experimental example of the latter is the observation that the tri-*t*-butylcycloheptatrienyl radical has a dimerization activation energy of 1.7 K cal/mol while the corresponding rate constant is only 3×10^5 M^{-1} s^{-1} at 22 °C.[10]

The electrodimerization of ANCN thus appears to follow a simple radical–radical coupling mechanism contrary to the conclusion of recent work which excludes such a mechanism and invokes the intermediacy of a dimeric complex where the two anion radicals are not covalently bonded to each other.[7] Two reasons were given for substantiating the latter conclusion. One is the observation, as in our case, that the pre-exponential factor is much smaller than the standard collision frequency. As discussed

above this is actually perfectly compatible with a radical–radical coupling mechanism. The other reason is that derivative cyclic voltammetry (DCV) mechanism analysis produced slopes which are significantly different from that predicted by the rate law. The DCV analysis is very similar in its principles and capabilities to the double potential step method we used, as described above. The considered slopes are those of $\ln R'_I$ *vs.* $\ln(1/v)$ plots, where R'_I is the ratio of the peaks on the backward and forward DCV scans and v the sweep rate. These plots are approximately linear within a large portion of the pertinent sweep rate range.[11a] The slopes were found to be –0.268, –0.264 and –0.219 for CHO, NO_2 and CN, respectively, instead of –0.307 as predicted for an *irreversible* $2ANX^{\cdot-} \rightarrow {}^{-}XNA\text{–}ANX^{-}$ dimerization. However, if we take the reversibility of the dimerization process into account, the data are perfectly compatible with the "simple" anion radical dimerization mechanism. This is shown in Fig. 3 in the case of ANCN. The experimental points (Tables 1 and 2 in Ref. 7) fall accurately on the working curve corresponding to a dimerization equilibrium constant $K=1.4\times10^4$ M^{-1} and to a dimerization rate constant $k=1.7\times10^5$ M^{-1} s^{-1}. It is clearly seen why the slope obtained, even with the four highest sweep rates (80, 60, 40, 20 V s^{-1}), is different, smaller in absolute value, than that predicted for an irreversible dimerization. The working curves were generated from the numerical resolution of the pertinent partial derivative equation system, depending upon two dimensionless parameters, $\kappa=KC_{ANX}$ and $\lambda=(RT/F)(k_1C_{ANX}/v)$ along already known and repeatedly applied procedures devised for the situations where the kinetics involve more than a single rate-determining step (see *e.g.* Ref. 12). Using the same set of working curves, it was found that dimerization is less reversible for $ANNO_2$ and ANCHO which matches the observation that the experimental slopes are then closer to the value predicted for irreversible dimerization. The failure to recognize the existence of a "simple" radical–radical coupling mechanism thus merely derived from an artefact in the treatment of the kinetic data. This illustrates the limitations of the "reaction order", "without calculation", approaches insistently advocated in recent publications (Refs. 6, 7, 11 and references therein). These approaches, known for a long time (see, *e.g.*, Ref. 13), should obviously be supplemented by further procedures involving some more

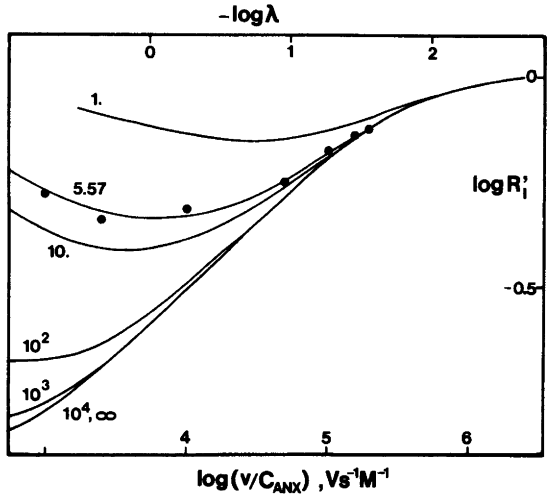

Fig. 3. Derivative cyclic voltammetric analysis of the electrodimerization mechanism of 9-cyanoanthracene in DMF containing Bu_4NBF_4 (0.1 M). Comparison between experimental data (●) [2b] and predicted kinetics for a reversible radical–radical dimerization (——). The number of each curve is the value of $\kappa=KC_{ANX}$, the dimensionless dimerization equilibrium constant. $\lambda=(RT/F)(k_1C_{ANX}/v)$ (k_1: dimerization rate constant, v: sweep rate). R'_I: ratio of the peaks on the backward and forward DCV scans.

algebra and computation when the kinetic scheme involves more than a single rate determining step.[12]

Our conclusion is thus that the answer to the question raised in the title of this section is *no*. 9-Cyanoanthracene anion radicals as well as other anthracene anion radicals substituted with electron withdrawing substituents are good candidates for "simple" radical–radical dimerization. The opposite conclusion recently drawn [7] arose from an artefact in the treatment of the kinetic data and from overlooking rather classical results of the chemical rate theory. The qualificative "simple" is, of course, rather subjective. More precisely, this reaction is to be viewed as an elementary step in the chemical sense. This, however, does not take place in vacuum and "complex" influences of the reaction medium (solvent, counter ions, water) on its thermodynamics and kinetics are anticipated.

THE ROLE OF WATER IN ORGANIC ELECTROREDUCTIVE DIMERIZATIONS IN APROTIC SOLVENTS. HOW GENERAL IS THE ANION RADICAL–WATER COMPLEX MECHANISM?

Addition of water often accelerates electroreductive dimerization of organic molecules in aprotic solvents. This has been observed with activated olefins [1a,2a,2b] as well as with carbonyl compounds.[14] This effect has been ascribed to specific solvation of the anion radicals by water molecules in the framework of a radical–radical coupling process.[2b] This interpretation was recently questioned and a more precisely specified reaction scheme accounting for the influence of water was proposed.[6b] It involves the coupling of an uncomplexed anion radical with an anion radical complexed by a single water molecule, resulting in an "anion radical–proton donor (water) complex mechanism" [6b] (eqns. (1)–(3)).

$$A + 1e \rightleftarrows A^{\cdot-} \quad (1)$$

$$A^{\cdot-} + H_2O \rightleftarrows A(H_2O)^{\cdot-} \quad (2)$$

$$A^{\cdot-} + A(H_2O)^{\cdot-} \longrightarrow \text{dimer} \quad (3)$$

As a matter of fact, electrokinetic data (using derivative cyclic voltammetry) obtained with diethyl fumarate in DMF for water concentrations ranging from 0.278 to 0.556 M do fit the kinetics predicted for such a reaction scheme.[6b]

We discuss in the following to what extent this "anion radical–water complex mechanism" is general and therefore should or should not replace the more general concept of specific solvation by water. The latter implies that two water complexed anion radicals may react together or that complexes involving more than a single water molecule may be involved.

As discussed above, 9-cyanoanthracene is a particularly attractive molecule for electrodimerization studies due to the fact that the dimerization process is reversible which allows the equilibrium constant as well as the forward and backward rate constants to be determined. For the present purposes, attractive features are that both the anion radical and the dimer dianion are resistant to protonation by water in a very extended concentration range. Complexation by water is also weak allowing the effect of water on the dimerization process to be investigated progressively.

The investigation described below was carried out at 20 °C in DMSO in the presence of 0.1 M NBu_4BF_4 as supporting electrolyte.

Linear sweep voltammetric peak shifts at low sweep rates (*i.e.*, under conditions where the reduction wave is chemically irreversible) with ANCN concentration and sweep rate (19 mV per log unit at 20 °C) indicate the occurrence of a radical–radical coupling dimerization process [2a,15] in the whole water concentration range (0–16 M). Chemical reversibility can be restored upon raising the sweep rate (this is obtained at 500 and 1000 V s^{-1} for the highest water concentration which corresponds to the largest dimerization rate). It was noticed that the standard potential, as determined as the middle of the cathodic and anodic reversible peak potential, does not vary appreciably with the water concentration. This shows that complexation of the anion radicals by water is rather weak, the equilibrium constants being less than 5×10^{-3} M^{-1} which would correspond to a 5 mV positive shift.

The overall rate constants for the forward and backward dimerization reactions, k_f and k_b, were determined using double potential step chronoamperometry. Their variations with water concentration are shown in Figs. 4 and 5. Concerning k_f, it is clearly seen that the $k_f[H_2O]$

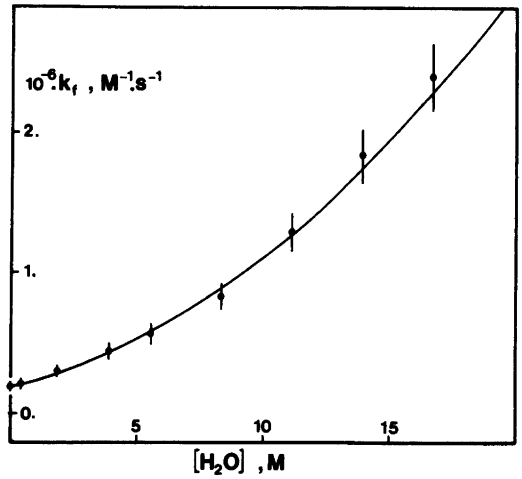

Fig. 4. Variations of the forward dimerization rate constant of 9-cyanoanthracene anion radical in DMSO (+0.1 M NBu_4BF_4) with the water concentration ($C°=10^{-3}$ M).

plot is not linear, ruling out the occurrence of the simple anion radical–water complex mechanism over the whole water concentration range. The data are compatible with the reaction scheme involving eqns. (4)–(10).

$$A+1e \rightleftarrows A^{\cdot-} \quad (4)$$

$$A^{\cdot-}+H_2O \rightleftarrows A(H_2O)^{\cdot-} \quad (K_A) \quad (5)$$

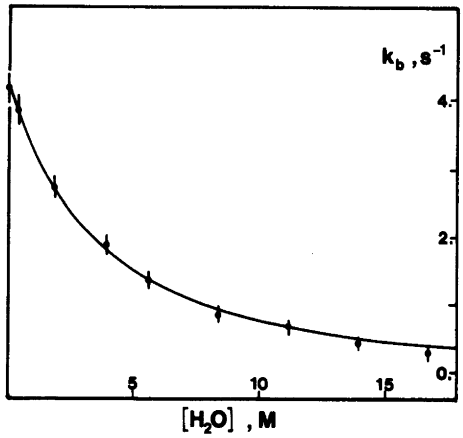

Fig. 5. Variations of the backward dimerization rate constant of 9-cyanoanthracene anion radical in DMSO (+0.1 M NBu_4BF_4) with the water concentration ($C°=10^{-3}$ M).

$$2A^{\cdot-} \underset{k_{-0}}{\overset{k_0}{\rightleftarrows}} {}^{-}A-A^{-} \quad (6)$$

$$A^{\cdot-}+A(H_2O)^{\cdot-} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} {}^{-}A-A(H_2O)^{-} \quad (7)$$

$$2A(H_2O)^{\cdot-} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} {}^{-}(OH_2)A-A(H_2O)^{-} \quad (8)$$

$${}^{-}A-A^{-}+H_2O \rightleftarrows {}^{-}A-A(H_2O)^{-} \quad (K_{D,1}) \quad (9)$$

$${}^{-}A-A(H_2O)^{-}+H_2O \rightleftarrows {}^{-}(OH_2)A-A(H_2O)^{-} \quad (K_{D,2}) \quad (10)$$

Assuming that the water complexation reactions are fast, thus remaining at equilibrium, as is very likely, the kinetics corresponding to the overall scheme amounts to a dimerization of $A^{\cdot-}$ having a forward rate constant given by eqn. (11).

$$k_f = k_0+k_1K_A[H_2O]+k_2(K_A[H_2O])^2/(1+K_A[H_2O])^2 \quad (11)$$

As the standard potential does not vary appreciably with water concentration it can be concluded that $K_A[H_2O] \ll 1$ in the above expression which depends then only on the values of k_0, k_1K_A and $k_2K_A^2$. Under these conditions, the best fit with the experimental data was obtained (see Fig. 4) for $k_0=1.9\times10^5$ M^{-1} s^{-1}, $k_1K_A=4.5\times10^4$ M^{-2} s^{-1}, $k_2K_A^2=4.9\times10^3$ M^{-3} s^{-1}. As mentioned earlier, K_A is not known but may be regarded as smaller than 5×10^{-3} M^{-1}. Taking this value one would obtain $k_1=0.9\times10^7$ M^{-1} s^{-1} and $k_2=2\times10^8$ m^{-1} s^{-1} which are minimal values of the two dimerization rate constants. It is seen that complexation by a water molecule greatly accelerates the dimerization process as compared to uncomplexed anion radicals.

The same scheme leads to the expression given by eqn. (12) for the backward rate constant:

$$k_b = \frac{k_{-0}+k_{-1}K_{D,1}[H_2O]+k_{-2}K_{D,1}K_{D,2}[H_2O]^2}{1+K_{D,1}[H_2O]+K_{D,1}K_{D,2}[H_2O]^2} \quad (12)$$

As shown by the experimental results in Fig. 5, k_b tends toward zero as the water concentration is increased. This is compatible with the dissocia-

tion rate constants for the mono- or disolvated dimers being small as compared to k_{-0}. Let us assume that these two rate constants are effectively negligible. This leads to a simplified expression for k_b given by eqn. (13).

$$k_b \simeq k_{-0}/(1+K_{D,1}[H_2O]+K_{D,1}K_{D,2}[H_2O]^2) \quad (13)$$

The two anionic centers in the $^-A-A^-$ dimer can be regarded as independent and chemically identical as far as complexation by water is concerned. Thus

$$K_{D,2} \simeq K_{D,1}/4$$

where the (1/4) factor results from a symmetry entropic term.[16] This leads to a simplified expression for k_b given by eqn. (14).

$$k_b = k_{-0}/(1+K_{D,1}[H_2O]+(K_{D,1}^2/4)[H_2O]^2) \quad (14)$$

which depends only on $K_{D,1}$. Fig. 2 shows that such an expression, with $k_{-0}=4.16$ s^{-1} and $K_{D,1}=0.27$ M^{-1} is in good agreement with the experimental data. It cannot, however, be excluded more than one water molecule complex for each anionic center of the $^-A-A^-$ dimer. A slightly better fit is indeed obtained if such reactions are taken into account.

The example of ANCN thus shows that the effect of water is not restricted to the "anion radical–water complex mechanism". The radical–radical coupling of two complexed anion radicals also interferes. At this point, it is worth recasting earlier data obtained with other organic molecules. With *p*-methylbenzylidene malonitrile (*p*-$CH_3-C_6H_4-CH=C(CN)_2$) a cyclic voltammetry investigation of the reduction in acetonitrile [2f] showed that the standard potential varies by more than 30 mV per log unit of water concentration between 0.17 and 5.7 M indicating a rather strong complexation of the anion radical by water. It, however, appears that the dimerization rate constant is practically insensitive to the increase in water concentration in this range. We may thus conclude, on the basis of the above equation giving k_f, that the prevalent dimerization pathway involves the coupling of complexed anion radicals, there predominating, at equilibrium, over the bare anion radicals. This is also compatible with the observation that at preparative scale concentrations (~0.1 M), a decrease of the rate of the dimerization process and a change of the overall kinetics are observed when the water concentration falls in the same range as the olefin concentration.[2e]

With acetophenone in acetonitrile,[14] the dimerization process is again accelerated by the addition of water. The standard potential becomes more positive upon addition of water by more than 90 mV per log unit of water concentration. This indicates the involvement of complexes containing more than one water molecule per anion radical.

The examples of 9-cyanoanthracene, *p*-methylbenzylidene malonitrile and acetophenone show that the "anion radical–water complex mechanism" is by no means a unique pathway accounting for the effect of water on the reductive electrodimerization of organic molecules in aprotic solvents. Other pathways such as dimerization of two complexed anion radicals or even dimerization of complexes involving more than one water molecule per anion radical may well be occurring depending upon the nature of the anion radical and the water concentration. *Specific solvation of the anion radicals by water* thus appears as a sounder and more general description of the effect of water addition on reductive electrodimerization processes.

METHODOLOGICAL ASPECTS. THE ELECTRODIMERIZATION OF 4-METHOXYBIPHENYL

The mechanistic investigation of electrodimerization reactions raises important methodological questions. One of these is the applicability of reaction order analysis. There is ample precedence that such an approach is valid for reactions kinetically controlled by a *single rate-determining step*. However, these approaches are not suited to more complex reaction schemes in which the kinetics is controlled by more than one rate-determining step. Erroneous mechanistic conclusions can then be drawn as illustrated by examples taken in the analysis of the reductive electrodimerization of 9-cyanoanthracene and of the oxidative electrodimerization of 4-methoxybiphenyl. Procedures involving some more algebra and computation, for which ample theoretical and experimental background exists, should instead be used for reaching sound conclusions.

Homogeneous chemical steps coupled with electrode electron transfers interfere in electrochemical reactions in a heterogeneous manner in the sense that they are coupled with the diffusion of reactants, intermediates and products to and from the electrode surface. The kinetic analysis of such reactions is thus somewhat more complex than for purely homogeneous processes. The time-dependent differential equations describing the homogeneous kinetics have to be replaced by time and space dependent partial derivative equations; boundary conditions at the electrode surface and in the bulk of the solution have to be taken into account. This explains why, in a first stage, analysis of this type of electrochemical kinetics and application to mechanism diagnosis and rate constant determination have been restricted to simple kinetic behaviours, *i.e.*, those in which the overall kinetics is controlled by a single rate-determining step. The latter does not necessarily follow or preceed immediately the electrode electron transfer, but may be separated from it by other chemical steps remaining at equilibrium. The corresponding homogeneous rate law is introduced into the diffusion partial derivative equations, the resolution of which provides the description of the kinetics of the overall electrochemical process.

Considering, for example, reaction schemes in which the initial electron transfer step, $A+e \rightleftarrows B$, is followed by homogeneous chemical steps involving B, situations of particular interest are met when the life-time of B is so short, with respect to diffusion, that "pure kinetic" conditions are achieved. These imply that a stationary state exists resulting from mutual compensation of the diffusion and chemical processes. The variations of the B concentration with time can then be neglected and the concentration profile of B is confined within a "reaction layer" vanishingly thin as compared to the diffusion layer. Under such conditions, the characteristics of the polarization curves are related in a rather simple manner to those of the homogeneous kinetics, namely reaction orders and overall rate constant, provided the initial electron transfer is sufficiently fast so as not to interfere kinetically. In cyclic voltammetry, these "pure kinetic" conditions give rise to totally irreversible waves. The peak potential, E_p, is then a linear function of the A/B standard potential and of the logarithms of the overall rate constant (k), sweep rate (v), initial substrate concentration (C°) and, according to the case, of the concentration of electroinactive reactants (Z°). The $\partial E_p/\partial \log v$, $\partial E_p/\partial \log C^\circ$, $\partial E_p/\partial \log Z^\circ$ are then simply related to the reaction orders of the homogeneous kinetic law. These expressions are obtained by integration of the space-dependent, time-independent, differential equations relative to B which do not require any numerical calculation. Mechanism analysis is then based upon the comparison between the experimental slopes and the values predicted for the limiting, single rate-determining step, kinetic behaviors. An early example of this type of mechanism analysis concerned radical–radical dimerization as illustrated experimentally by the reduction of benzaldehyde in a protic medium.[13] Since then, the method has been extended to a number of other reaction schemes [12,15,17] and applied to various electrochemical reactions such as electrodimerization of tropylium [18a] and immonium cations,[18b] carbonyl compounds,[2e] imines,[18c] activated olefins,[2a,2b] polyenic compounds,[3d,3e] electrocyclisations,[19] electrohydrogenations.[17c,18c,20] Systematic mechanism analysis thus involves the comparison of the measured slopes with those predicted for all the limiting kinetic behaviors of all the possible mechanisms (see, *e.g.*, Refs. 2a, 2b). Numerical calculations are necessary for deriving the overall rate constant from the peak potential knowing the A–B standard potential independently. They are also required if one wants to use the information contained in the whole polarization curve rather than in the peak potential only. This allows more efficient discrimination between the various limiting kinetic behaviors. Measurement of the peak width or fitting of the whole polarization curve may be used in this connection as well as other procedures such as convolution of the current with the linear diffusion characteristic $(\pi t)^{-1/2}$ function.[2f,21] Numerical calculation then consists in the computation of the convolution integral from the experimentally determined current.[2f,21]

The relationship between the homogeneous kinetics and the electrochemical response is somewhat more complex when, still dealing with a single rate-determining step, the chemical process is not sufficiently fast as compared to diffusion for the "pure kinetic" conditions to be achieved. The partial derivative equations can no

longer be simplified into space-dependent, time-independent, derivative equations. The dependence of the electrochemical response upon substrate concentration, electroinactive reactant concentration, time-scale (*i.e.*, sweep rate in cyclic voltammetry) can however be predicted for any given limiting behavior by dimension analysis of the system.[12b,12c,17a,17c,17e] It thus appears than the interplay between diffusion and chemical processes is dependent upon a single dimensionless parameter λ. If, for example, the reaction orders of the homogeneous process are α for A, β for B and ζ for an electroinactive reactant, Z, $\lambda=(RT/F)k(C^\circ)^{(\alpha+\beta-1)}C_z^\zeta/\nu$ in cyclic voltammetry [12b,12c,17a,17c,17e] (or, equivalently, $\lambda=k(C^\circ)^{(\alpha+\beta-1)}(C_z^\circ)^\zeta t$, in potential step chronoamperometry) where k is the overall rate constant, ν the sweep rate and t the time. When looking for the reaction mechanism, a first test is thus to check that the electrochemical response does follow the concentration and sweep rate (or time) dependence predicted, through λ, for the hypothesized limiting behavior: since the electrochemical response depends only upon λ, it should remain the same when varying C°, C_z° and ν so as $(C^\circ)^{(\alpha+\beta-1)}(C_z^\circ)^\zeta/\nu$ remains constant. This is, however, not sufficient in most cases since different limiting kinetic behaviors may well give rise to the same concentration and sweep rate (or time) dependence. A further step will be then to compare the experimental and predicted shapes of the electrochemical response=f(λ) relationship. This requires the calculation of the corresponding working curve by algebraic or numerical integration of the partial derivative equation system. For one-electron processes, such as electrodimerizations, adequate electrochemical responses are obtained from current ratios in cyclic techniques such as cyclic voltammetry,[22a] derivative cyclic voltammetry [11c] or double potential step chronoamperometry.[1a,22b]

An early extension of the capabilities of mechanism analysis beyond the above described cases, was provided by the treatments in which the reversibility of the coupled chemical reaction was taken into account involving the participation of both the forward and the backward reactions to kinetic control.[12b,12c,23] More recently, a number of more complex reaction schemes have been analyzed involving the competition between two or even three rate-limiting steps. Methods were thus described which allow to predict the influence of the operational parameters, sweep rate (or time, or diffusion layer thickness), concentrations, on the overall kinetics, particularly how their variations shift the system toward limiting behaviors. This has been applied to a variety of electrochemical processes, under various micro- and macroelectrolysis regimes: two-electron processes involving competing heterogeneous and homogeneous electron transfers,[24] electrocatalysis of chemical reactions,[25] homogeneous catalysis of electrochemical reactions,[26] competition between H-atom and electron transfers,[27] between radical–radical and radical substrate dimerization and between dimerization and further electron transfer.[28] These treatments can be extended to other reaction schemes and/or other electrochemical techniques. Ample background is thus available for analyzing rather complex mechanisms.

This reminder of the previously acquired knowledge in the field of electrochemical mechanism analysis seemed necessary in view of the recently proposed "reaction order" approach of the problem.[6,7,11,29] As far as limiting kinetic behaviors are concerned, this approach is essentially the same as the previously described methods mentioned above. Being actually based on the rigorous relationships established in previous work by other authors, its application to practical situations is anticipated to lead to correct conclusions as far as their range of applicability is respected, *i.e.*, as far as single rate-determining step kinetic behaviors are dealt with. However, the use of this approach has also been repeatedly advocated for more complex reaction schemes and applied to such experimental situations.[6,7,11c,29b–29d] Overlooking the abundant previous work relative to this type of situations,[24–28] the kinetic interplay between diffusion and homogeneous chemical reactions was then either ignored, as if the homogeneous process was functioning by itself,[29b–29d] or taken into account on questionable intuitive grounds.[11c] It is anticipated that this may lead to erroneous mechanism diagnostics. This was shown above to be the case for the mechanism analysis of the electrodimerization of anthracenes substituted with electron-withdrawing substituents. We give hereafter a second example concerning the anodic electrodimerization of 4-methoxybiphenyl in acetonitrile [11c] which is an illustrative example of

the general problem of the competition between radical–radical and radical–substrate coupling in electrodimerization reactions.

The kinetic data obtained using derivative cyclic voltammetry (DCV) [11c] are summarized in Fig. 6 under the form of a log-log plot of the variations of $v_{1/2}$ with the substrate concentration C°, $v_{1/2}$ being the value of the sweep rate necessary for the ratio of the derivative peaks on the backward and forward scans of the cyclic voltammogram to equal 0.5. It is seen that $v_{1/2}$ is proportional to C° at high values of C°, and to $(C^\circ)^2$ at low C° values. It was concluded [11c] from these observations that the dimerization mechanism involves the competition between a radical–radical coupling (rrc) and a radical–substrate coupling (rsc) reaction (eqn. 0′–4′).

$$R-e \rightleftarrows R^{\cdot+} \qquad (0')$$

(rrc):

$$2R^{\cdot+} \overset{k_1}{\rightleftarrows} {}^{+}R-R^{+} \qquad (1')$$

(rsc):

$$R+R^{\cdot+} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} {}^{\cdot}R-R^{+} \quad (K_2=k_2/k_{-2}) \qquad (2')$$

$$^{\cdot}R-R^{+}+R^{\cdot+} \overset{k_3}{\rightleftarrows} {}^{+}R-R^{+}+R \qquad (3')$$

$$^{+}R-R^{+} \longrightarrow 2H^{+}+\text{final dimer} \qquad (4')$$

(R=4-methoxybiphenyl). More precisely, the forward reaction (1′) was regarded as the rds in the rrc reaction which fits the C° proportionality found at high C°'s, while forward reaction (3′) was regarded as the rds of the rsc reaction with reaction (2′) remaining at equilibrium which fits the $(C^\circ)^2$ proportionality observed at low C°'s. Intuitively, it is predicted that the corresponding limiting rrc behavior will predominate at low C°'s and the rsc behavior at high C°'s, opposite to what is found experimentally concerning the variations of $v_{1/2}$ with C°. This discrepancy was explained by hardly controllable qualitative reasoning on the differences existing between the purely homogeneous process and the electrochemical process where the homogeneous reactions are coupled with diffusion. What is the actual answer to the question as results from a rigorous treatment of the hypothesized competition between the two mechanisms in the context of diffusion to and from the electrode? The problem can be formulated in dimensionless terms as described by eqns. (15)–(17).

$$\frac{\partial a}{\partial \tau} = \frac{\partial^2 a}{\partial y^2} \cdot \frac{\partial b}{\partial \tau} = \frac{\partial^2 b}{\partial y^2} - \frac{2RT}{F}\frac{k_1 C^\circ}{v} b^2 \left(1+\frac{K_2 k_3}{k_1} C^\circ a\right) \qquad (15)$$

$$\tau=0, y\geq 0 \text{ and } y\to\infty, \tau>0: a=1, b=0 \qquad (16)$$

$$y=0, \tau>0: (\partial a/\partial y)+(\partial b/\partial y)=0, \quad a=b\exp[-(F/RT)(E-E^\circ)], \qquad (17)$$

$E=E_i+vt$ for $0\leq t\leq\theta$, $E=E_f-v(t-\theta)$ for $t\geq\theta$

the current-time function being obtained as:

$$t2i=-FSD^{1/2}C^\circ(Fv/RT)^{1/2}(\partial a/\partial y)_0$$

with $\tau=Fvt/RT$, $y=x(Fv/RTD)^{1/2}$, $a=[R]/C^\circ$, $b=[R^{\cdot+}]/C^\circ$; t: time, x: distance from the electrode, C°: bulk conc. of R, E: electrode potential, E°: $R/R^{\cdot+}$ standard potential, E_i: initial potential, E_f: scan inversion potential, θ: scan inversion time, S: electrode surface area, D: diffusion coefficient of R and $R^{\cdot+}$. The calculations were carried out for $(F/RT)(E^\circ-E_i)=10$, E_i being then sufficiently negative to E° for having practically no influence of the cyclic voltammogram. E_f was taken 300 mV positive to E°, at 22 °C.

Since it is assumed that the rrc mechanism predominates at high C°'s, k_1 is found as equal to 2.76×10^4 M^{-1} s^{-1} (right-hand side asymptote in Fig. 6). Similarly $K_2k_3=1.28\times10^8$ M^{-2} s^{-1} as derived from the rsc behavior assumed to predominate at low C°'s (left-hand side asymptote in Fig. 6). The above system and thus the ratio of the derivative peaks are thus functions of only two parameters, v and C°. For each value of C°, the value of v, $v_{1/2}$, for which this ratio equals 0.5 was numerically obtained from an explicit finite difference resolution of the above system.[30] The resulting $v_{1/2}-C^\circ$ working curve is shown in Fig. 6 (dashed-line) in comparison with the experimental data.

It is clearly seen that the predicted kinetics is in complete disagreement with the experimental data, showing that the proposed rrc–rsc competi-

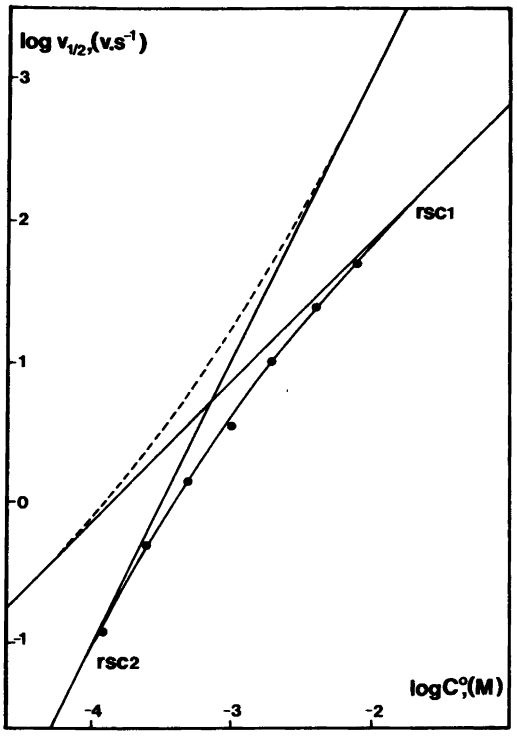

Fig. 6. Anodic electrodimerization of 4-methoxybiphenyl. Comparison between experimental kinetic data (●) (from Table 1 in Ref. 11c) and predicted kinetics for an rrc–rsc2 competitive mechanism (– – –) and an rsc mechanism (——). $v_{1/2}$: sweep rate value necessary for the ratio of the derivative peaks on the backward and forward scans to equal 0.5. $C°$: 4-methoxybiphenyl bulk concentration.

tive mechanism is certainly wrong. This casts severe doubts on the contention that the process going on at high concentrations involves the radical–radical coupling of two cation radicals.[11c] Important consequences concerning the chemistry of electrodimerization in general ensue. The dimerization reaction occurring at high concentration in the oxidation of 4-methoxybiphenyl was indeed taken as the prototype of radical–radical coupling in the ion-radical series, a reaction claimed to occur very scarcely [7, 11c] in contrast with the conclusions of past investigations. In this connection, the activation energy of the reaction, 10.6 K cal/mol, was regarded as a minimum value for the rrc of all ion radicals, serving as a piece of evidence against the reality of previously postulated rrc processes for which the activation energies are smaller.[11c] All these reasonings now become very doubtful since the very existence of an rrc process in the oxidation of 4-methoxybiphenyl in the high concentration range appears itself as doubtful. On the other hand, rrc of ion radicals may well involve activation energies significantly smaller than 10 Kcal/mol as discussed above.

Since the rrc–rsc competitive mechanism is clearly ruled out by proper analysis of the experimental data, the question arises of the actual nature of the mechanism of the oxidative electrodimerization of 4-methoxybiphenyl. Let us consider that the rrc pathway is negligible, *i.e.*, that dimerization totally proceeds along the rsc mechanism. In this context, we remove the restriction that (3′) is the rds with (2′) remaining at equilibrium. In other words, it is considered that both steps may participate to the kinetic control of the overall rsc process. There are then two limiting kinetic behaviors, one in which (2′) is the rds and another in which (3′) is the rds with (2′) acting as a pre-equilibrium. These limiting behaviors will be named rsc1 and rsc2, respectively. For rsc1, $v_{1/2}$ is proportional to $C°$ while for rsc2 it is proportional to $(C°)^2$. Passing from rsc2 to rsc1 implies that reaction (3′) becomes more and more efficient as opposed to backward reaction (2′). An increase of $C°$ is anticipated to produce this effect since reaction (3′) is second order and backward reaction (2′) is first order. This is similar to what occurs in disproportionation processes where the chemical step interposed between the electrode electron transfer and the solution electron transfer is a first order reaction in both directions.[24a] In more quantitative terms, the overall process is described by the following system (eqns. (18), (19)) which involves the stationary state assumption concerning $^{\cdot}R-R^{+}$.

$$\frac{\partial a}{\partial \tau} = \frac{\partial^2 a}{\partial y^2} \tag{18}$$

$$\frac{\partial b}{\partial \tau} = \frac{\partial^2 b}{\partial y^2} - \frac{2RT}{F}\,\frac{k_2 c°}{\nu}\,\frac{ab^2}{b+\dfrac{k_{-2}}{k_3 C°}} \tag{19}$$

with the same initial and boundary conditions as for the rrc–rsc2 mechanism and the same symbolism. The rsc1 behavior is obtained for $k_3 C° \gg k_{-2}$,

i.e., at the high concentration limit. It ensues that k_2 can be estimated as $k_2=3.55\times10^4$ M^{-1} s^{-1}. The rsc2 behavior is reached when $k_3C^\circ \ll k_{-2}$, *i.e*, at the low concentration limit. We then obtain an estimation of k_2k_3/k_{-2}, $k_2k_3/k_{-2}=1.28\times10^8$ M^{-2} s^{-1}. The system and thus the ratio of the derivative peaks are thus a function of the two parameters ν and C°. For each value of C°, the value of ν, $\nu_{1/2}$, for which this ratio equals 0.5 was then numerically obtained according to the same computation procedure as for the rrc–rsc2 case. The resulting $\nu_{1/2}-C^\circ$ working curve is shown in Fig. 6 (solid line) in comparison with the experimental data; the best fitting with the experimental data leads to the following values: $k_2=4.07\times10^4$ M^{-1} s^{-1} and $k_3k_2/k_{-2}=1.65\times10^8$ M^{-2} s^{-1}.

It is seen that the agreement between the predicted and experimental kinetic behaviors is now excellent. The rsc mechanism is thus compatible with the DVC data. For the following reasons, some caution should, however, be exerted as to the conclusion that this is the actual mechanism of the reaction. We attempted to check that the linear sweep voltammetric peak variations with sweep rate and concentration predicted for such a mechanism are indeed followed. In the low range of concentrations ($C^\circ \simeq 0.1$ mM) this implied the use of rather low sweep rates ($\nu<0.01$ V s^{-1}) in order that the wave becomes irreversible, *i.e.*, that "pure kinetic" conditions be achieved. It was also of interest to raise the concentration so as to attempt to reach the region where the rsc1 behavior predominates which corresponds to $\partial E_p/\partial \log \nu=-30$ mV and $\partial E_p/\partial \log C^\circ=30$ mV as opposed to -20 and 20 mV, respectively, for the rrc mechanism. In our hands, these experimental conditions led to poor accuracy and reproducibility of peak potential measurements, resulting most probably from product adsorption. It was suspected that the deprotonated dimer could itself be oxidized in the same potential region as the starting compound possibly leading to polymeric products. Whether these reactions are or are not a part of the process taking place under DCV conditions is not clear at present. More generally, the exact nature of the reaction products formed under these conditions is not ascertained as compared to what is obtained under preparative scale conditions.[31]

In conclusion, it appears worthwhile to again emphasize the dangers of using "reaction order, without calculations" approaches to mechanism analysis outside of their actual range of validity, *i.e.*, in the case of reactions that are kinetically controlled by more than one step. On a plea of avoiding mathematical complexity, these approaches thus ignore the availability of sound theoretical background which would allow the right mechanistic conclusions to be drawn. In this sense, they amount to a regression with regard to the present capabilities of mechanism analysis of electrochemical reactions.

CONCLUDING REMARKS

1. Contrary to recent contention,[7] radical–radical coupling of anion radicals does not appear as an uncommonly followed pathway. The reductive electrodimerization of 9-substituted anthracenes do follow this type of mechanism. The results obtained with these molecules showed that steric factors should be taken into account when comparing the activation energy with the rate constant at a given temperature. Erroneous conclusions recently drawn in this respect were founded on the misconception that the activation energy of the reaction should be compared to the activation energy of the diffusion limiting process.[7,11c] This implicitly assumes that the pre-exponential factors are the same for both processes since, actually, the rate constants should be compared through:

$$\frac{1}{k_{obs}} = \frac{1}{k_{dif}} + \frac{1}{k_{act}}$$

and not directly the activation energies. In other words, such a procedure amounts to the neglecting of steric factors in all cases, taking the perfect gas collision frequency as pre-exponential factor.

In the case of activated olefins, radical–radical coupling also appears as a rather common pathway except in very dry aprotic media. In the latter case, some evidence exists that a radical–substrate coupling pathway prevails although this is still not a fully demonstrated conclusion.

2. Acceleration of the dimerization reaction by addition of water appears to be related in a number of cases to specific solvation of the anion radicals rather than to protonation by water. The "anion radical–proton donor complex mechanism",[6b] is a possible mode of the role of

water under particular circumstances. It is by no means a unique pathway accounting for the effect of water. Other pathways such as dimerization of two complexed anion radicals or even dimerization of complexes involving more than one anion radical may well occur, depending upon the nature of the starting molecule and the water concentration.

3. The danger of using "reaction order, without calculation" approaches to the analysis of electrochemical mechanisms not controlled by a single rate-determining step should be emphasized. Far from being "capable of unravelling many of the complexities of ion radical reactions" [29d] or of showing "their true power in such situations".[29c] these oversimplified approaches actually lead to erroneous mechanistic conclusions. They should be replaced by correct kinetic analyses for which a large background is now available as illustrated by the above discussion. Besides the reduction of 9-substituted anthracenes, the oxidative dimerization of 4-methoxy biphenyl [11c] is a typical illustration of the shortcomings of such oversimplified treatments of the kinetic data. Important consequences as to the chemistry of cation radicals ensue.

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Are Low or Negative Activation Enthalpies Consistent with the Alleged One Step Mechanism for the Dimerization of 9-Substituted Anthracene Anion Radicals?

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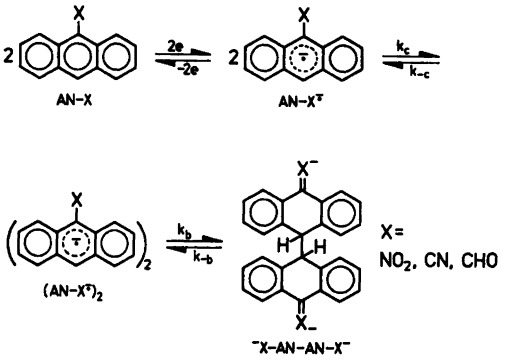
9-Cyano- and 9-nitroanthracene anion radicals undergo dimerization in DMF and DMSO without the interference of acetic acid at concentrations up to about 0.1 M. This allows for the study of the dimerization mechanisms under conditions where the reactions are irreversible. Detailed comparisons of derivative cyclic voltammetric and double potential step chronoamperometric experimental and theoretical data showed the data to fit the dimerization mechanisms, either a simple one step or one involving an association of two anion radicals before bonding occurs. Water at concentrations below about 0.2 M was observed to have a negligible effect on the reaction rates and a competing mechanism becomes significant at higher [H_2O]. Very low enthalpies of activation (1.2 and −0.2 kcal/mol) were observed for the reactions of 9-cyano- and 9-nitroanthracene anion radicals, respectively. The data are discussed in terms of a two step mechanism involving first a reversible association of two anion radicals before the bond forming reaction. The conclusions are compared with current thought in reactive intermediate chemistry where low and negative activation enthalpies are frequently observed.

We recently arrived at the following conclusions,[1] based upon kinetic studies of the *reversible* dimerization of 9-substituted anthracene anion radicals. "Since the only species that we have been able to identify by the voltammetric and kinetic studies involved in the anion radical–dimer dianion equilibria are the ions, we find no alternative than to postulate that the reactions involve two coupled equilibria. The first (Scheme 1) could be the formation of a complex involving two anion radicals which are not covalently bonded to each other and the second involves the reversible bond formation".



Scheme 1.

Amatore, Pinson and Savéant [2] reinvestigated the reactions of 9-cyanoanthracene anion radical and presented a critique of our work. Their conclusions were that a simple dimerization mechanism (1) is followed and that the experimental data, theirs *as well as ours,* are compatible with theoretical data assuming that model. Our conclusions were attributed to artefacts in the kinetic treatment and to the neglect of

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results from classical chemical kinetics. Comments were also made [2,3] concerning related work.[4-6]

$$2\ AN{-}X^{\cdot-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} {}^{-}X{-}AN{-}AN{-}X^{-} \qquad (1)$$

A number of comments are in order at the outset regarding the method in which Amatore, Pinson and Savéant [2] have disputed the mechanism that we proposed.[1] Their case lies entirely in the fact that experimental data can be fitted to theoretical data for mechanism (1). What they *do not* consider is that theoretical data are not unique for a particular reaction mechanism and fit equally well for another mechanism giving an identical rate law. The rate law for reaction (1) is (2). This is identical to that for the mechanism in Scheme 1 with the exception that the constants have different meanings as is evident from rate law (3).

$$\text{Rate} = k_1 C^2_{\text{AN-X}^{\cdot-}} - k_{-1} C_{(\text{X-AN-AN-X})^{2-}} \qquad (2)$$

$$\text{Rate} = k_b K_c C^2_{\text{AN-X}^{\cdot-}} - k_{-b} C_{(\text{X-AN-AN-X})^{2-}} \qquad (3)$$

Therefore, theoretical working curves are not capable of distinguishing between the two possibilities. The latter should have been emphasized, but was not even mentioned. Because the two mechanisms are indistinguishable from the kinetic point of view some other evidence must be relied upon. We concluded that the apparent activation energies for the reactions were much lower than expected and in the case of $AN{-}NO_2^{\cdot-}$ there may even be an inverse temperature effect.[1] Amatore, Pinson and Savéant [2] discuss only the $AN{-}CN^{\cdot-}$ case which we found to have the highest activation energy and they conclude that this is consistent with reaction (1). They do not mention the $AN{-}NO_2^{\cdot-}$ results which were our strongest point of evidence regarding activation energies. They claim that one of our arguments against the simple one step mechanism was that the Arrhenius pre-exponential factor for the reaction of $AN{-}CN^{\cdot-}$ was much smaller than the standard collision frequency. The Arrhenius *A* factor was *not* evaluated in our work.[1] In fact, we did not discuss the possible significance of the *A* factors for any of the compounds in our study. The *A* factors that can be calculated from our data [1] (Table 1) are *not* particularly small when compared to values which have been reported for a number of similar reactions in solution.[7] Here we would like to emphasize that in solution the collision process may differ considerably from that in the gas-phase and the use of an *A* factor based on the collision theory for bimolecular gas-phase reactions as a standard for data obtained in solution is highly questionable.[8] In this context it should also be stressed that because of the accumulative nature of errors, the evaluation of *A* factors is, as a rule, encumbered with a high degree of uncertainty. For these reasons, along with the fact that the mechanism must be known with certainty before the *A* factor has significance, we have not based our arguments on the latter. Furthermore, it is surely not justifiable to base mechanistic conclusions on a single *A* value close to 10^9 M^{-1} s^{-1} as was done.[2]

In this paper, we show that the reactions can be studied without the complications of the reverse

Table 1. Summary of rate constants and activation parameters for the dimerization of 9-substituted anthracene anion radicals in aprotic solvents.

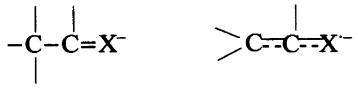
Compound Data from Solvent	9-Cyano Ref. 1 DMF	9-Cyano Ref. 2 DMSO	9-Formyl Ref. 1 DMF	9-Nitro Ref. 1 DMF
k/M^{-1} s^{-1}	$1.3 \cdot 10^5$	$1.9 \cdot 10^5$ [a]	$3.1 \cdot 10^5$	$1.6 \cdot 10^6$
T/°C	22	20.5	22	22
E_a/kcal mol^{-1}	4.2	4.6	3.2	<1.8 [b,c]
$\Delta H^{\ddagger}$/kcal mol^{-1}	3.6 [c]	4.0 [c]	2.6 [c]	<1.2 [b,c]
A/M^{-1} s^{-1}	$2 \cdot 10^8$ [c]	$7.9 \cdot 10^8$	$<3 \cdot 10^7$ [b,c]	
$\Delta S^{\ddagger}$/cal K^{-1} mol^{-1}	−23 [c]	−21 [c]	−25 [c]	<−26 [b,c]

[a] k_1. [b] Estimated maximum value from a non-linear Arrhenius plot. [c] Calculated from the data in Refs. 1 and 2.

reactions by having acetic acid present to rapidly protonate the dianions. We support our proposal of a two step mechanism with additional experimental data and show how it is consistent with other recent discussions on similar behaviour observed in reactive intermediate chemistry.

RESULTS AND DISCUSSION

The effect of acetic acid on the dimerization. It has recently been shown [9,10] that some dimerization reactions of anion radicals are kinetically unaffected by the presence of acetic acid in low concentrations. In general, anions with structural element *1* will be more basic and protonated more rapidly than the corresponding anion radical with structural element *2*. This is the situation in



1 *2*

such reactions as protonation or dimerization of anion radicals. We have employed these observations to design kinetic experiments under conditions that the dimerization reactions of $AN-X^{\cdot-}$ are irreversible. Under these conditions the last terms in rate laws (2) and (3), due to dissociation of the dimers, are insignificant due to the rapid protonation of the dimer dianions.

The data in Table 2 show the effect of acetic acid concentration on the apparent rate constants for the dimerization of $AN-CN^{\cdot-}$ in DMF at 20.6 °C. The point of importance is that the rate constant is independent, $1.95(\pm 0.13)\times 10^5$ M^{-1} s^{-1}, of the acetic acid concentration up to 40 mM. The rate constant measured by double potential step chronoamperometry (DPSC) [11] is slightly larger than the one we reported earlier,[1] 1.32×10^5 M^{-1} s^{-1}, based on derivative cyclic voltammetry (DCV) studies. A comparable set of experiments indicated that the rate constant for dimerization of $AN-NO_2^{\cdot-}$ is equal to 2.3×10^6 M^{-1} s^{-1} as compared to 1.6×10^6 M^{-1} s^{-1} in the absence of acetic acid. If these small differences are real, the lower values in the absence of the acid could reflect the contribution of the dissociation of the dimer dianions. That the influence of the dissociations is small is also illustrated by the data in Table 1, which summarizes the results from previous work.[1,2] Only in the evaluation of the data reported in Ref. 2 was the contribution from the dissociation taken into account. In spite of this, the data from the two independent studies are almost identical. The lack of influence of the dissociation on observed rate constants is even more clearly brought out by the data in Tables 7 and 8 discussed later.

Table 2. The effect of acetic acid concentration on the rate of dimerization of 9-cyanoanthracene anion radical.[a]

C_{HOAc}/mM	$\tau_{1/2}$ [b]/ms	k [c]/10^5 M^{-1} s^{-1}
8	7.76	2.14
12	8.95	1.85
20	8.76	1.89
40	8.71	1.91
		Av. 1.95 (0.13)

[a] Measurements by DPSC in DMF containing Bu_4NBF_4 (0.1 M) at 20.6 °C. [b] Pulse width. [c] Rate constant assuming the simple dimerization mechanism.

It was not only necessary to show that the reaction rates for the dimerizations are essentially independent of the acetic acid concentration but also that the reactions remain second order in anion radical as had been demonstrated earlier in the absence of acid.[1] The data in Tables 3 and 4 serve this purpose. The data for $AN-CN^{\cdot-}$ were

Table 3. Reaction order study of the dimerization of 9-cyanoanthracene anion radical in the presence of acetic acid.[a]

C_{AN-CN}/mM	T/°C	$\tau_{1/2}$ [b]/ms	k [c]/10^5 M^{-1} s^{-1}
0.5	30.0	8.85	1.88
1.0	30.0	4.86	1.71
2.0	30.0	2.48	1.67
			Av. 1.78 (0.11)
0.5	−29.8	20.6	0.806
1.0	−29.8	11.1	0.748
2.0	−29.8	5.61	0.740
			Av. 0.765 (0.036)

[a] Measurements by DPSC in DMF containing Bu_4NBF_4 (0.1 M) and acetic acid (0.004 M). [b] Pulse width. [c] Rate constant assuming the simple dimerization mechanism.

Table 4. Reaction order study of the dimerization of 9-nitroanthracene anion radical in the presence of acetic acid.[a]

C_{AN-NO_2}/mM	T/°C	$v_{1/2}$[b]/V s^{-1}	k[c]/10^6 M^{-1} s^{-1}
0.1	39.9	50.3	2.19
0.2	39.9	99.0	2.15
0.1	28.8	50.0	2.26
0.2	28.8	95.4	2.15
0.1	10.4	49.7	2.39
0.2	10.4	95.3	2.29
0.1	−0.6	44.5	2.22
0.2	−0.6	80.5	2.01

[a] Measurements by DCV in DMF containing Bu_4NBF_4 (0.1 M) and acetic acid (0.004 M). [b] Sweep rate. [c] Rate constant assuming the simple dimerization mechanism.

obtained by DPSC in the concentration range, 0.50 to 2.00 mM, at two widely separated temperatures, −30 and +30 °C. The apparent rate constants were constant to ±6 % indicating a good fit to second order kinetics. The faster dimerization of $AN-NO_2^{\cdot-}$ was studied by DCV at lower concentrations, 0.1 and 0.2 mM. Because of the low concentrations necessary to hold the rate low enough to make measurements it was not possible to get DPSC data in this case. Apparent rate constants were measured at four temperatures and at each temperature were found to vary by less than ±10 %, again showing a very good fit to second order kinetics.

Thus, we conclude that the dimerization of the anion radicals are unaffected by the presence of low concentrations of acetic acid. In order to test for reversibility, experiments were conducted by DCV at low sweep rates in the manner described earlier.[1] In these cases, the derivative peak current ratio decreased with decreasing v (voltage sweep rate) in the manner expected for an irreversible reaction of the anion radicals. This is in contrast to the opposite behaviour observed in the absence of acetic acid. Therefore, in the presence of the acid the dissociation of the dimer dianion can be neglected. This point will be discussed in more detail in the next section.

Table 5. Normalized DPSC working curve analysis of the dimerization of 9-cyanoanthracene anion radical.[a]

R_I	$(kC_A\tau)_{theory}$	$(yC_A\tau)^b_{exp}$	$(yC_A\tau)^c_{exp}$
0.80	0.20	0.21	0.20
0.75	0.26	0.27	0.27
0.70	0.35	0.35	0.35
0.65	0.43	0.43	0.43
0.60	0.54	0.54	0.55
0.55	0.66	0.67	0.67
0.50	0.83	0.83	0.83
0.45	1.03	1.06	1.03
0.40	1.26	1.30	1.30
0.35	1.58	1.63	1.70
0.30	2.07	2.08	2.12
0.25	2.67	2.73	2.66
m_1=		0.97	1.00
m_2=		1.00	1.01
m_T=		1.00	1.01

[a] Measurements in DMF containing Bu_4NBF_4 (0.1 M) and acetic acid (0.004 M). C_{AN-CN}=0.5 mM. [b,c] Two independent data sets.

Comparison of experimental data for the dimerization reactions to theoretical data. Experimental DPSC data for the dimerization of AN−CN anion radical are compared to theoretical data for irreversible dimerization in Table 5. As mentioned earlier, a fit of experimental and theoretical data in this case does not differentiate between the two mechanisms in question. However, a comparison of the data does give a measure of the compatibility of the experimental data to the mechanisms. The method used for the comparison is that recently reported.[12] For the second order dimerization mechanism the basis of the method is eqn. (4)

$$(yC_A\tau)_{R_I} = (kC_A\tau)_{R_I} \quad (4)$$

which states that at any particular value of R_I, the normalized current ratio, the pulse width τ multiplied by the substrate concentration C_A and a constant y is equal to the theoretical value of $kC_A\tau$ for a perfect fit of experimental to theoretical data. If the theoretical data apply to the mechanism being investigated, y is equal to the rate constant k for the process. We evaluated y by assuming relationship (4) when R_I is equal to 0.50 and then calculated $yC_A\tau$ for each value of R_I given in Table 5. The next step in the analysis involves evaluating three linear regression slopes,

in this case m_1 (R_I=0.80–0.50), m_2 (R_I=0.50–0.25) and m_T (R_I=0.80–0.25). For a fit of experimental and theoretical data, $m_1=m_2=m_T=1.00$. In the two independent sets of data we observed 0.97, 1.00, 1.00 and 1.00, 1.01, and 1.01 for the three slopes. This represents an acceptable fit of data for the dimerization mechanisms. Data at τ less than 2 ms were observed to deviate from the theoretical values and were not included in the correlations. We do not consider this deviation serious since low C_A (0.5 mM) were involved which can give rise to interference by the double layer charging process at short times. Theoretical data for different mechanisms usually do not differ much for high values of R_I. The most significant deviations are expected at long times and would be reflected in m_2.

The data in Table 6 are for a comparable DCV analysis of the dimerization of AN–$NO_2^{\cdot-}$ in DMF both in the presence (4 mM) and absence of acetic acid. In both cases m_1 (R'_I=0.70–0.50) and a slope encompassing R'_I from 0.70 to 0.40 were reasonably close to unity, 1.01 and 1.04 (in the presence of HOAc) and 0.97 and 1.07 (in the absence of HOAc). At lower R'_I yC_A/a where a is Fv/RT deviated significantly from the theoretical values of kC_A/a. Analysis of the AN–$CN^{\cdot-}$ dimerization using CV and a concentration of 0.5 mM gave similar results. The data fit the working curve at short times, *i.e.* down to R'_I of about 0.4, but deviate at long times. We do not have a good explanation of this behaviour but have good reason to believe that it is not due to a deviation from the reaction mechanism at long times since an excellent fit of data to the DPSC working curve was observed under comparable conditions for AN–$CN^{\cdot-}$ as demonstrated in Table 5. Any electrode surface complicatons would influence the data most at long times and low concentrations. The fact that the data for the two experiments in Table 6 are identical within experimental error illustrates that the dissociation of the dimer dianion does not influence the data even when acetic acid is not present.

What we can conclude from the data in Tables 5 and 6 is that in the presence of small amounts of acetic acid experimental data for the reactions of both AN–$CN^{\cdot-}$ and AN–$NO_2^{\cdot-}$ are consistent with theoretical data for the dimerization mechanisms.

The role of water in the dimerization of 9-cyanoanthracene anion radical in DMF and DMSO. The effect of added water on the apparent rate constants for the dimerization of AN–$CN^{\cdot-}$ in the absence and the presence of HOAc (4.4 mM) in DMF and DMSO is shown by the data in Tables 7 and 8, respectively. In all cases, the data show that water has a negligible effect at concentrations lower than about 0.2 M. The data were treated in the same manner as in a recent study on the dimerization of benzaldehyde anion radical in aqueous ethanol.[13] Since the reaction is second order in anion radical regardless of the water concentration, the apparent rate constant k_{app} must consist of a term for the reaction not involving water (k_0) and that involving water, k_W. If the latter is first order in water, k_{app} is described by eqn. (5).

$$k_{app} = k_0 + k_W[H_2O] \quad (5)$$

If (5) is an adequate description of k_{app}, dividing the $k_W[H_2O]$ term by the concentration of added water should result in a constant which is the third order rate constant for the reaction second order in AN–$CN^{\cdot-}$ and first order in water. On the other hand, if there is any contribution from a reaction second order in water, k_W will not be a constant and will increase with increasing $[H_2O]$. In DMF rate constants were determined at $[H_2O]$ as great as 2.5 M while

Table 6. Normalized DCV working curve analysis of the dimerization of the 9-nitroanthracene anion radical.[a]

R'_I	$(kC_A/a)_{theory}$	$(yC_A/a)^b_{exp}$	$(yC_A/a)^c_{exp}$
0.70	0.039	0.036	0.039
0.65	0.048	0.050	0.052
0.60	0.065	0.066	0.067
0.55	0.087	0.087	0.084
0.50	0.118	0.118	0.118
0.45	0.165	0.165	0.162
0.40	0.24	0.25	0.26
0.35	0.38	0.43	0.42
0.30	0.62	0.86	1.01
0.25	1.12	2.48	1.79

[a] The analysis is described in the text. Measurements in DMF containing Bu_4NBF_4 (0.1 M). [b] C_{AN-NO_2}=0.1 mM, C_{HOAc}=1.6 mM. [c] As *b*, but without HOAc.

Table 7. The effect of water on the rate of dimerization of 9-cyanoanthracene anion radical in DMF.[a]

C_{H_2O}/M	C_{HOAc}/mM	$k^b/10^5$ M^{-1} s^{-1}	$k_W C_{H_2O}/10^5$ M^{-1} s^{-1}	$k_W/10^5$ M^{-2} s^{-1}
0	0	2.00	–	–
0.19	0	2.03	0.03	0.16
0.37	0	2.22	0.22	0.60
0.55	0	2.23	0.23	0.42
0.73	0	2.31	0.31	0.42
1.09	0	2.45	0.45	0.41
				Av. 0.40 (0.16)
0	4.4	2.06	–	–
0.19	4.4	2.16	0.10	0.54
0.37	4.4	2.18	0.12	0.33
0.55	4.4	2.34	0.28	0.51
0.73	4.4	2.35	0.29	0.40
1.09	4.4	2.52	0.46	0.42
1.79	4.4	2.88	0.82	0.46
2.48	4.4	3.20	1.14	0.46
				Av. 0.45 (0.07)

[a] Measurements by DCV in solvent containing Bu_4NBF_4 (0.1 M) and 9-cyanoanthracene (0.50 mM) at a mercury electrode at 19 °C. [b] Apparent rate constant evaluated from the voltage sweep rate necessary for the ratio of the derivative peaks to equal 0.700.

Table 8. The effect of water on the rate of dimerization of 9-cyanoanthracene anion radical in DMSO.[a]

C_{H_2O}/M	C_{HOAc}/mM	$k^b/10^5$ M^{-1} s^{-1}	$k_W C_{H_2O}/10^5$ M^{-1} s^{-1}	$k_W/10^5$ M^{-2} s^{-1}
0	0	2.60	–	–
0.19	0	2.71	0.11	0.59
0.37	0	2.82	0.22	0.60
0.55	0	2.89	0.29	0.53
0.73	0	2.99	0.39	0.53
1.09	0	3.14	0.54	0.50
1.79	0	3.40	0.80	0.45
2.48	0	3.75	1.15	0.46
3.47	0	4.24	1.64	0.47
				Av. 0.52 (0.06)
0	4.4	2.66	–	–
0.19	4.4	2.72	0.06	0.32
0.37	4.4	2.83	0.17	0.46
0.55	4.4	2.84	0.18	0.33
0.73	4.4	2.95	0.29	0.40
1.09	4.4	3.16	0.50	0.46
1.79	4.4	3.54	0.88	0.49
2.48	4.4	3.86	1.20	0.48
3.47	4.4	4.34	1.68	0.48
				Av. 0.43 (0.07)

[a,b] See Table 7.

water concentrations as high as 3.5 M were employed in the experiments in DMSO. The last columns in Tables 7 and 8 give k_W. In all cases, k_W is constant within experimental error and no trends are evident with increasing $[H_2O]$. Also, all four series of experiments give values of k_W identical within the error limits. The values observed range from 0.40 to 0.52×10^5 M^{-2} s^{-1}. Another interesting feature of the data are that the rate constants obtained are independent of whether or not acetic acid was present in either solvent. The rate constants measured in DMSO are about 30 % larger than those obtained in DMF.

In the course of the rate constant measurements, the peak potential for the reduction of AN−CN was measured at 100 V s^{-1}. At this sweep rate the ratio of the derivative peaks of the cyclic voltammogram was of the order of 0.65−0.85 so that the peak potential measurement at 100 V s^{-1} was very nearly the reversible value. The peak potentials were observed to be independent of $[H_2O]$ to ±1 mV up to about 1 M and then a slight shift in the negative direction was observed at higher concentrations. The latter is most likely due to some adsorption which was not serious enough to hamper the kinetic measurements. Under normal circumstances, phase selective second harmonic a.c. voltammetry is the method of choice for reversible potential measurements.[14] However, in this case the peak potential measurements discussed above appear to be more reliable. This is evident from the data in Table 9 for the potentials measured by phase selective second harmonic a.c. voltammetry. The potentials listed are for the quadrature component and even greater shifts were observed for the in phase component. The data indicate that some adsorption process is causing a phase shift so that the measured potentials are not the reversible values. But since we could not detect any shift in the peak potentials measured at 100 V s^{-1} at $[H_2O]$ less than 1 M, we conclude that the reversible potential is independent of the water concentration. Thus, $AN-CN^{-\cdot}$ *is not specifically solvated by water* in either DMF or DMSO.

Table 9. Phase selective second harmonic *a.c.* reversible potential measurements on the reduction of 9-cyanoanthracene in aqueous DMSO.[a]

C_{H_2O}/M	$-E_{zc}$(300 Hz)[b]/mV	ΔE_{zc}/mV
0	286.4(0.5)	0
0.19	287.4(0.3)	−1.0
0.37	288.8(0.4)	−2.4
0.55	290.1(0.3)	−3.7
0.73	291.7(0.2)	−5.3
1.09	293.2(0.2)	−6.8

[a] In solvent containing Bu_4NBF_4 (0.1 M) and HOAc (4.4 mM). DC voltage sweep rate was 40.0 mV/s. [b] The zero current crossing potentials of the quadrature component referred to a bias potential of −1.273 V *vs.* Ag/Ag^+ in acetonitrile. The numbers in parentheses are the standard deviations in 5 replicate measurements.

The general effect of water then is that at $[H_2O]$ greater than about 0.2 M, a competing mechanism begins to become important. The reaction is second order in $AN-CN^{-\cdot}$ and first order in water. The most likely mechanism is the pre-equilibrium mechanism previously found for the dimerization of diethyl fumarate anion radical.[4,15] In the present case this mechanism consists of equilibrium (6) followed by reaction (7) and is described by rate law (8).

$$AN-CN^{-\cdot}+H_2O \overset{K_6}{\rightleftharpoons} AN-CN^{-\cdot}/H_2O \qquad (6)$$

$$AN-CN^{-\cdot}/H_2O+AN-CN^{-\cdot} \xrightarrow{k_7} \text{dimer} \qquad (7)$$

$$\text{Rate} = k_7K_6[AN-CN^{-\cdot}]^2[H_2O] \qquad (8)$$

Although it would be possible to gain more definitive information about this reaction by carrying out deuterium kinetic isotope effect studies as has been done in related cases,[4,13] this was deemed unnecessary since the reaction only takes place when substantial amounts of water are added which is aside from the conditions of interest to this study.

Amatore, Pinson and Savéant [2] have commented on the pre-equilibrium mechanism [4] and suggest that specific solvation by water is a safer and more general formulation. In fact, more recently the same authors have suggested that the dimerization of $AN-CN^{-\cdot}$ in DMSO in the presence of water involves specific solvation by water and can involve coupling of either one solvated anion radical with an unsolvated one or of two solvated anion radicals.[16] The first possibility is just a restatement of the pre-equilibrium mechanism. The second possibility implies that

the reaction should become second order in water at the higher water concentrations where it is alleged to take place. We do not observe this mechanism even when [H_2O] in DMSO is 3.5 M. The fact that k_6 is apparently very small argues that it would not be possible to observe this mechanism under conditions where the measurements can be made.

It is interesting to note that the pre-equilibrium mechanism [4,15] is now being called specific solvation by water.[16] This is admittedly an unimportant point but one that cannot pass without comment. The use of this terminology is meant to imply that this is what was meant by specific solvation in earlier work.[17] Just to set the record straight we quote from the paper discussing the effect of water on the electrodimerization of anion radicals.[17] The following two paragraphs are taken directly from that paper.

"If, thus, $A^{\cdot-}$ is assumed to be solvated and not protonated by the added water, the only possible reaction sequence is: $A+1e^- \rightleftarrows A^{\cdot-}$, $2A^{\cdot-}$ (solvated by water)$\rightarrow D^{2-}$ (rate determining step), $D^{2-}+2\ H_2O \rightarrow DH_2+2\ OH^-$ as in the case where E_p is independent of the water addition.

Now, the variation of E_p with the amount of water present can be attributed to the fact that the coupling of two anion radicals is easier when they are surrounded by more water molecules."

In our opinion, this unwillingness to accept that the pre-equilibrium mechanism is distinctly different from what is implied by specific solvation and the preceding paragraphs leads to confusion and does little for the task at hand, *i.e.* to advance the knowledge on how anion radicals couple. An example of the confusion caused is the effect of water on the rate of dimerization of $AN-CN^{\cdot-}$. We have shown that water has a negligible effect at concentrations less than 0.2 M. A different reaction begins to take place at higher [H_2O] which competes with the mechanism that is being examined in drier media. Then we ask the following question. Why add water in the first place? The overall result is that it confuses the entire issue. If the reason to add water is to study the competing reaction, *i.e.* mechanism (6)+(7), AN−CN is a poor choice of substrate since K_6 is so small that it is not detected by voltammetric measurements. A much better case, the dimerization of diethyl fumarate anion radical, has been studied rather thoroughly.[4,15] In this case it was possible to not only determine K for the association reaction but also to evaluate $\Delta H°$.

The effect of [Bu_4N^+] *on the rate of dimerization of* $AN-CN^{\cdot-}$. Our kinetic data for the dimerization of $AN-CN^{\cdot-}$ and $AN-NO_2^{\cdot-}$ suggest a pre-equilibrium mechanism which we have proposed to consist of the reactions in Scheme 1. An alternative possibility, the water complex mechanism (6)+(7), has been ruled out. A third possibility is that the supporting electrolyte cation, Bu_4N^+, pairs with $AN-CN^{\cdot-}$ in a pre-equilibrium (9) before rate determining coupling reaction (10). In order to test for this mechanism, [Bu_4NBF_4] was varied by a factor of 8 in a series of rate measurements on the dimerization of $AN-CN^{\cdot-}$.

$$AN-CN^{\cdot-}+Bu_4N^+ \overset{K_9}{\rightleftharpoons} AN-CN^{\cdot-}/Bu_4N^+ \qquad (9)$$

$$AN-CN^{\cdot-}/Bu_4N^+ + AN-CN^{\cdot-} \xrightarrow{k_{10}} \text{dimer} \qquad (10)$$

The data are summarized in Table 10. The apparent rate constant does depend upon the supporting electrolyte concentration but increases by only 50 % as [Bu_4NBF_4] was increased from 0.025 to 0.20 M. Mechanism (9)+(10) could account for our data if Bu_4NBF_4 was significantly associated in DMF. However, association constants for tetraalkylammonium salts in DMF are too small [18] to account for the data. We think that it is more likely that the increase in rate caused by the increase in the supporting electrolyte concentration is simply a salt effect. The transition state leading to the formation of the dimer dianion is highly polarized and would be

Table 10. The effect of Bu_4N^+ concentration on the rate of dimerization of 9-cyanoanthracene anion radical in DMF.[a]

$C_{Bu_4NBF_4}$/M	$v_{0.7}$/V s^{-1}	$k/10^5$ M^{-1} s^{-1}
0.025	47.1	1.46
0.050	57.4	1.78
0.100	66.8	2.07
0.200	71.6	2.22

[a] Measurements by DCV at 19 °C at a mercury electrode. The difference in switching and reversible potentials was 300 mV.

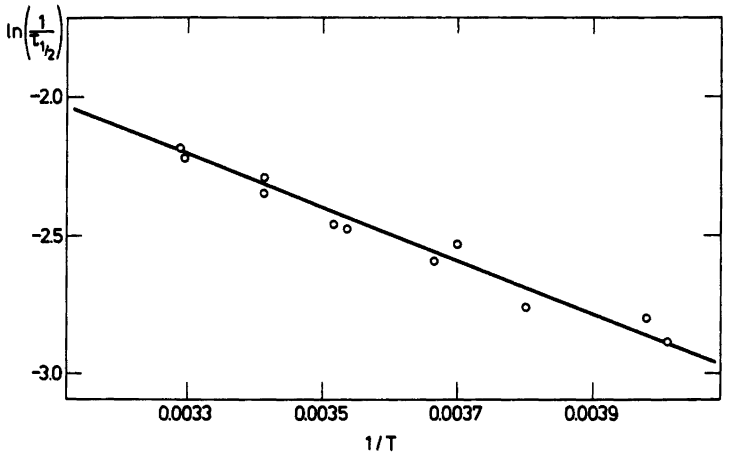


Fig. 1. Arrhenius plot for the dimerization of 9-cyanoanthracene anion radical (0.5 mM) in DMF containing Bu_4NBF_4 (0.1 M) and acetic acid (4 mM).

stabilized by a more polar environment. However, we cannot rule out that mechanism (9)+(10) represents a competing reaction.

The effect of temperature on the kinetics and the mechanistic implications of the results. The kinetics were studied at temperatures ranging from −27 to 31 °C by DPSC for the reaction of $AN-CN^{-}$ and by DCV for the faster reactions of $AN-NO_2^{-}$ in DMF containing HOAc. Activation energies were obtained using eqn. (11) for DCV and eqn. (12) for DPSC.[19]

$$\ln (v_{1/2}/T) = -E_a/RT+\text{constant} \quad (11)$$

$$\ln (1/\tau_{1/2}) = -E_a/RT+\text{constant} \quad (12)$$

The subscripts (1/2) indicate that v or τ necessary for the normalized current ratios R'_I (DCV) or R_I (DPSC) to equal 0.500 were evaluated at each temperature. It is necessary to divide $v_{1/2}$ by T for the activation energy determination since the normalized sweep rate is a ($=Fv/RT$). The results from these measurements are presented in Figs. 1 and 2. In both cases satisfactory linear relationships according to eqns. (11) and (12) were observed. The activation energies, E_a, and hence enthalpies, $\Delta H^{\neq}$,

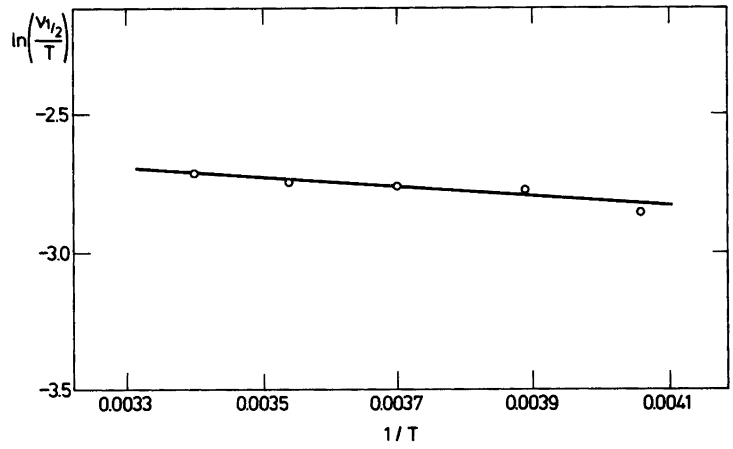


Fig. 2. Arrhenius plot for the dimerization of 9-nitroanthracene anion radical (0.1 mM) in DMF containing Bu_4NBF_4 (0.1 M) and acetic acid (0.8 mM).

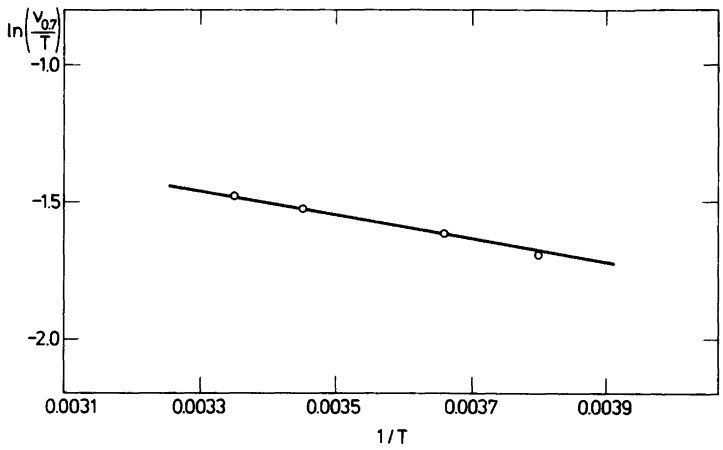


Fig. 3. Arrhenius plot for the dimerization of 9-cyanoanthracene anion radical (0.5 mM) in DMF in the presence of H_2O (0.07 M) and Bu_4NBF_4 (0.1 M).

were calculated from the slopes. The values of the activation enthalpies were found to be 1.2 kcal/mol ($AN-CN^{\cdot-}$) and −0.2 kcal/mol ($AN-NO_2^{\cdot-}$) with activation entropies of −30 cal/K mol in both cases. The values observed in the presence of HOAc are somewhat smaller than those resulting from the measurements in the strictly aprotic solvent.

In order to determine whether or not the presence of acetic acid is responsible for the lower activation energies observed in this study as compared to that reported earlier,[1] we carried out a series of experiments under conditions comparable to those reported in Table 7. Since water does not affect the rate at concentrations lower than about 0.2 M, the experiments were carried out with [H_2O] equal to 0.07 M. To minimize any possible effect of the reversibility of the reaction in the absence of acetic acid, $v_{0.7}$ was taken as the measure of the apparent rate constant. This is equivalent to the measurement of initial rates and involves a time scale about 30 % as long as when $v_{0.5}$ is measured. Measurements were made at temperatures ranging from −10 to 25 °C. The data are illustrated in Fig. 3. The apparent enthalpy of activation was observed to be equal to 0.3 kcal/mol, *i.e.* somewhat smaller that obtained for the data in Fig. 1. Thus, it appears that in the presence of low concentrations of either water or acetic acid the activation enthalpies are very low while the rate of dimerization at room temperature is virtually unaffected.

The important point to be made regarding the activation enthalpies is that they are significantly smaller than those expected for reactions controlled by translational diffusion. Such deviations from "normal behaviour" have been reported in a number of cases including the dimerization of phenoxy radicals [7,20–22] and nitroxide radicals,[23] the proton transfer between 2,4-dinitrophenol and aliphatic tertiary amines,[24] several photochemical reactions,[25–27] an example of the Diels-Alder reaction [28] and the reaction of methyl phenyl ketene with 1-phenyl ethanol.[29] The general conclusion in *all* these chemically very different cases is that the results can only be satisfactorily explained if it is assumed that the reaction in question proceeds through at least one intermediate, in many cases called a complex, which is present in low concentration and in equilibrium with starting material (eqns. (13) and (14)). This mechanism gives rise to rate law (15).

$$A+B \text{ (or A)} \underset{k_{-13}}{\overset{k_{13}}{\rightleftharpoons}} C \qquad (13)$$

$$C \xrightarrow{k_{14}} D \qquad (14)$$

$$\text{Rate} = \frac{k_{13}k_{14}}{k_{-13}+k_{14}}[A][B] \text{ or } \frac{k_{13}k_{14}}{k_{-13}+k_{14}}[A]^2 \qquad (15)$$

Because the heat of formation for the complex, ΔH°_{13}, is a negative term, the overall activation enthalpy, $\Delta H^{\ddagger}$, which is made up of two terms (eqn. (16)), may become very small and even negative.

$$\Delta H^{\ddagger} = \Delta H^{\circ}_{13} + \Delta H^{\ddagger}_{14} \quad (16)$$

Similarly the activation enthropy, $\Delta S^{\ddagger}$, consists of two terms (eqn. (17)).

$$\Delta S^{\ddagger} = \Delta S^{\circ}_{13} + \Delta S^{\ddagger}_{14} \quad (17)$$

These are both negative resulting in $\Delta S^{\ddagger}$ values typically in the range -15 to -40 cal/K mol, but values as low as -70 cal/K mol have been reported in special cases.[23,29] As a result of this, $\Delta G^{\ddagger}$ may take appreciable positive values and consequently the reactions appear as relatively slow. The values we find for the activation enthropies, with or without HOAc present, are all between -23 and -30 cal/K mol and thus in excellent agreement with our view on the mechanism, *i.e.* that the reaction passes through an intermediate, or in other words that the potential energy surface for the reaction has at least one energy minimum.[30,31] We do not wish to speculate on the nature or the life-time of these intermediates. However, in the light of the view expressed [3] that the relative slowness of the reactions could be explained, as an alternative to our proposal, by assuming that a large fraction of the collisions between the anion radicals are inefficient and thus not leading to products, we do want to point out an important feature of collisions in solution. Compared to the gas-phase where the life-time of an encounter complex is only that of one collision, an encounter complex in solution may survive as many as 1000 collisions between the reactants before they separate again or go to products, the so-called cage-effect.[8,32–35] The interactions between the molecules in the solvent cage are rather weak and not strictly directional and do not hinder rotational or translational events within the cage, events which finally may lead to the right geometry for bond formation. Whether or not such a complex will manifest itself kinetically as an intermediate is a question of how deep the energy minimum is and the relative importance of the contributions from enthalpy and entropy terms. Obviously we do not believe that all such collisions are efficient in the present case, but the steric requirements for the bond formation are in no way related to the question of the number of elementary steps in the reaction.

Thus, although our experimental results are unusual they are *not* sufficiently peculiar that their interpretation requires that we have to "overlook rather classical results of chemical rate theory".[2] On the contrary. As it is evident from the discussion in the preceding paragraphs our observation of $\Delta H^{\ddagger}$-values significantly smaller than those for diffusion controlled reactions is perfectly compatible with classical rate theory even without the necessity to speculate on the nature of the molecular collisions or to argue by help of non-specific steric factors.[2,3] The way in which the criticism of our work is based on such arguments is reminiscent of the following section from North's book:[8] "One of the most unsatisfactory features of the collision theories of chemical reactions is the so-called steric factor. This factor is incorporated into the rate expression for a chemical reaction in order to include the probability that the orientation of the molecules during a collision may or may not be favourable for chemical reaction to take place. It therefore is very often of an empirical nature, and as such used as a cloak for ignorance in fitting experimentally observed rate constants to so-called "normal theoretical" values".

Comments on the "reaction order" approach. Amatore, Pinson and Savéant [2] imply that the kinetic methods developed in one of our laboratories [36,37] and for simplicity described as the "reaction order" approach have been known for a long time. To substantiate the latter, reference was made to an older paper.[38]

Before rebutting these implications we must examine what is meant by the "reaction order" approach and some of the circumstances which led to its development in the way it is meant to be applied. During the study of two different electrode processes, the oxidation of hexamethylbenzene and 4-bromoanisole in acetonitrile, good fit of experimental data to theoretical working curves for first order processes were observed.[39] In both cases, the rate constants observed were strongly dependent upon the substrate concentrations showing that the fit of experimental and theoretical data was coincidental in both cases. In addition, unusual temperature effects were observed. In fact, apparent negative activation

energies of the order of −4 kcal/mol were observed for the hexamethylbenzene and the perdeuterated isomer cation radical reactions. Following this, it was pointed out that apparent activation energies can be derived from experimental voltammetric data without doing any calculations or without being aware of the mechanism of the process in question.[19] The only requirement for the application of this method is that the electrode response can be described by eqn. (18)

$$\text{Observable} = f(k \cdot \text{Variables}) \qquad (18)$$

in which the Observable is the measured quantity such as the peak current ratio in cyclic voltammetry and the variables are the time of the experiment (sweep rate in CV), concentration, *etc.* Eqn. (18) then is the basis for the "reaction order" approach. The method was amplified [36] and it was shown that it could be used to define the rate law for an unknown process without doing any theoretical calculations. A limitation of the method, which was clearly pointed out,[36] is that the reaction orders in substrate (A) and primary intermediate (B) defined in eqn. (19)

$$A \pm e^- \rightleftarrows B \qquad (19)$$

are not separable and were expressed as $R_{A/B}$. It was suggested that this uncertainty should be taken into account by examining possible mechanisms with the appropriate combinations of the individual reaction orders R_A and R_B.

Amatore, Pinson and Savéant attempt to discredit the approach and imply that it fails when more than a single rate determining step is involved in a mechanism. It is a simple matter to test this conclusion. Three theoretical working curves are presented in Ref. 2 for such a case where the "reaction order" approach is alleged to fail. The curves are constructed according to relationship (20) where R is the normalized current ratio in DPSC and θ

$$R = f(kC_A\theta) \qquad (20)$$

is the pulse width. It is clear that this representation fits general relationship (18). What the calculations add is that it is possible to determine the two parameters k_1 and K_1 if the mechanism assignment is correct. *However, working curves are not unique to a particular mechanism.* This particular example is an excellent demonstration of this. The results presented here show that the working curves apply to the mechanism in Scheme 1 as well and that the activation parameters are inconsistent with eqn. (1). Amatore, Pinson and Savéant have made the fundamental mistake of assuming that a data fit to a theoretical working curve is sufficient to establish a mechanism. What this does show is that there are definite limitations to the use of theoretical calculations in mechanism assignment. This is precisely the fact that led to the development of the "reaction order" approach.[19,36,37,39]

We can now examine the implication [2] that the "reaction order" approach was known in 1963.[38] The paper cited deals with the peak potential shift in linear sweep voltammetry brought about by reactions (21) and (22).

$$O \overset{(ne)}{\rightleftarrows} R \qquad (21)$$

$$mR \xrightarrow{k} P \qquad (22)$$

The effect of the kinetics on the peak potential was summarized by the following sentence. "Le potentiel de pic varie linéairement avec log v et log C, les pentes étant respectivement, à 25 °C, de $60/n(m+1)$ mV/unité de log v et $(m-1)/(m+1) \cdot 60/m$ mV/unité de log C". We agree that the latter represents the use of a *reaction order* but has no bearing whatsoever on the DCV and DPSC methods that we use in kinetic studieds. In the primary paper on this subject [36] reference is made to the discussion by Bezilla and Maloy on reaction orders in electrode measurements.[40] The latter was in conjunction with the use of theoretical working curves. It is difficult to find justification for the implications made by Amatore, Pinson and Savéant.[2,3]

Conclusions. We conclude that the question raised by Amatore, Pinson and Savéant [2] relating to whether or not a simple dimerization of anion radicals ever takes place has not been answered. Our data clearly show that it does not take place during the dimerization of 9-substituted anthracene anion radicals as they emphatically concluded. In this paper we have addressed the question as to whether or not low or negative activation enthalpies are consistent with the one

step mechanism. Our conclusion is that they are *not* and that such evidence is of great value in treating reactions for which kinetic data are consistent with either one step or complex reaction schemes.

EXPERIMENTAL

The instrumentation, electrodes, cells and data handling procedures were those described earlier.[41] Reagent grade DMF and DMSO containing the supporting electrolyte (Bu_4NBF_4) were passed through a column containing neutral alumina before use. 9-Nitroanthracene (Fluka, *purum*) and 9-cyanoanthracene (EGA-Chemie) were recrystallized from 1-propanol prior to use. DPSC was carried out by potential steps from about 300 mV less negative than E_{rev} to about 300 mV more negative at 50 s intervals between measurements. Derivative cyclic voltammetry experiments were carried out as previously described.[42]

NOTE

After the writing of this paper was completed another note appeared by Amatore, Pinson and Savéant dealing with the effect of water on the dimerization of $AN-CN^-$ in DMSO.[43] At very high water concentrations (up to 16 M) they found that the apparent rate was greater than can be accounted for by our eqn. (5) and they suggest some contribution of the dimerization of $AN-CN^-/H_2O$. Although this is a natural consequence of going to high enough $[H_2O]$ so that equilibrium (6) provides high enough concentrations of the complex, we do not agree that their data prove this effect. The bulk properties of aprotic solvents like DMSO do not change appreciably by addition of water up to about 5 % but do change significantly at higher proportions of water.[44-46] It was for this reason that we limited our water concentration to about 5 %. The transition state for the formation of the dimer dianion, regardless of the exact mechanism, is highly polar since there is a localization of charge in the two monomer entities. Therefore, going to a more polar solvent system is likely to cause an increase in rate. Thus, it is surely not safe to conclude that a mechanism change takes place in going to the very high water concentrations. The effect can just as well be explained by the change in the bulk properties of the solvent. In any event, the authors [43] neglect to consider this possibility and therefore their mechanistic conclusions must be viewed with suspicion. These suspicions have been confirmed by a reinvestigation of the linear sweep voltammetry and DCV behaviour of AN−CN in both DMF and DMSO with water concentrations as high as 16 M.[47]

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Mechanisms of the Electrohydrodimerization of Activated Olefins. VII.* The Validity of Rate–Activation Energy Relationships for Dimerization Reactions of Ion Radicals

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Diethyl fumarate (DEF) anion radical undergoes dimerization in wet dimethylformamide (DMF) by a two-step mechanism involving pre-equilibrium (i) and coupling reaction (ii)

$$DEF^{\cdot-} + Z \overset{K_i}{\rightleftarrows} DEF^{\cdot-}/Z \qquad (i)$$

$$DEF^{\cdot-}/Z + DEF^{\cdot-} \xrightarrow{k_{ii}} \text{dimer} \qquad (ii)$$

in which Z has been identified as either water or alkali metal cations. The association reaction (i) can have negative $\Delta H°$. Since the rate law for these processes is (iii), the apparent rate constant is equal to $k_{ii}K_i$.

$$\text{Rate} = k_{ii}K_i[DEF^{\cdot-}]^2[Z] \qquad (iii)$$

Thus, the apparent activation energies (E_a) reflect the contributions of both k_{ii} and K_i and can be very small and even negative. When Z is H_2O, E_a was observed to be 1.9 kcal/mol $\Delta H_i°$ was found to be −5.9 kcal/mol which results in E_a for reaction (ii) equal to 7.8 kcal/mol. When Z is Na^+, E_a was observed to be 4.1 kcal/mol and K_{ii} at 298 K was estimated to be of the order of 0.2 M^{-1} or less giving a minimum estimate of k_{ii} to be 10^7 $M^{-1}s^{-1}$. A maximum value of 10 $M^{-1}s^{-1}$ (at 298 K) was estimated for the rate constant for the dimerization of uncomplexed $DEF^{\cdot-}$ under the reaction conditions. Assuming that the entropy factors for the latter reaction are similar to reaction (ii) leads to estimations of E_a for the dimerization in the range, 11–12 kcal/mol. It was concluded that anion radical dimerization reactions do not show unusual rate constant–activation energy relationships.

In a preliminary communication of this work,[1] the kinetics of the dimerization of diethyl fumarate (DEF) anion radical in wet dimethylformamide (DMF) were investigated. The reaction was observed to be first order in H_2O and a very small kinetic isotope effect k_H/k_D was observed.

A previous investigation [7] had resulted in a second order rate constants of 44 $M^{-1}s^{-1}$ attributed to reaction (1)

$$2\ DEF^{\cdot-} \xrightarrow{k_1} \text{dimer} \qquad (1)$$

with an Arrhenius activation energy (E_a) of about 4 kcal/mol. On the basis of the new kinetic data along with the apparent incompatibility of the rate constant–activation energy data, a two-step mechanism was proposed involving pre-equlibrium (1) followed by coupling reaction (2).

$$DEF^{\cdot-} + H_2O \overset{K_2}{\rightleftarrows} DEF^{\cdot-}/H_2O \qquad (2)$$

$$DEF^{\cdot-}/H_2O + DEF^{\cdot-} \xrightarrow{k_3} \text{dimer} \qquad (3)$$

Amatore, Pinson and Savéant [8] have commented on this work and imply that this adds little new information to the mechanisms of anion radical dimerization since $DEF^{\cdot-}/H_2O$ is still an anion radical. Therefore, they recommend viewing the

* See Refs. 1–6 for other parts in this series.

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water effect as one of specific solvation as had previously been advocated.[9] The latter view would seem to be totally inconsistent with the reasons for studying the mechanism of a reaction. We find it much more important to identify the various steps in a reaction rather than be satisfied with an overall rate constant for the process. Thus, we consider mechanism (2)+(3) to differ significantly from mechanism (1) with specific solvation of the anion radicals by water.

One of the primary consequences of a pre-equilibrium such as (1) is that the observed activation parameters do not reflect the contributions from a single step and are thus of limited value in discussions of the energetics of the reactions. On the other hand, the observation of unusual effects of the temperature upon observed rate constants can be of importance in assigning mechanisms to reactions. Such studies are common in physical organic chemistry [10] but have been used to a very limited extent in the study of electrode processes. This was recently pointed out and examples were discussed where inverse temperature effects were observed.[12] Savéant and coworkers [8,13] have attacked our use of low activation energies as an indication of complex reaction mechanisms and have implied that we have overlooked classical results of chemical rate theory. We have invoked arguments relating to temperature effects a number of times and thus this issue is of considerable importance to our work. The evidence and conclusions arrived at for a number of processes with unusual temperature behaviour are summarized in Table 1.

In order to gain a better understanding of rate constant–activation energy relationships for ion radical dimerization reactions, two known cases involving pre-equilibria were reinvestigated. The reactions studied were the anion radical water complex reaction (2)+(3) and the ion association reaction (4) and (5)

$$DEF^{\cdot-}+M^+ \overset{K_4}{\rightleftarrows} DEF^{\cdot-}/M^+ \quad (4)$$

$$DEF^{\cdot-}/M^+ + DEF^{\cdot-} \xrightarrow{k_5} \text{dimer} \quad (5)$$

previously studied in DMF by Hazelrigg and Bard [19] and in DMSO by Ryan and Evans.[20]

RESULTS

The effect of water on the rate of dimerization of diethyl fumarate anion radical. In the preliminary report of this work the water concentration was varied up to 0.55 M in DMF and an approximate first order relationship was observed.[1] Rate

Table 1. Unusual temperature effects reported for electrode processes.

Process	Evidence	Conclusions	Ref.
Dehalodimerization of 4-halo-*N,N*-dimethyl aniline cation radicals	$E_a \sim 0$	Reversible dimerization	13
Deprotonation of hexamethylbenzene cation radicals	Inverse temperature effect $E_a \sim -4$ kcal/mol	Complex kinetic scheme	11
Dimerization of dimethoxystilbene cation radicals	$E_a \sim 0$	Complex mechanism	14
Coupling of 4-methoxybiphenyl cation radicals	$E_a \sim 0$ at low concentration	Complex mechanism	15
Dimerization of 9-substituted anthracene anion radicals	$E_a < 1$ kcal/mol for the 9-nitro derivative	Pre-equilibrium followed by dimer formation	16–17
Dimerization of 9-diazofluorene anion radicals	Non-linear Arrhenius plot, $E_a \sim 2$ kcal/mol	Pre-equilibrium followed by dimer formation	18
Dimerization of *p*-methylbenzylidene malononitrile anion radicals	Concentration dependent E_a as low as 1 kcal/mol	Pre-equilibrium followed by dimer formation	6

Table 2. Effect of water on the dimerization of diethyl fumarate anion radical in DMF.[a]

$[H_2O]$/mM	Lit.[b] $k_{obs}/M^{-1}s^{-1}$	This work $k_{obs}/M^{-1}s^{-1}$	$k^c/M^{-2}s^{-1}$	
139	121	—		
278	242	235	845	
417	349	—		
550	458	412	749	
820		618	754	
1090		817	750	743±13
1355		980	723	
1618		1193	737	
Intercept[d]/$M^{-1}s^{-1}$	11	30		
Corr. coef.[d]	0.9997	0.9995		

[a] Measurements by derivative cyclic voltammetry at a gold electrode at 20.6 °C. [b] The rate constants reported in Ref. 1 are too large by a factor of 1.42. The correct relationship between $v_{1/2}$ and k at 295 °C is $k=4.64\ v_{1/2}/C_A$. [c] Third order rate constants obtained by dividing k_{obs} by the water concentration. [d] From correlation of k_{obs} *vs.* C_{H_2O}.

constants as a function of C_{H_2O} are gathered in Table 2. The data are from this work as well as from Ref. 1 and include C_{H_2O} up to 1.6 M. The observed rate constants (k_{obs}) correlate linearly with C_{H_2O} and give intercepts which should be related to the second order rate constant in the absence of added water. At C_{H_2O} greater than 0.5 M the third order rate constants obtained by dividing k_{obs} by C_{H_2O} were observed to be constant to ±2 % with a value of 743 $M^{-2}s^{-1}$. The extrapolated rate constants C_{H_2O} (added water)=0 correspond to those expected if the residual water introduced in the solvent-electrolyte was 15 (11 $M^{-2}s^{-1}$) and 40 (30 $M^{-2}s^{-1}$) mM assuming all the reaction goes according to mechanism (2)+(3).

The effect of water on the reversible potential for the reduction of diethyl fumarate in DMF. The phase selective second harmonic *a.c.* zero crossing potentials (E_{zc}) gathered in Table 3 were obtained on a solution of DEF (0.1 mM) in DMF. The reason for the low concentration of DEF was to diminish the effect of the second order reaction of the anion radical on the electrode potential measurements. Measurements were made at frequencies of 100 and 300 Hz. Any kinetic complications would cause E_{zc} at the two frequencies to differ by a degree depending upon the rate of the reaction. At all C_{H_2O} the two E_{zc} are within 0.5 mV of each other. This suffices to show that the potentials listed are for the reversible charge transfer (6).

$$DEF + e^- \overset{E_{rev}}{\rightleftarrows} DEF^{\cdot-} \qquad (6)$$

At C_{H_2O} of 0.14 M or greater, correlation of ΔE_{rev} *vs.* C_{H_2O} resulted in a correlation coefficient of 0.9998 and an intercept of −34 mM. After adding 34 mM to all C_{H_2O}, linear regression over all of the data (including the "dry" entry) resulted in a correlation coefficient of 0.9999 with an intercept of 3.7 mM. Finally, a correlation assuming the "dry" solution to be 30 mM in water and adding 30 mM to all C_{H_2O} gave a correlation coefficient of 0.9999 and an intercept of only −0.3 mM. From this it can be concluded that the residual water introduced in the solvent-electrolyte was present at a concentration of about 30 mM for the experiments described in Table 3. If the association of $DEF^{\cdot-}$ with H_2O can be described by equilibrium (7), the shift in E_{rev} with increasing C_{H_2O} is related to K_7 by eqn. (8).

$$DEF^{\cdot-} + m\ H_2O \overset{K_7}{\rightleftarrows} DEF^{\cdot-}/(H_2O)_m \qquad (7)$$

$$\Delta E_{rev} = (RT/F) \ln (1+K_7 C_{H_2O}{}^m) \qquad (8)$$

The last column in Table 3 shows that the data fit eqn. (8) for $m=1$ reasonably well and omitting the values for the two highest water concentrations results in a value for K_7 of 0.57±0.07 M^{-1}. The increasing trend at the higher water concentration may be due to contributions where $m=2$.

Table 3. The effect of water concentration in DMF on the reversible potential for the reduction of diethyl fumarate.[a]

$[H_2O]$/mM	$-E_{zc}$(100 Hz) [b]	$-E_{zc}$(300 Hz) [b]	ΔE_{rev}/mV	K^d/M^{-1}
Over Al_2O_3	342.98(0.08)	342.72(0.00)	0	—
"Dry" [c]	342.57(0.08)	342.15(0.00)	0.49	0.66
28	342.12(0.08)	341.97(0.00)	0.74	0.52
70	341.66(0.10)	341.66(0.10)	1.20	0.49
139	340.76(0.10)	340.46(0.00)	2.24	0.55
278	338.84(0.10)	338.96(0.18)	3.95	0.55
550	335.01(0.00)	334.63(0.13)	8.03	0.65
1090	327.56(0.10)	327.82(0.25)	15.2	0.74
2020	315.26(0.00)	315.78(0.08)	27.3	0.96

[a] Measurements by phase selective second harmonic a.c. voltammetry at a mercury electrode in solvent containing DEF (0.1 mM) and Bu_4NBF_4 (0.1 M) at 18.9 °C. [b] The quadrature zero current crossing potentials referred to a bias potential of −1.46 V *vs.* Ag/Ag^+ in acetonitrile. The numbers in parentheses refer to the standard deviation in 5 measurements. [c] No added water. [d] The equilibrium constant for the association of $DEF^{\cdot-}$ with water assuming that $[H_2O]$ in the "dry" solution was 30 mM, which was added to all other water concentrations.

The effect of sodium ion on the reversible potential for the reduction of diethyl fumarate in DMF. The data in Table 4 were obtained by phase selective second harmonic *a.c.* voltammetry measurements on a solution containing DEF (0.1 mM) and H_2O (27 mM added) at 18.3 °C. It was only possible to make reliable measurements of C_{NaI} up to 6.6 mM. At C_{NaI} of 13.2 mM the *a.c.* current was greatly diminished and the E_{zc} were not reproducible. At higher C_{NaI} the signal for the quadrature component completely disappeared. This behaviour is most likely due to a phase shift caused by adsorption on the electrode and was not further investigated.

In the C_{NaI} range where E_{rev} measurements were possible, E_{zc} was constant to ±0.2 mV which is about the experimental error in the measurements. The numbers in parentheses in Table 4 refer to the standard deviation in 5 replicate measurements without disturbing the cell. The error becomes larger when the cell is disturbed by the addition of a reagent. The relationship between ΔE_{rev} and K_4 is defined in eqn. (9).

$$\Delta E_{rev} = (RT/F) \ln (1+K_4 C_{Na^+}) \quad (9)$$

Since we conclude that ΔE_{rev} is no greater than 0.2 mV when C_{NaI} is 6.6 mM and assuming that Na^+ is not associated with supporting electrolyte anions as is indicated from other work,[19] we obtain a maximum value of K_4 (where M^+ is Na^+) of about 0.5 M^{-1}.

The reaction order in sodium ion during the dimerization of diethyl fumarate anion radical in DMF. Kinetic measurements were carried out using either derivative cyclic voltammetry (DCV) or double potential step chronoamperometry (DPSC). Second order rate constants obtained at C_{NaI} ranging from 4.2 to 110 mM are summarized in Table 5. Dividing k_{obs} by C_{NaI} gives a third order rate constant which should be independent of C_{NaI} if the reaction is first order in sodium ion. The DCV measurements gave a value of $1.04\pm0.07\times10^6$ $M^{-2}s^{-1}$ while a value of $1.53\pm0.11\times10^6$ $M^{-2}s^{-1}$ was obtained from the

Table 4. The effect of sodium ion concentration on the reversible potential for the reduction of diethyl fumarate in DMF.[a]

[NaI]/mM	$-E_{zc}$(300 Hz) [b]
0	328.1(0.1)
3.3	327.8(0.1)
6.6	328.2(0.1)
13.2	diminished signal [c]

[a] Measurements by phase selective second harmonic *a.c.* voltammetry at a mercury electrode in solvent containing Bu_4NBF_4 (0.1 M), DEF (0.1 mM) and water (27 mM added) at 18.3 °C. [b] Zero current crossing potential of the quadrature component referred to a bias potential of −1.47 V *vs.* Ag/Ag^+ in acetonitrile. The numbers in parentheses refer to the standard deviation in 5 measurements. [c] At this concentration the *a.c.* signal became very small and completely disappeared at higher concentrations.

Table 5. The reaction order in sodium ion in the dimerization of diethyl fumarate anion radical in DMF.[a]

[NaI]/mM	Method [b]	$k_{obs}/M^{-1}s^{-1}$	$10^{-1}k_{obs}/[Na^+]/M^{-2}s^{-1}$
4.18	DCV	4.18×10^3	1.00
8.35	DCV	8.07×10^3	0.967
16.7	DCV	1.66×10^4	0.994
55.0	DCV	6.19×10^4	1.12
110	DCV	1.21×10^5	1.10
			1.04±0.07
16.7	DPSC	2.33×10^4	1.40
55.0	DPSC	8.68×10^4	1.58
110	DPSC	1.61×10^4	1.61
			1.53±0.11

[a] In solvent containing Bu_4NBF_4 (0.1 M), water (70 mM) and DEF (2.0 mM) at 16.3 °C. [b] Measurements by either derivative cyclic voltammetry or double potential step chronoamperometry at an Hg electrode.

DPSC data. The deviations observed are about those expected from experimental error. We can conclude that under the conditions of our measurements, the reaction is first order in sodium ion.

The rate constants obtained by DCV are about 0.67 those from DPSC. We have no explanation for this discrepancy but note that it is not uncommon that absolute values of rate constants obtained by various electrochemical techniques differ somewhat.

Apparent activation energies for the dimerization of diethyl fumarate in DMF. Apparent second order rate constants for the dimerization of $DEF^{\cdot-}$ under various conditions were obtained over temperatures ranging from −6 to 40 °C. Arrhenius activation energies along with k_{298}, apparent second order rate constants at 298 K, obtained from the correlations are summarized in Table 6. The correlation coefficients are for correlation of the rate constants measured at five different temperatures. The point of most interest is that E_a is about 1.9 kcal/mol when H_2O but no metal ions are present, about 4.4 kcal/mol in the presence of Li^+ and 4.1 kcal/mol in the presence of Na^+ ions.

In order to determine the degree of precision in the E_a values, three determinations were carried out by DPSC on the same solution containing DEF (2.0 mM), NaI (16.7 mM) and water (70 mM added). The data are shown in Table 7. The correlations resulted in E_a equal to 4.1±0.1 kcal/mol and k_{298} equal to $2.95\pm0.08\times10^4$ $M^{-1}s^{-1}$.

Table 6. Apparent activation energies and rate constants for the dimerization of diethyl fumarate anion radical in DMF.[a]

[DEF]/mM	$[H_2O]$/mM	$[M^+]$/mM	Method [b]	$k_{298}/M^{-1}s^{-1}$	E_a/kcal mol^{-1}	r [c]
2.0	1090	0	DCV	1.14×10^3	1.9	0.994
9.6	1090	0	DCV [d]	0.86×10^3	1.8	0.989
2.0	dry [e]	4.7(Li^+)	DPSC	1.88×10^4	4.4	0.997
2.0	dry [e]	11.8(Li^+)	DPSC	4.10×10^4	4.3	0.999
1.9	dry [e]	8.0(Na^+)	DPSC	1.10×10^4	4.0	0.985
1.9	dry [e]	8.0(Na^+)	DCV	7.59×10^3	4.2	1.000
1.9	dry [e]	3.3(Na^+)	DCV	3.12×10^3	3.9	1.000

[a] Measurements at 5 different temperatures ranging from −6 to 40 °C. [b] Derivative cyclic voltammetry (DVC) and double potential step chronoamperometry (DPSC). [c] Correlation coefficient of the Arrhenius plot. [d] At a gold electrode, all other measurements were at mercury electrodes. [e] No added water.

Table 7. Precision of activation energy measurements for the dimerization of diethyl fumarate anion radical in DMF.[a]

Determination [b]	E_a/kcal mol^{-1}	$10^{-4}k_{298}$/M^{-1}s^{-1} [c]
1	4.2	2.86
2	4.0	3.00
3	4.1	2.98
	4.1±0.1	2.95±0.08

[a] In solvent containing Bu_4NBF_4 (0.1 M), water (70 mM added), DEF (2.0 mM) and sodium iodide (16.7 mM). [b] Replicate measurements on the same solution at five temperatures ranging from −6 to 40 °C by DPSC at a mercury electrode. [c] Apparent second order rate constant at 298 K.

Table 9. The effect of temperature on the equilibrium constant for the association of diethyl fumarate anion radical with water in DMF.[a]

T/K	K [b]/M	$-\Delta S°$ [c]/cal K^{-1}mol^{-1}
302.4	0.44	21.1
292.1	0.56(0.01) [d]	21.4
272.9	1.24(0.42) [e]	21.2

[a] Measurements by phase selective second harmonic a.c. voltammetry at 300 Hz and d.c. sweep rate of 40.0 mV/s. [b] Calculated from ΔE_{rev} at water concentrations ranging rom 0.1 to 0.6 M. [c] Calculated using the observed value of $\Delta H°$ equal to −5.9 kcal/mol (correlation coefficient=0.994). [d] Two independent determinations. [e] Four independent determinations.

The effect of water on the rate and activation energy of the anion radical–sodium ion pair reaction. The data in Table 8 were obtained by DCV on solutions containing DEF (2.0 mM) and NaI (8.7 mM). The value of E_a was found to be nearly independent of C_{H_2O} and equal to 3.6±0.2 kcal/mol while a slight trend was found in k_{obs} with increasing water concentration. The column labeled k_{corr} is k_{obs} corrected for the contribution of the water reaction with a second order rate constant of 743 M^{-1}s^{-1} (Table 2). The decreasing trend is quite apparent in k_{corr}. What can be concluded from this data is that the product k_5K_4 decreases slightly with increasing water concentration. It seems likely that K_4 is decreased and k_5 is either unaffected or increased.

A further point concerning the data in Table 8 is that E_a measured by DCV are about 0.5 kcal/mol less than those obtained by DPSC. A possible reason for this is that the DCV data are somewhat more sensitive to uncompensated solution resistance than are the DPSC data. We have observed that some adsorption takes place in the presence of sodium ion (Table 4). This could bring about uncompensated resistance problems and contribute to some error in DCV activation energies. For this reason we regard the DPSC values in Table 7 to be more reliable than those in Table 8.

The thermodynamic parameters for the association of diethyl fumarate anion radical with water. Equilibium constants for reaction (7) where $m=1$ were determined at three temperatures with C_{H_2O} ranging from 0.1 to 0.6 M. The data are summarized in Table 9. Significant variations in the K_7 values were observed at the lowest temperature and 4 independent determinations were made. Correlation of ln K_7 *vs.* T^{-1} resulted in an estimate of −5.9 kcal/mol for $\Delta H°_7$ and a correlation coefficient of 0.994. The last column gives the values of $\Delta S°_7$ and the small degree of variation shows that the data are linearly related.

Table 8. The effect of water concentration on the kinetics of the dimerization of diethyl fumarate anion radical in DMF in the presence of sodium iodide.[a]

$[H_2O]$/mM	E_a/kcal mol^{-1}	$10^{-4}k_{obs}$/M^{-1}s^{-1}	$10^{-4}k_{corr.}$ [b]/M^{-1}s^{-1}
0	3.5	1.11	1.11
69.5	3.8	1.02	1.02
139	3.2	0.994	0.984
278	3.4	1.020	1.000
550	3.7	0.973	0.932
1090	3.7	0.955	0.874

[a] Measurements by DCV in solvent containing Bu_4NBF_4 (0.1 M), sodium iodide (8.7 mM) and DEF (2.0 mM) at a mercury electrode. Data at five temperatures ranging from −6 to 40 °C. [b] k_{obs} (at 298 K) corrected by subtracting $743C_{H_2O}$.

DISCUSSION

For the general case of an anion radical dimerization consisting of pre-equilibrium (10) followed by coupling reaction (11),

$$A^{\cdot-} + Z \overset{K_{10}}{\rightleftarrows} A^{\cdot-}/Z \quad (10)$$

$$A^{\cdot-}/Z + A^{\cdot-} \xrightarrow{k_{11}} \text{dimer} \quad (11)$$

the apparent rate constants and activation energies are expected to depend strongly on K_{10}. If K_{10} is much greater than 1 and Z is in excess, essentially all of $A^{\cdot-}$ will exist as $A^{\cdot-}/Z$ in solution and both the rate of the reaction and the apparent E_a will be independent of K_{10} and C_Z. Under these circumstances reaction (11) would probably be insignificant compared to the dimerization of $A^{\cdot-}/Z$. (12).

$$2\ A^{\cdot-}/Z \xrightarrow{k_{12}} \text{dimer} \quad (12)$$

On the other hand, if K_{10} and C_Z are sufficiently small that $A^{\cdot-}$ is the predominant species in solution the rate law will be (13)

$$\text{Rate}_{10,11} = k_{11}K_{10}C_{A^{\cdot-}}{}^2C_Z \quad (13)$$

and the observed kinetic parameters will depend upon both k_{11} and K_{10}. The apparent activation energy will depend not only on E_a for (11) but also on $\Delta H°$ for (10). Association reactons such as (10) are usually exothermic as has been well established for ion pair formation between alkali metal cations and anion radicals in ethereal solvents.[21] The exothermicity of such interactions is expected to increase with increasing solvent polarity.[21] Thus, it is reasonable to expect $\Delta H°_2$ (complexing with water) and $\Delta H°_4$ (ion pairing with Na^+ and Li^+) to be negative for the reactions of $DEF^{\cdot-}$ in DMF.

Before discussing the kinetic parameters for the two reactions it is necessary to establish that the observed rate laws conform to the pre-equilibrium mechanisms. That the dimerizations are clearly second order in $DEF^{\cdot-}$ has been firmly established in the presence of water [1,7] and in the presence of alkali metal ions.[19] It is evident from the data in Table 2 that the reaction is first order in water in the absence of alkali metal ions. Thus, the rate law in the presence of water is (14).

$$\text{Rate}_{2,3} = k_3K_3C_{DEF^{\cdot-}}{}^2C_{H_2O} \quad (14)$$

Since our reaction order data for Li^+ is limited to evaluation from only two rate constants we will limit our discussion of reactions (4)+(5) to the case where M^+ is sodium ion. The data in Table 5 show that the reaction order is 1.04±0.07 up to C_{Na^+} as great as 0.11 M. The rate law in the presence of Na^+ is (15).

$$\text{Rate}_{4,5} = k_5K_4C_{DEF^{\cdot-}}{}^2C_{Na^+} \quad (15)$$

Hazelrigg and Bard [19] found a small increasing trend in the observed rate constants with increasing C_{NaI} and attributed this to the contribution of reaction (16).

$$2\ DEF^{\cdot-}/Na^+ \xrightarrow{k_{16}} \text{dimer} \quad (16)$$

However, electrode filming limited their studies to C_{NaI} up to 8 mM. The reason that we were able to use much higher C_{NaI} is apparently that our solutions contained more water (C_{H_2O},added=70 mM). In any event, it is clear from the data in Table 5 that there is either no or negligible contribution from reacton (16) under our conditions. It could be that K_4 is sufficiently reduced by the inclusion of water to preclude the occurrence of reaction (16).

The most important consequence of the observation that the reactions follow rate laws (14) and (15) is that the observed E_a will be minimum values since both $\Delta H°_2$ and $\Delta H°_4$ are expected to be negative. Indeed, $\Delta H°_2$ was observed to be equal to −5.9 kcal/mol (Table 9). In the case of reaction (4) no shift in E_{rev} could be found at sodium ion concentrations where meaningful measurements could be carried out (Table 4) and K_4 at 292 K was estimated to be less than 0.5 M^{-1}.

The values of E_a observed were 1.9 and 4.1 kcal/mol for reactions (2)+(3) and (4)+(5), respectively. At 298 K, K_2 is of the order of 0.5. The apparent value of k_3K_2 was estimated to be equal to 743 $M^{-2}s^{-1}$ (Table 2) which then results in a value of 1500 $M^{-1}s^{-1}$ for k_3. The activation energy for reaction (3) can be estimated by substracting the contribution of $\Delta H°_2$, *i.e.* −5.9 kcal/mol, from the apparent E_a which results in a value of 7.8 kcal/mol. The value of k_5K_4 at 298 K can be derived from the data in Table 5 and is

equal to 1.8×10^6 $M^{-2}s^{-1}$ at C_{H_2O} of 70 mM (added). Since K_4 was estimated to be <0.5 M^{-1} at C_{H_2O} of 27 mM (added) and it is expected to be even lower at higher C_{H_2O}, an estimate of 0.2 M^{-1} would not be unreasonable. Thus, k_5 is most likely of the order of 10^7 $M^{-1}s^{-1}$ or greater and it is surely greater than 5×10^6 $M^{-1}s^{-1}$ under the conditions of the measurements.

The residual water in the DMF–Bu_4NBF_4 solvent-electrolyte system was estimated to be equal to 30 mM (Results section) and the apparent rate constant in the absence of added water (and alkali metal ions) was estimated by extrapolation to be 30 $M^{-1}s^{-1}$ in this work (Table 2) while k_3K_2 was equal to 743 $M^{-2}s^{-1}$. This implies that if reaction (1) takes place at all under the conditions of this study, k_1 is equal to or less than about 10 $M^{-1}s^{-1}$. Why are reactions (3) and (5) so much more favorable than reaction (1)? The obvious explanation is that the complexing of $DEF^{\cdot-}$ with water (2), most likely by hydrogen bonding, or pairing with alkali metal ions (4) reduces the charge repulsion in the dimer forming transition states and results in decreases in the activation energies of reactions (3) and (5) relative to reaction (1). The degree of the reduction of charge repulsion must depend upon how effectively the charge in $DEF^{\cdot-}$ is neutralized by the complex formation. Clearly, ion pairing must be more effective in charge neutralization than is hydrogen bonding. Therefore, it appears safe to conclude that $(E_a)_1>(E_a)_3>(E_a)_5$ where the subscripts refer to the eqn. numbers for the reactions.

The value of $(E_a)_3$ was determined to be 7.8 kcal/mol. From this value and the estimated value of k_3 at 298 K, *i.e.* 1500 $M^{-1}s^{-1}$, the Arrhenius log A [eqn. (17)]

$$\log k = \log A - E_a/(\ln 10RT) \qquad (17)$$

can be estimated to be 9. Assuming the same log A and that k_1 is equal to 10 $M^{-1}s^{-1}$ at 298 K, we arrive at a value of 10.9 kcal/mol for reaction (1). A conservative estimate of $(E_a)_5$ can be taken to be 4.1 kcal/mol. This is surely a minimum value since ΔH°_4 is most likely negative and a greater apparent E_a (4.4 kcal/mol) was obtained when M^+ was Li^+. This conservative value of $(E_a)_5$ can be used to get another estimate of $(E_a)_1$. Assuming a value of 10^7 $M^{-1}s^{-1}$ for k and 4.1 kcal/mol for E_a results in log A equal to 10. This value of log A then results in an estimate of 12.3 kcal/mol for $(E_a)_1$. Accordingly, it is reasonable to conclude that $(E_a)_1$ probably falls somewhere in the range between 11 and 12 kcal/mol.

From the data discussed in the previous paragraphs it is obvious that the dimerization reactions of $DEF^{\cdot-}$ in DMF have activation parameters in the range expected for normal second order reactions. There does not appear to be anything unusual about the rate constant–activation energy relationship for these reactions. Furthermore, there does not appear to be any reason to believe that the situation should differ for other related dimerizations of anion radicals having similar structural features. The use of the apparent activation energy as a guide in proposing complex reaction mechanisms (Table 1) [6,11,13–18] appears to be a safe procedure in ion radical reactions as well in other second order reactions.[10] The comments of Savéant [12] as well as those by Amatore, Pinson and Savéant regarding our use of low activation energies as well as the implication [8] that we have overlooked classical results of chemical rate theory appear to be without justification.

EXPERIMENTAL

The instrumentation, electrodes, cells and data handling procedures were those described earlier.[22] Reagent grade DMF containing the supporting electrolyte (Bu_4NBF_4) was passed through a column containing neutral alumina before use. Reagent grade DEF was used without further purification. Double potential step chronoamperometry was carried out by potential steps from about 300 mV less negative than E_{rev} to about 300 mV more negative at about 50 s intervals between measurements. Derivative cyclic voltammetry experiments were carried out as previously described.[23]

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The Mechanisms of the Dimerization of Benzaldehyde Anion Radical in Ethanol and Aqueous Ethanol Buffers

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Benzaldehyde anion radical undergoes dimerization in ethanol and aqueous ethanol containing Bu_4NOH (>10 mM) by a mechanism consisting of the reversible dimerization (i) followed by protonation reaction (ii). This mechanism was demonstrated by showing the applicability of rate law (iii) to the data.

$$2\,PhCHO^{\cdot-} \overset{K_i}{\rightleftharpoons} PhCH(O^-)-CH(O^-)Ph \qquad \text{(i)}$$

$$PhCH(O^-)CH(O^-)Ph + ROH \overset{k_{ii}}{\longrightarrow} PhCH(OH)CH(O^-)Ph + RO^- \qquad \text{(ii)}$$

$$\text{Rate} = k_{ii}K_i[PhCHO^{\cdot-}]^2[ROH] \qquad \text{(iii)}$$

A primary deuterium kinetic isotope effect was observed and the rate of the reaction was observed to be independent of $[Bu_4NOH]$ as long as the latter was greater than about 20 mM. At lower $[Bu_4NOH]$ or in the presence of HOAc another mechanism competes which is first order in benzaldehyde anion radical. The latter was demonstrated by derivative cyclic voltammetry reaction orders approaching 1 and by linear sweep voltammetry in the presence of HOAc. The coulometric n value was observed to be 1 regardless of the conditions indicating that dimerization takes place under all conditions studied. The most likely first order reaction mechanism was suggested to be protonation (iv) followed by attack on the substrate (v) and further reduction.

$$PhCHO^{\cdot-} + ROH \overset{k_{iv}}{\longrightarrow} Ph\dot{C}HOH + RO^- \text{ (rate determining)} \qquad \text{(iv)}$$

$$Ph\dot{C}HOH + PhCHO \rightarrow PhCH(OH)CH(O^{\cdot})Ph \qquad \text{(v)}$$

An alternative mechanism involving reaction (vi) instead of (v) was deemed less likely since the rate of (vii) is expected to be near the diffusion controlled limit and this would at least compete with (vi) and result in n values greater than unity.

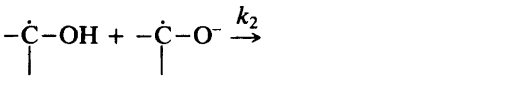
$$Ph\dot{C}HOH + PhCHO^{\cdot-} \rightarrow PhCH(OH)CH(O^-)Ph \qquad \text{(vi)}$$

$$Ph\dot{C}HOH + PhCHO^{\cdot-} \rightarrow Ph\bar{C}HOH + PhCHO \qquad \text{(vii)}$$

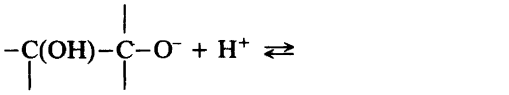
A number of aromatic carbonyl compounds are reduced in ethanolic buffers in one electron processes accompanied by rapid dimerization of the anion radicals.[1,2] On the basis of extensive linear sweep voltammetry (LSV) studies, Nadjo and Savéant [3] concluded that the dimerization mechanism consists of reactions (1)–(3), which may be described by rate law (4) where the apparent rate constant (k_{app}) is equal to k_2K_1.

0302-4369/83 $2.50

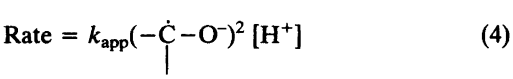
(1)



(2)



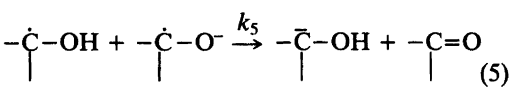
(3)



(4)

The reaction was also studied by *a.c.* polarography and the data were found to be consistent with the second order dimerization but no attempt was made to verify the proton donor term in rate law (4).[4] Nadjo and Savéant's justification for assuming the reaction to be first order in proton donor was based on the observation that the apparent rate of dimerization was dependent upon the pH of the buffer used decreasing in the order, veratrol (pH ~13.6), phenol (pH ~15.7) and Bu_4NOH.

The most significant aspect of this mechanism is that reaction (2) must take place with the exclusion of the thermodynamically favourable electron transfer reaction (5).



(5)

We find this possibility unlikely * since (5) may be predicted to take place at very near the diffusion controlled limit. This implies that the maximum possible value of k_2 is very close to that of k_5 and it is more likely that it is considerably smaller. Furthermore, in a related system the protonation of methyl cinnamate in phenolic buffers, where the first step is reaction (1), only reaction (5) follows and results in an overall 2e reduction.[5]

The question which should be answered before accepting Nadjo and Savéant's mechanism is as follows. Is the dimer-forming reaction preceded by protonation equilibrium (1)? To answer this question one should consider the complete equation which takes into account the conjugate base of the proton donor (6).

(6)

(7)

Neither of the previous kinetic studies [3,4] resulted in data pertaining to the reaction order in A^-. Thus, the mechanism of the dimerization of anion radicals of carbonyl compounds in ethanolic buffers was not known at the outset of this study.

In this paper we report the results of kinetic studies of the dimerization of benzaldehyde anion radical in ethanolic and aqueous ethanolic buffers. Our data were found to be incompatible with the Nadjo-Savéant mechanism and we conclude that proton transfer does not take place before the formation of the dimer dianion.

RESULTS

The reaction of benzaldehyde anion radical in basic ethanol. The Nadjo-Savéant [3] mechanism (1)–(3) requires, according to rate law (7), that the rate of the reaction be inversely proportional to the concentration of the conjugate base of the strongest acid in the medium. In ethanol containing small amounts of water the latter is hydroxide ion. The data in Table 1 show that at $[OH^-]$ up to about 12 mM the apparent rate constant, proportional to $v_{0.4}$, depends upon the base concentration. The quantity, $v_{0.4}$, is the voltage sweep rate necessary for the ratio of the derivative of the peak currents during derivative cyclic voltam-

* It is possible that reaction (5) is slow but in general this second step of the ECE_h mechanism takes place preferentially over other possible reactions of the neutral radical.

Table 1. The effect of base on the rate of reaction of benzaldehyde anion radical in ethanol.[a]

$[OH^-]$/mM [b]	$v_{0.4}$/V s^{-1} [c]	$v_{0.4}[OH^-]$/V mM s^{-1}
1.0	125.9	125.9
2.0	90.9	182
4.0	57.9	232
8.0	45.1	361
12.0	37.7	452
16.0	34.6	554
20.0	34.1	682

[a] In solvent containing Bu_4NBF_4 (0.1 M). Measurements by DCV at a mercury electrode at 18.7 °C. [b] Added as a 25 % solution of Bu_4NOH in methanol. [c] The voltage sweep rate necessary for the derivative current ratio to equal 0.400.

metry (DCV) to equal 0.400.[6] However, the product $v_{0.4}[OH^-]$ is not constant as rate law (7) requires. At $[OH^-]$ greater than 12 mM, $v_{0.4}$ appears to be independent of the base concentration.

A further requirement of rate law (7) is that the reaction be second order in $PhCHO^{\cdot-}$. In terms of DCV reaction orders,[6] this requirement calls for the ratio $v_{1/2}$/[PhCHO] to be constant at a given buffer concentration. The substrate concentration was varied in five different buffers. The results are shown in Table 2. Only in the most concentrated buffer (20 mM) is a reasonably good fit to second order kinetics observed. The data indicate that the reaction order in $PhCHO^{\cdot-}$ is greater than 1 but less than 2 in the other buffers. For example, in the 4 mM buffer a 10-fold increase in substrate concentration resulted in only a 4-fold increase in $v_{1/2}$.

Since the data for the 20 mM buffer approximates that expected for a second order dimerization, a temperature study was carried out on the rate of reaction of benzaldehyde anion radical in this medium. The kinetics were studied by double potential step chronoamperometry (DPSC) and $\tau_{1/2}$ is the pulse width at which the ratio of the normalized current ($=i_b/i_f(1-2^{-1/2})$ is equal to 0.500. The observed apparent second order rate constants were evaluated using eqn. (8).[7]

$$k_{app} = 0.830([PhCHO]\tau_{1/2})^{-1} \quad (8)$$

Table 2. Effect of substrate concentration on the rate of reaction of benzaldehyde anion radical in ethanol containing base.[a]

$[OH^-]$/mM	[PhCHO]/mM	$v_{1/2}$/V s^{-1}	$v_{1/2}$/[PhCHO]
2.0	0.10	62.2	622
2.0	0.20	72.0	360
2.0	0.40	104.1	260
4.0	0.10	31.3	313
4.0	0.20	47.8	239
4.0	0.40	73.9	185
4.0	1.00	123.3	123
8.0	0.10	17.6	176
8.0	0.20	29.2	146
8.0	0.40	48.3	121
8.0	1.00	86.1	86
12.0	0.10	14.6	146
12.0	0.20	20.7	103.5
12.0	0.40	39.1	97.8
12.0	1.00	73.3	73.3
20.0	0.10	8.65	86.5
20.0	0.20	21.3	106.5
20.0	0.40	36.9	92.3
20.0	1.00	67.2	67.2

[a] Measurements by DCV at a mercury electrode at 18.7 °C in solvent containing Bu_4NBF_4 (0.1 M).

Table 3. The effect of temperature on the rate of dimerization of benzaldehyde anion radical in basic ethanol.[a]

T/K	$\tau_{1/2}$/ms [b]	k_{app}[c]/$M^{-1}s^{-1}$
272.3	3.14	2.64×10^5
291.3	2.37	3.50×10^5
303.6	2.02	4.11×10^5
311.8	1.75	4.74×10^5

[a] Measurements by DPSC on a solution containing benzaldehyde (1.0 mM), Bu_4NOH (20 mM) and Bu_4NBF_4 (0.1 M). [b] The pulse width necessary for the normalized current ratio to equal 0.500. [c] Calculated from theoretical data for the simple dimerization mechanism, $k=0.830/C_{PhCHO}\tau_{1/2}$. The Arrhenius activation energy was equal to 2.5 kcal/mol with a correlation coefficient of 0.998.

Measurements at 4 temperatures ranging from 273.2 to 311.8 K, resulted in an Arrhenius activation energy of 2.5 kcal/mol with a correlation coefficient of 0.998 (Table 3).

The reaction of benzaldehyde anion radical in aqueous ethanolic buffers. Since previous studies [1-4] were carried out in ethanol containing appreciable quantities of water, the effect of $[H_2O]$ on the apparent second order rate constants was determined. The data in Table 4 show the effect of water added to solutions 20 mM in Bu_4NOH on the apparent rate constants. In the two independent sets of experiments, approximately 50 % increases in k_{app} were observed in going from added water of 0 to 1.12 M. The reaction order in water was determined from eqn. (9)

$$k_{app} = k_0 + k_w[H_2O] \quad (9)$$

where k_0 is the apparent rate constant in the absence of *added* water. If eqn. (9) is an adequate description of k_{app}, dividing the $k_w[H_2O]$ term by the concentration of added water should result in a constant which is the third order rate constant for the reaction second order in $PhCHO^{\cdot-}$ and first order in water. The last column in Table 4 shows that the data fit eqn. (9) remarkably well and that k_w is equal to $1.18(\pm0.13)\times10^5$ $M^{-2}s^{-1}$.

The effect of $[Bu_4NOH]$ at 3 different water concentrations was determined. The data in Table 5 show that $\nu_{1/2}$ measured at buffer concentrations of 50, 25 and 10 mM are essentially independent of $[Bu_4NOH]$. Once again, the apparent second order rate constants increased with increasing water concentration.

Another feature of the Nadjo-Savéant mechanism [3] is that it predicts a small deuterium *equilibrium* isotope effect when the proton donor in reaction (6) is labelled with deuterium. In general, the values of K_H/K_D are normally close to unity while primary deuterium kinetic isotope effects k_H/k_D are generally >2.[8] In order to test for an equilibrium or a kinetic isotope effect, the kinetics were studied in solutions where either H_2O or D_2O were added. Since the Bu_4NOH was available as a 25 % solution in methanol, it was not possible to have solvent in which all hydroxylic groups contained D. The data in Table 6 show the effect of increasing the proportion of deuter-

Table 4. The effect of water concentration on the rate of dimerization of benzaldehyde anion radical in basic ethanol.[a]

$[H_2O]$	$\nu_{1/2}$/V s^{-1}	$10^{-5}k_{app}$/$M^{-1}s^{-1}$	10^{-5} k_w/$M^{-2}s^{-1}$ [b]
0	37.2	3.50	0
0.28	40.8	3.84	1.21
0.56	43.2	4.07	1.02
1.12	51.8	4.88	1.23
0	33.0	3.10	0
0.28	35.9	3.38	1.00
0.56	41.0	3.85	1.34
0.84	43.3	4.07	1.15
1.12	48.2	4.53	1.28
k_w/$M^{-2}s^{-1}$			1.18×10^5

[a] At 18.7 °C in solutions containing benzaldehyde (0.5 mM), Bu_4NOH (20 mM) and Bu_4NBF_4 (0.1 M) at a mercury electrode. [b] Apparent third order rate constant due to the water added to the system.

Table 5. The effect of water and base on the rate of dimerization of benzaldehyde anion radical in aqueous ethanol.[a]

$[Bu_4NOH]$/mM	$[H_2O]$/M	$v_{1/2}$/V s^{-1}	
50	0.56	48.9	50.1±1.9 ($k_{app}=2.35\times10^5$ M^{-1} s^{-1})
25	0.56	49.2	
10	0.56	52.3	
50	1.39	64.8	64.9±3.7 ($k_{app}=3.05\times10^5$ M^{-1} s^{-1})
25	1.39	61.2	
10	1.39	68.6	
50	2.78	98.5	92.3±4.7 ($k_{app}=4.39\times10^5$ M^{-1} s^{-1})
25	2.78	89.3	
10	2.78	92.0	

[a] Measurements by DCV in solvent containing benzaldehyde (1.0 mM) and Bu_4N^+ (0.1 M) at 18.2 °C at a mercury electrode.

Table 6. Deuterium kinetic isotope effect studies on the dimerization of benzaldehyde anion radical in aqueous ethanol.[a]

[PhCHO]/mM	$[H_2O]$/M	$10^{-6}k_{app}$/M^{-1}s^{-1}	$(k_H/k_D)_{app}$	Active [b] H/D
0.50	5.56 (H_2O)	1.62	1.03	1.38
0.50	5.56 (D_2O)	1.57		
0.25	11.2 (H_2O)	2.23	1.57	0.61
0.25	11.2 (D_2O)	1.42		
0.20	13.9 (H_2O)	2.85	3.50	0.47
0.20	13.9 (D_2O)	0.813		

[a] In solvent containing Bu_4OH (0.04 M), methanol (0.9 M) and Bu_4NBF_4. [b] The ratio of hydroxylic H to hydroxylic D in the solvent to which D_2O was added.

ated hydroxylic groups in the solvent. The last column gives the ratio of hydroxylic H to hydroxylic D in the solvent after D_2O was added. The most interesting feature of the data is that at high active H/D ratio (1.38) a low value of the apparent kinetic isotope effect was observed but when D became more abundant. $(k_H/k_D)_{app}$ increased significantly to 1.57 when the H/D ratio was 0.61 and finally to 3.50 at H/D equal to 0.47. These data lead to two conclusions; (i) the D is mostly present as CH_3OD and CH_3CH_2OD in the solvent with excess hydroxylic H and (ii) a relatively large primary deuterium kinetic isotope effect is evident as the amount of hydroxylic D is increased. No attempt was made to calculate the intrinsic k_H/k_D since the exact distribution of D in the hydroxylic groups of solvent containing ethanol, methanol and water is not known.

The reaction order in $PhCHO^{\cdot-}$ was determined in solvent containing water (2.78 M) and Bu_4NOH (40 mM). The data in Table 7 show the fit to second order kinetics over a 10-fold

Table 7. The effect of substrate concentration on the apparent rate constants for the dimerization of benzaldehyde in aqueous ethanol.[a]

[PhCHO]/mM	$v_{1/2}$/V s^{-1}	$10^{-5}k_{app}$/M^{-1}s^{-1}
0.10	13.3	10.7
0.20	21.1	8.58
0.40	41.4	8.42
0.60	60.7	8.23
1.00	86.6	7.04

[a] In solvent containing water (2.78 M), Bu_4NOH (0.04 M) and Bu_4NBF_4 (0.09 M).

Table 8. The effect of temperature on the rate of dimerization of benzaldehyde anion radical in aqueous ethanol.[a]

T/K	$[H_2O]$/M	$v_{1/2}$/V s^{-1}	$10^{-6}k_{app}$/ $M^{-1}s^{-1}$
300.6	16.7	117.3	4.62
291.2	16.7	95.9	3.90
282.5	16.7	120.4	5.04
272.1	16.7	75.1	3.27
301.7	5.56	50.2	1.97
291.2	5.56	47.1	1.92
283.2	5.56	42.0	1.76
272.6	5.56	23.6	1.03

[a] In solvent containing benzaldehyde (0.20 mM), Bu_4NOH (0.04 M) and Bu_4NBF_4.

Table 9. Linear sweep voltammetry study of the dimerization of benzaldehyde anion radical in ethanol containing acetic acid.

	mV/decade	Reaction order
$dE^p/d\log v$ [a]	29.2±1.1	1.0 in $PhCHO^{-}$
$dE^p/d\log C_{PhCHO}$ [b]	−5.3±0.4	~0.2 in PhCHO
$dE^p/d\log C_{HOAc}$ [c]	−25.0±0.5	~0.8 in HOAc

[a] Measurements at 0.25, 0.50, 0.75, 1.00 and 2.00 mM in PhCHO in ethanol at [HOAc]=10 mM at 11.1 °C. [b] Measurements at 100, 200, 400 and 1000 mV s^{-1} under the same conditions as *a*. [c] Measurements at [HOAc]=4, 8, 12, 16, 24 and 32 mM at 18.3 °C.

Table 10. Determination of the apparent number of electrons transferred during the reduction of benzaldehyde in ethanol.[a]

Conditions	Relative peak current [b]	n_{app} [c]
EtOH	1.01(±0.0)	1.01
EtOH–HOAc (4 mM)	0.99(±0.2)	0.99
EtOH–Bu_4NOH (20 mM)	1.00(±0.1)	1.00

[a] Measurements by LSV in solvent containing PhCHO (0.5 mM) and Bu_4NBF_4 (0.1 M) at 18.3 °C at a mercury electrode. [b] LSV peak current measured at 200 mV s^{-1}. [c] The apparent number of electrons per molecule of PhCHO transferred assuming a value of 1.00 for the reaction in the presence of Bu_4NOH.

concentration range. The data fit is relatively good with k_{app} equal to $8.60(\pm1.32)\times10^5$ $M^{-1}s^{-1}$. The value measured at the lowest substrate concentration is responsible for much of the deviation.

The data in Table 8 were obtained to determine the effect of temperature on the apparent rate constants for the dimerization of $PhCHO^{-}$ in aqueous ethanol. At a very high water concentration (16.7 M) the Arrhenius plot was not linear and the apparent activation energy was close to 0. However, if the data measured at 282.5 K are omitted the linearity is reasonably good and E_a is about 1.9 kcal/mol. At $[H_2O]$ of 5.56 M the linearity was better with E_a about 3.9 kcal/mol.

The reaction of benzaldehyde anion radical in ethanol containing acetic acid. The reaction under these conditions was too rapid to study using DCV or DPSC. The results of an LSV study are summarized in Table 9. The reaction orders in $PhCHO^{-}$ (*b*), PhCHO (*a*) and HOAc (*x*) were calculated from experimental LSV data using eqns. (10), (11) and (12), respectively.[9]

$$dE^p/d\log v = \ln 10(RT/F)/(b+1) \quad (10)$$

$$dE^p/d\log C_A = \ln 10(RT/F)(a+b-1)/(b+1) \quad (11)$$

$$dE^p/d\log C_X = \ln 10(RT/F)x/(b+1) \quad (12)$$

Values of $dE^p/d\log v$ were observed to be equal to 29.2(±1.1) mV/decade over an eightfold range of substrate concentration. The theoretical value for a first order reaction of primary intermediate $PhCHO^{-}$ at 11 °C is 28.1 mV/decade which is within the limits of error of the experimental value. The reaction orders in PhCHO and HOAc were observed to be 0.2 and 0.8, respectively.

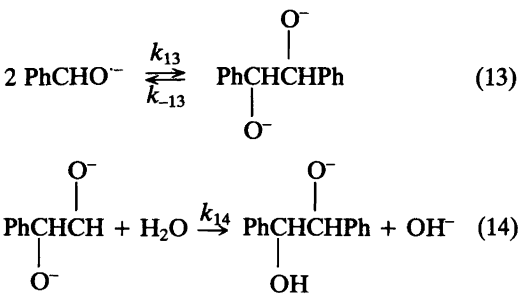
The observation of kinetics first order in $PhCHO^{-}$ in media containing HOAc suggested that protonation accompanied by further reduction might be taking place under these conditions. In order to test for this possibility the coulometric *n* value was determined by LSV measurements. For a rapid reaction following charge transfer, the height of the LSV wave at low *v* is independent of the rate of the reaction and is proportional to the number of electrons per molecule reacting. The data in Table 10 were obtained on solutions of benzaldehyde in ethanol and in ethanol containing either HOAc or

Bu_4NOH. Since it is established that the reaction is a dimerization under the latter conditions,[1-4] this can serve as the standard for $n=1.00$. The relative peak currents for all three solutions containing benzaldehyde (0.50 mM) measured at 200 mVs^{-1} were within a ±1 % range. This means that the overall reaction under all conditions involves le reduction and hence dimerization in all three cases.

DISCUSSION

The most significant kinetic evidence which requires discarding the Nadjo-Savéant mechanism [3] is the failure to observe inverse first order in hydroxide ion and the observation of a *kinetic* rather than an *equilibrium* deuterium isotope effect in media containing hydroxylic D and H. This demonstrates a general trend. The Nadjo-Savéant paper [3] was published in 1971. At that time most mechanistic papers published in the area of organic electrochemistry were based on measurements by a single technique and usually at a single concentration and a single temperature. Nadjo and Savéant used only linear sweep voltammetry which is an indirect kinetic technique since the response of the primary intermediate is not observed. The latter was in spite of the fact that the rate of the reaction is low enough for the use of direct kinetic techniques such as DPSC and cyclic voltammetry. Nadjo and Savéant used only two substrate concentrations in the study of a second order reaction. Today, this type of mechanistic evidence is not acceptable. The importance of temperature [10] and concentration [6,10] effects in the study of electrode mechanisms has recently been emphasized. In reactions involving proton transfers, deuterium kinetic isotope effects have recently been employed in the study of organic electrode processes.[10-16] The determination of apparent activation energies during the study of electrode processes has become common.[5,6,10,13,14,16-36] In short, acceptable electrode process mechanistic evidence today is comparable to that generally presented in physical organic reaction mechanism studies.[37]

Most of the previous work has been carried out in aqueous ethanol.[1-4] The evidence is strong that in aqueous ethanolic buffers the reaction is a second order dimerization of $PhCHO^{\cdot-}$. Second order behaviour was observed over a tenfold range of substrate concentration (Table 7). In three different hydroxide ion buffers (Table 5) the apparent rate constant was observed to be independent of $[OH^-]$. In these media there is a primary deuterium kinetic isotope effect when hydroxylic H is replaced by hydroxylic D (Table 6). The apparent activation energy is dependent on $[H_2O]$ and was observed to be about 3.9 kcal/mol at $[H_2O]$ equal to 5.6 M and about 1.9 kcal/mol at $[H_2O]$ of 16.7 M (Table 8). A significant portion of the reaction goes through a mechanism that is first order in water (Table 4). When $[H_2O]$ is 1.12 M about 50 % of the reaction goes by that mechanism and at $[H_2O]$ equal 16.7 M greater than 90 % of the rate can be attributed to the mechanism first order in water and second order in $PhCHO^{\cdot-}$. Thus, under the latter conditions the results suggest mechanism (13)+(14) and the corresponding rate law (15).



$$2\ PhCHO^{\cdot-} \underset{k_{-13}}{\overset{k_{13}}{\rightleftarrows}} PhCH(O^-)CHPh(O^-) \quad (13)$$

$$PhCH(O^-)CH(O^-) + H_2O \xrightarrow{k_{14}} PhCH(O^-)CHPh(OH) + OH^- \quad (14)$$

$$\text{Rate} = k_{14}K_{15}[PhCHO^{\cdot-}]^2[H_2O] \quad (15)$$

It is necessary that proton transfer takes place in an irreversible step to account for the independence on $[OH^-]$ of the observed rate constants as well as the primary k_H/k_D. This mechanism also predicts that the apparent activation energy $(E_a)_{app}$ should depend upon $[H_2O]$ and decrease with increasing water concentration. This is obvious from expression (16)

$$(E_a)_{app} = (\ln A - \ln k_{app})RT \quad (16)$$

which takes into account that k_{app} is a *pseudo* second order rate constant directly proportional to the water concentration.

We are forced to be somewhat more speculative for the reaction second order in $PhCHO^{\cdot-}$ at lower $[H_2O]$. When $[OH^-]$ is 20 mM and water is not intentionally added the kinetic data approximately fit rate law (17).

$$\text{Rate} = k_{app}[\text{PhCHO}^{\cdot-}]^2 \quad (17)$$

What must be kept in mind is that the solvent is a potential proton donor and that methanol is introduced since the Bu_4NOH was a 25 % solution in that solvent. The [MeOH] in solutions 20 mM in Bu_4NOH was of the order of 0.5 M. Thus, it is likely that the same mechanism is followed under conditions where water is not added with EtOH and MeOH taking the place of water in reaction (14). As in all reactions involving the solvent it is impossible to determine the reaction order in ethanol. The reaction order in methanol was not determined since the uncertainty of the role of ethanol would still be present.

When water was not intentionally added and [OH^-] was <12 mM, the reaction order in $PhCHO^{\cdot-}$ was observed to be >1 but <2 (Table 2). Under these conditions the apparent number of electrons transferred was still 1.0 which is indicative of a dimerization mechanism (Table 10). The apparent rate constant under these conditions decreases with increasing [OH^-] but not in a well-defined manner (Table 2). These results suggest that proton transfer to the anion radical occurs under these conditions and is consistent with either the Nadjo-Savéant mechanism or with (18)+(19) and rate law (20).

$$\text{PhCHO}^{\cdot-} + \text{ROH} \underset{k_{-18}}{\overset{k_{18}}{\rightleftharpoons}} \text{PhĊHOH} + \text{RO}^- \quad (18)$$

$$\text{PhĊHOH} + \text{PhCHO} \xrightarrow{k_{19}} \text{PhCH(O)CH(OH)} \quad (19)$$

$$\text{Rate} = k_{18}k_{19}[\text{PhCHO}^{\cdot-}][\text{PhCHO}][\text{ROH}]/(k_{-18}[\text{RO}^-] + k_{19}[\text{PhCHO}]) \quad (20)$$

The data in Table 2 do not distinguish between these two possibilities. However, as mentioned earlier, the thermodynamically favored electron transfer reaction (5) is expected to be very close to diffusion controlled and should compete effectively with reaction (2). The overall result expected for these competing mechanisms is that a significant fraction of the two electron reduction product would be formed and the observed apparent n values should be greater than 1. This is not observed, either in this study or in previous work.[1-4] On this basis, mechanism (18)+(19) is consistent with the data while the Nadjo-Savéant mechanism is not.

In order to gain a little more insight into the dimerization mechanism when the reaction order in $PhCHO^{\cdot-}$ is less than 2, studies were carried out in ethanol containing low concentrations of acetic acid (HOAc). The reaction remains a one electron reduction (Table 10) under these conditions. The results of the LSV study were revealing. The reaction order in $PhCHO^{\cdot-}$ was observed to be 1.0 within experimental error (Table 9) while that in HOAc was 0.8 and in PhCHO was 0.2. These results are once again consistent with mechanism (18)+(19), where ROH is HOAc, being the major reaction pathway.

From the results discussed in the previous paragraphs, it appears safe to conclude that two mechanisms account for the dimerization of benzaldehyde anion radical in ethanol. In the presence of water and hydroxide ion, the predominant mechanism is (13)+(14). In less basic solvent mechanism (18)+(19) becomes important and predominates in acidic solvent.

EXPERIMENTAL

Absolute ethanol was used without further purification. Tetrabutylammonium hydroxide was Fluka (practical) and was available as a 25 % solution in methanol. The instrumentation, electrodes, cells and data handling procedures were those described earlier.[38]

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Electrochemistry in Liquid Ammonia. VII. Halonitrobenzenes

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The reduction of halonitrobenzenes (RX) (*o*-, *m*-, *p*-Cl; *m*-Br; *o*-, *m*-, *p*-I) in liquid ammonia, 0.1 M KI at a platinum electrode was investigated by cyclic voltammetric and controlled potential coulometric techniques. In this medium the radical anions, $RX^{\cdot-}$, were much more stable compared to other nonaqueous systems; for example, the *m*-Cl and *p*-Cl radical anions were stable on the coulometric time scale (lifetimes $\geqslant$30 min). The dianions of most of these (RX^{2-}) underwent rapid loss of halide ion; however, the dianion of *m*-chloronitrobenzene was stable. Estimates of the rate constants for the loss of halide by the dianions are given.

The electrochemical reduction of halogenated aromatic compounds has been discussed in numerous reports [1–14] and especially exhaustively in Ref. 1. In general, the reduction process of aryl halides can be represented by the following reaction scheme:

$$RX + e \longrightarrow RX^{\cdot-} \quad (1)$$

$$RX^{\cdot-} \xrightarrow{k} R^{\cdot} + X^{-} \quad (2)$$

$$R^{\cdot} + e \longrightarrow R^{-} \quad (3)$$

$$R^{-} + HS \longrightarrow RH + S^{-} \quad (4)$$

where HS is a solvent.

The rate of the halogen cleavage process depends upon the solvent, the nature of the halogen and its position on the aromatic ring, and the presence of various other substituents on the molecule. The stability of the radical anions formed by electron uptake is enhanced by increasing the odd electron's delocalization through the presence of electron-withdrawing groups ($-NO_2$, –CN). The potential for reduction of the neutral radical ($R^{\cdot}$) is usually more positive than that of the parent (RX) leading ultimately to the formation of a carbanion which is rapidly protonated by the solvent. In dimethylformamide (DMF), acetonitrile (MeCN), and dimethyl sulfoxide (DMSO), there has been evidence that $R^{\cdot}$ (the product of reaction 2) can undergo a hydrogen abstraction reaction involving the solvent:

$$R^{\cdot} + HS \longrightarrow RH + S^{\cdot} \quad (5)$$

The hydrogen abstraction mechanism usually results in an apparent number of electrons passed in a coulometric reduction of less than 2. This also leads to formation of solvent-substituted products produced by a variety of reaction paths involving $R^{\cdot}$ and $S^{\cdot}$. In certain cases, where the radical anion of the halogenated parent ($RX^{\cdot-}$) is more stable, other reaction pathways, including disproportionation, are possible:

$$R^{\cdot} + RX^{\cdot-} \longrightarrow R^{-} + RX \quad (6)$$

$$R^{-} + HS \longrightarrow RH + S^{-} \quad (7)$$

This overall reaction pathway is indistinguishable from the mechanism represented by reactions (1)–(4). The final product is the same and involves the addition of 2 electrons and 1 proton per molecule.

The formation of stable radical anions of certain members of a series of halogenated benzophenones and fluorenones in DMF, DMSO, and ACN have been reported.[5,11,15] In fact, such radicals were sufficiently long-lived to

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permit their detection by ESR and the measurement by cyclic voltammetry and polarography of the standard potential, $E°$, for the $RX/RX^{\cdot-}$ couple. The stability of the $RX^{\cdot-}$ can be measured by lowering the temperature, thus slowing down the halogen cleavage rate and retarding side reactions with impurities and solvent.[6,16] Liquid NH_3 has been shown to be a suitable solvent to investigate the halogen cleavage process in the absence of possible hydrogen abstraction. The reduction of a series of halobenzophenones in NH_3 has been reported.[10] The enhanced stability of the generated radical ions observed in liquid NH_3 was attributed to the low temperature (–40 °C) conditions of the experiment. However, stable dianions were also easily formed in this solvent. This has not been possible in other nonaqueous solvents, such as DMF and MeCN.

The electrochemical reduction of halonitrobenzenes in ACN, DMF and DMSO has been described.[4,14] In all cases, the reduction process involved the formation of the corresponding radical anion and halide ion. Nitrobenzene, which is the main reaction product, is formed by hydrogen atom abstraction from either the solvent or the supporting electrolyte. The proposed reaction mechanism was consistent with an ECE-type mechanism (chemical reaction between electron transfer processes). ESR experiments involving *in situ* generation of halogenated nitrobenzene radical anions in CD_3CN confirmed that the abstraction of the hydrogen atom is, in fact, from the solvent and not from the supporting electrolyte.[6] The relative decomposition rates of the radical anions increased in the order:

o-iodo>*o*-bromo>>*p*-iodo>*m*-iodo.

The fast kinetics associated with the halogen cleavage step at room temperature prevented the investigation of the electrogenerated radical anion. For this reason, low temperature electrochemical studies were performed in an attempt to isolate the one-electron transfer reduction. At –19 °C, the rates of follow-up chemical reactions were sufficiently slowed that a cyclic voltammogram of *p*-iodonitrobenzene, for example, indicated a one-electron reversible wave. Information about the lifetimes of unstable radical anions of aromatic halides has also recently been obtained by utilizing homogeneous redox catalysis reactions for generation of $RX^{\cdot-}$.[2]

The previous studies produced little or no information regarding the stabilities of the dianions. In fact, protonation was so rapid that dianions were not detected even at relatively low temperatures. Past investigations from this laboratory of the reduction of several compounds (nitrobenzene, nitrosobenzene and benzophenone) in liquid NH_3 demonstrated that this solvent was useful for the formation of stable radical anions and dianions.[17–20] In the study reported here, we extend this solvent to halonitrobenzenes to probe their reduction behavior and compare the reactivity of the radical anions with other nonaqueous solvent systems.

EXPERIMENTAL

The apparatus and experimental techniques for solvent handling and purification were described in detail in earlier papers.[18–21] The supporting electrolyte used in the experiments, reagent grade potassium iodide, was dried at 120 °C in an oven and then stored in a desiccator. The cell used for electrochemical measurements was a standard three-compartment cell with a Pt disk (0.04 cm^2) working electrode for voltammetry and a large Pt-screen working electrode for coulometry. Details of components and design of a similar cell have been previously described.[18,21] The electrochemical experiments were carried out with a PAR Model 173 potentiostat incorporating a Model 179 digital coulometer equipped with positive feedback for IR compensation and a Model 175 Programmer (Princeton Applied Research Corporation, Princeton, NJ). A Model 2000 X–Y recorder (Houston Instruments, Austin, Texas) was used to record the current-potential curves. For fast scan rates, a Tektronix Model 564 storage scope was used. The experiments were performed at –40 °C.

RESULTS AND DISCUSSION

o-Chloronitrobenzene. The cyclic voltammetric, CV, results for the reduction of *o*-chloronitrobenzene at a Pt-electrode in liquid NH_3 containing 0.1 M KI at –40 °C shows three reduction waves at –0.4, –1.1 and –1.22 V *vs.* $Ag/AgNO_3$ (0.1 M). A typical cyclic voltammogram is shown in Fig. 1. The reversible wave at –0.4 V had a peak separation of 50 mV (46 mV theoretically for a one electron reversible process at –40 °C). The current ratio i_{pa}/i_{pc} was 1.01 (i_{pa} –

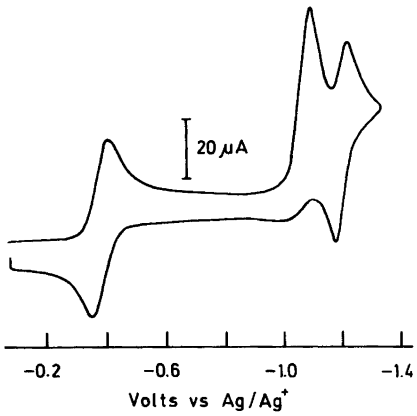
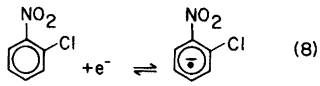


Fig. 1. Cyclic voltammogram for the reduction of 2.10 mM *o*-chloronitrobenzene in liquid NH_3 at –40 °C. Sweep rate 200 mV/s.

anodic peak current, i_{pc} – cathodic peak current). Controlled potential coulometry at –0.60 V resulted in the passage of 0.96 F/mol. The electrogenerated radical anion had a bright orange color. A cyclic voltammogram of the solution following coulometry and initiated at –0.6 V indicated the presence of the initial reversible wave. Coulometric oxidation at –0.1 V resulted in an n_{app} = 0.93 (where n_{app} is the number of F/mol). The CV and coulometric results indicate that the first reduction wave corresponds to the production of a stable radical anion of *o*-chloronitrobenzene:



(8)

This species was stable on both the CV and coulometric time scale as indicated by the coulometric oxidation data.

These can be compared with those obtained by Fujinaga and coworkers (Ref. 21) from ESR studies using *in situ* generation of various halogenated radicals of nitrobenzene in DMF. The ESR data indicated that the electrogenerated radical anion of *o*-chloronitrobenzene was stable by the observation of its spectrum. However, if the radical anion was generated coulometrically, it was stable only for about 15 min. The dehalogenation was evident by the appearance of lines for nitrobenzene radical anion in the ESR spectrum. The data presented for *o*-chloronitrobenzene show an enhanced stability of the radical anion in liquid NH_3 over other nonaqueous solvents, which could be attributed to the low-temperature conditions of the reaction (–40 °C). No decomposition was observed even when the electrogenerated radical anion of *o*-chloronitrobenzene was allowed to stand at –40 °C for 30 min as evident by oxidative coulometry, where essentially complete regeneration of parent with the same number of coulombs as in the previous reduction was found.

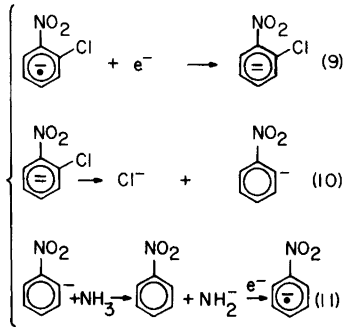
Table 1. The effect of scan rate on peak potential of the second reduction wave of *o*-chloronitrobenzene in liquid NH_3. C=2.1 mM, t=–40 °C.

Scan rate (V/s)	$-E_{pc}$ (Volts)
0.05	1.10
0.10	1.10
0.20	1.11
0.50	1.14
1.00	1.14

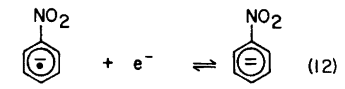
The second reduction wave at –1.1 V as shown in Fig. 1 was irreversible even at scan rates up to 10 V/s. The peak potential of this wave shifted towards negative values by about 40 mV for each 10-fold increase in scan rate, Table 1. The peak current, i_{pc}, at a scan rate of 200 mV/s was about 1.8 times as large as that of the current of the first reduction wave. Although the peak potential of the third reversible wave was independent of scan rate, its peak height varied with v. When the scan rate was slow, the peak was slightly larger. The electrolysis of the solution at –1.12 V following the first electrolysis resulted in a dark brown solution and an n_{app} of 2.1. The potential was carefully monitored for any possible shift during the electrolysis so as not to include the wave at –1.22 V which could result in higher-than-predicted n_{app}-values. A potential scan performed following coulometry and initiated at –1.12 V resulted in the appearance of a new reversible wave at potentials slightly negative of the first reduction wave. The reversible wave at –1.22 V remained. The introduction of small amounts of nitrobenzene to the solution produced a cyclic voltammogram identical to that obtained after coulometry except that the waves were much larger. The analysis of the previous data clearly shows that the radical anion of *o*-chloroni-

trobenzene is reduced at potentials more negative than –1.0 V to produce ultimately nitrobenzene radical anion with the uptake of 2 electrons and a proton. The nitrobenzene radical anion is further reduced at –1.22 to the dianion. A possible reaction mechanism is:

at the second wave



at the third wave



The rate constant for the halogen cleavage process, reaction (10), can be estimated from CV data to be about 1.2 s^{-1}.

The slight increase in peak height of the third reduction wave with decreasing scan rate can be explained by the fact that as the scan rate is decreased, more time is available for the radical anion of *o*-chloronitrobenzene to dehalogenate. On the other hand, at high scan rates, the decomposition of the radical anion is slowed which inhibits nitrobenzene production. The absence of anodic current for the second wave, even at relatively high scan rates, implies that the halogen cleavage, reaction (10), is very fast and that the rate-determining step is the protonation of the carbanion, reaction (11).

m-*Chloronitrobenzene.* The electrochemical reduction of *m*-chloronitrobenzene in liquid NH_3 resulted in two reversible one-electron waves at –0.3 and –1.09 V *vs.* $Ag/AgNO_3$ (0.1 M) as shown in Fig. 2. The peak potential for both waves showed no dependency on scan rate. The coulometric reduction of the solution at –0.5 V produced the orange radical anion and an n_{app}-value of 0.95. The radical anion was very stable on the coulometric time scale, and could be oxidized back to the parent with an n_{app}-value of 0.92. Controlled potential electrolysis at –1.12 V

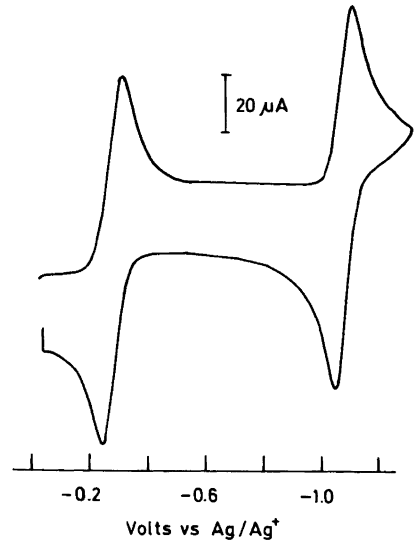


Fig. 2. Cyclic voltammogram for the reduction of 4.1 mM *m*-chloronitrobenzene in liquid NH_3 at –40 °C. Sweep rate 200 mV/s.

gave a dark red solution after coulometry initiated at –1.12 and swept in a positive direction resulted in the production of two new reversible waves at –0.42 V and at –1.25 V (Fig. 3). The height of these waves was about 10 % of that of the initial reversible waves. From the time

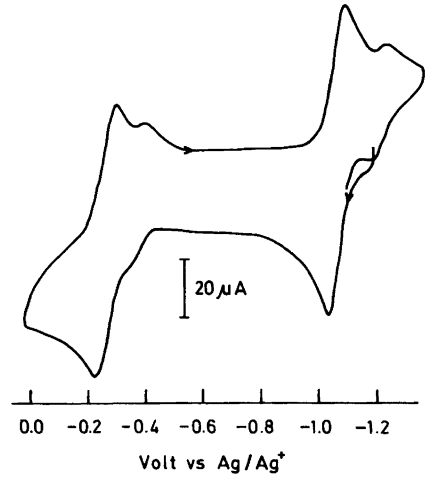
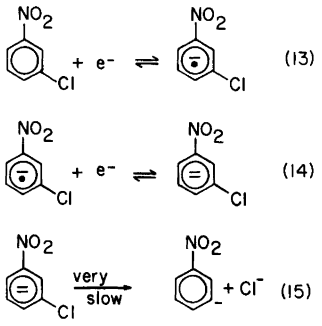


Fig. 3. Cyclic voltammogram for *m*-chloronitrobenzene following coulometric reduction at –1.12 V. Sweep rate 200 mV/s. C=4.1 mM, t=–40 °C.

required to complete the electrolysis, 15 min, and the information regarding nitrobenzene production from cyclic voltammetry, the rate constant for the dehalogenation of the dianion was estimated to be $>10^{-4}$ s^{-1}.

The results indicate that the mechanism of the reduction of *m*-chloronitrobenzene in liquid NH_3 involves the transfer of single electron to form a stable radical anion of the parent. This species was extremely stable as it can be quantitatively reconverted to the parent compound. The dianion of *m*-chloronitrobenzene, although it was stable on the CV time scale, underwent slight decomposition after bulk electrolysis. This decomposition did not exceed 15 %, even when the generated dianion was allowed to stand for 30 min before coulometric oxidation was initiated. From the previous data, we conclude that the reduction of *m*-chloronitrobenzene in liquid NH_3 involves two one-electron transfer steps to form a stable anion radical and a dianion. The dehalogenation of the dianion is very slow, so that a mechanism of the reaction can be written as:



Although the dianion of *m*-chloronitrobenzene was very stable, stepping the potential to the solvent cathodic limits resulted in a noticeable increase in nitrobenzene production. In fact, if the potential was held at –2.2 V for a few minutes, the decomposition of halogenated dianion to nitrobenzene dianion was complete. This observation, although unusual, is not surprising, since the cathodic limit of NH_3 involves the production of solvated electrons. This very strong reducing species probably causes electron addition to the halogenated dianion forming a trianionic species which rapidly cleaves a halide.

p-*Chloronitrobenzene.* The CV for the reduction of *p*-chloronitrobenzene is given in Fig. 4. The voltammogram indicates two successive one-

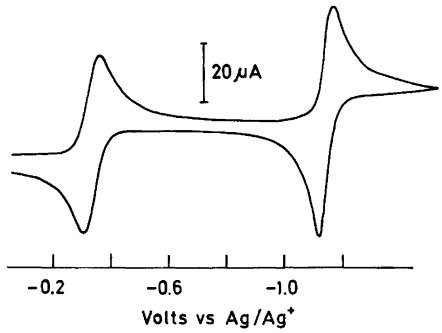


Fig. 4. Cyclic voltammogram for the reduction of 2.05 mM *p*-chloronitrobenzene in liquid NH_3. Sweep rate 200 mV/s, t=–40 °C.

electron processes at –0.36 and –1.16 V. The peak potentials of these waves were not affected by changes in sweep rate. In addition, no new waves appeared even at relatively slow scan rates, *e.g.*, 20 mV/s. Controlled potential experiments were performed to investigate the stability of the radical anion and resulted in an n_{app} = 1.06 for the first wave. No evidence of dehalogenation was observed, since 98 % of the radical anion was converted to the parent by coulometric oxidation. Coulometric reduction at –1.18 V produced an n_{app} = 1.85 and a dark brown solution. A potential scan in the positive direction from –1.18 V showed that the original reversible wave at –0.36 V had disappeared and a new reversible wave appeared at –0.42 V. When the potential was swept negatively from –1.18 V, another reversible wave was present at –1.22 V (Fig. 5). To confirm that these two new waves

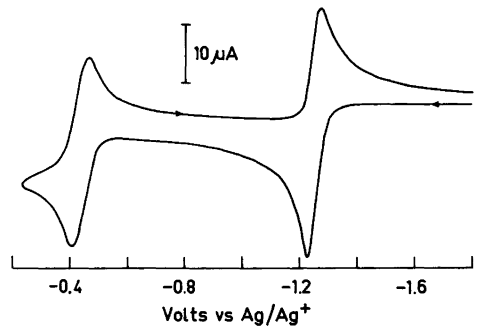


Fig. 5. Cyclic voltammetry following coulometric reduction at –1.18 V of a 4.1 mM solution of *p*-chloronitrobenzene in liquid NH_3. Sweep rate 200 mV/s, t=–40 °C.

were those of nitrobenzene, a 2 μl sample of pure nitrobenzene was introduced into the cell. The cyclic voltammogram of the resulting solution was identical to that obtained before the addition of nitrobenzene with the exception that the peak currents were larger. The previous experimental data clearly demonstrate that *p*-chloronitrobenzene was reduced initially to a stable radical anion in liquid NH_3. The addition of a second electron results in the formation of the dianion of the halogenated parent. This species, although stable on the CV time scale, undergoes dehalogenation and complete conversion to nitrobenzene upon electrolysis.

m-*Bromonitrobenzene.* The CV behavior of *m*-bromonitrobenzene in liquid NH_3 is shown in Fig. 6. Two one-electron reversible waves were observed at –0.30 and –1.08 V, with peak separations of 49±3 mV and 43±3 mV, respectively. The peak potentials for both waves were independent of scan rate, characteristic of nernstian reactions. The extension of the potential sweep beyond –1.2 V resulted in the appearance of a new wave at –1.25 V and on scan reversal a second small oxidation wave at –0.38 V. A typical cyclic voltammogram of this system at different scan rates is shown in Fig. 7. At potential sweeps of less than 100 mV/s, the wave at –1.08 V began to lose its reversible appearance and, at the same time, the one at –1.25 V increased in height. At sweeps >500 mV/s, however, the wave at –1.08 V was totally reversible and the wave at –1.25 V had completely disappeared. The data represents an ECE type process. The slow sweeps allow sufficient time for the dehalogenation and formation of nitrobenzene dianion. The electrolysis of the solution at –0.40 V resulted in the formation of an orange radical anion with n_{app} = 1.10. This species was stable to permit its quantitative conversion to the parent molecule. On the other hand, coulometric reduction at –1.1 V resulted in the passage of 1.95 F/mol and a deep red solution. The CV results following electrolysis indicated the presence of the two nitrobenzene waves discussed previously and observed for other halonitrobenzenes. The overall reaction sequence thus can be represented by:

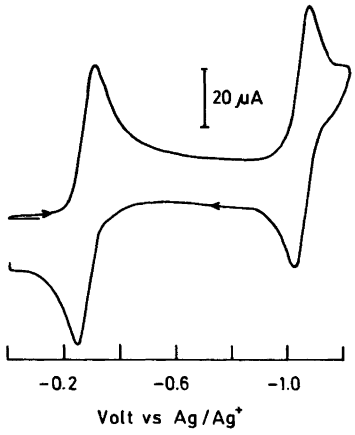


Fig. 6. Cyclic voltammogram for the reduction of a 3.75 mM solution of *m*-bromonitrobenzene in liquid NH_3. Sweep rate 200 mV/s, *t*=–40 °C.

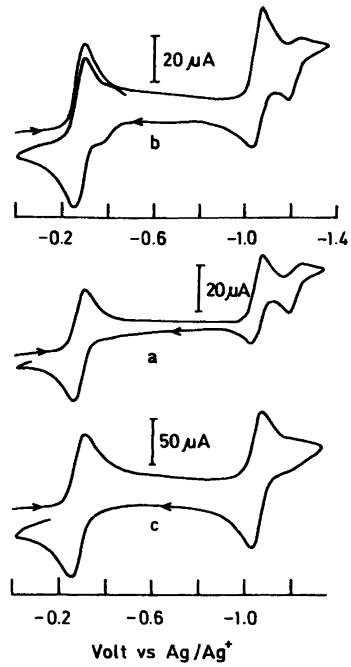
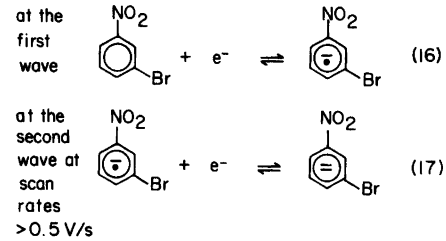
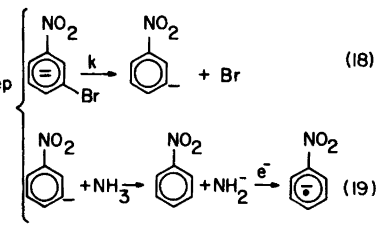


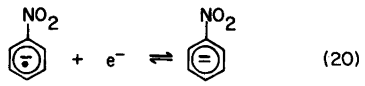
Fig. 7. Cyclic voltammogram for the reduction of 3.75 mM *m*-bromonitrobenzene at various sweep rates: (a) 50 mV/s; (b) 100 mV/s; (c) 500 mV/s.



at the second wave at slow sweep rates



at the third wave



The CV data permits the estimation of a rate constant, k, for the halide elimination step, reaction (18), of the order of 10^{-2} s^{-1}.

o-*Iodonitrobenzene.* The reduction of *o*-iodonitrobenzene in liquid NH_3 occurred in three successive waves. Two reversible waves are observed at –0.40 V and –1.22 V and an irreversible wave at –0.84 V. The E_{pc} for the first reduction wave was completely independent of scan rate; the same was true for the third wave.

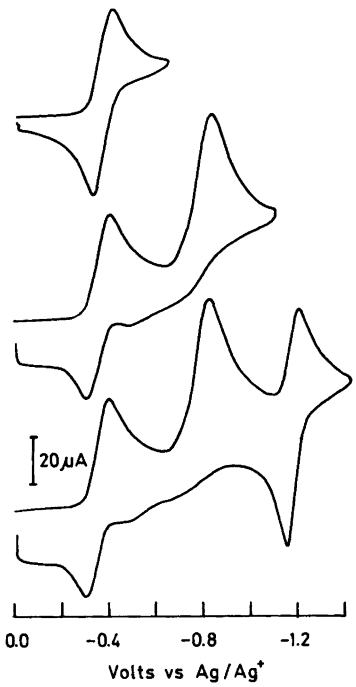


Fig. 8. Cyclic voltammogram for the reduction of 3.78 mM *o*-iodonitrobenzene in liquid NH_3. Sweep rate 200 mV/s, t=–40 °C.

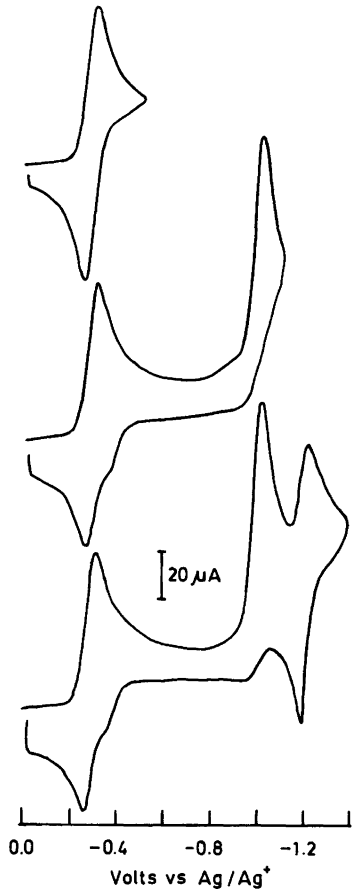


Fig. 9. Cyclic voltammogram for the reduction of 5.12 mM *m*-iodonitrobenzene in liquid NH_3. Sweep rate 200 mV/s, t=–40 °C.

The irreversible wave at –0.84 V was almost twice as large as the other two waves. The peak potential of this wave was dependent on scan rate where a 10-fold increase in sweep rate resulted in a 20 mV negative shift in peak potential. No anodic current was observed for this wave even when the sweep rate was increased to 50 V/s. The cyclic voltammogram of the system is given in Fig. 8. Coulometry at –0.6 V produced an n_{app} = 1.6. The cyclic voltammogram following electrolysis and initiated at –0.6 V resulted in the appearance of two reversible waves at –0.38 and –0.20 V, respectively. Addition of a 2 μl of pure nitrobenzene caused an increase in the wave at –0.2 V. The second electrolysis experiment at –0.90 V resulted in n_{app} = 1.51 and a voltammo-

gram showing only nitrobenzene waves at –0.28 V and –1.10 V. The voltammetric results lead to the conclusion that the reduction of *o*-iodonitrobenzene in liquid NH_3 proceeds by the initial production of a stable radical anion. The coulometric experiments, however, show that this radical anion is partially decomposed to nitrobenzene in about 20 min, the duration of the electrolysis. When the electrolyzed solution was allowed to stand at –40 °C for an additional 15 min, no decrease in the height of the wave corresponding to $I–C_6H_4–NO_2^-$ was observed.

m-*Iodonitrobenzene.* The CV results for the reduction of *m*-iodonitrobenzene in liquid NH_3 were very similar to those obtained from the ortho isomer. However, the halogen cleavage process was much more rapid for the ortho derivative. The voltammograms given in Fig. 9 show three well-defined waves at –0.32, –1.05 and –1.24 V, respectively. As previously, a stable radical anion is formed as seen by the reversibility of the first wave on the CV time scale. The irreversible wave at –1.05 V was about 1.7 times as large as the first reduction wave. Moreover, its peak potential shifted toward negative values with increasing scan rate. Evidence of dehalogenation of *m*-iodonitrobenzene species was also detected by a multicycle voltammogram which indicated that repeated cycling produced a new reversible wave at the same potential as that of the formation of the radical anion of nitrobenzene. The electrolysis of the solution at –0.45 V resulted in the passage of 0.93 F/mol. The solution was orange and showed a CV behavior identical to the parent. A second coulometric electrolysis was performed at –1.10 V and an n_{app} = 1.78 was obtained. The cyclic voltammogram of this solution following coulometry revealed two reversible waves at –0.42 and –1.24 V corresponding to the two-step reduction of nitrobenzene. The results indicate that the reduction of *m*-iodonitrobenzene involves the production of a radical anion which is stable even on the coulometric time scale. The dianion, however, rapidly loses iodide ion to produce, ultimately, nitrobenzene. The CV data at varying sweep rates permit the calculation of a rate constant for the dehalogenation process as 2 s^{-1}.

p-*Iodonitrobenzene.* Three reduction waves were observed for the reduction of *p*-iodonitrobenzene: a reversible wave at –0.30 V, an

Table 2. Summary of the cyclic voltammetric and coulometric data for the reduction of a series of halonitrobenzenes in liquid NH_3 containing 0.1 M KI at –40 °C.[a]

Compound	$-E_p$ (RX/RX$^\cdot$) Volts	n	n_{app}[b]	$-E_p$ ($RX^{\cdot-}$/RX^{2-}) Volts	n	n_{app}	$-E_p$ ($R^{\cdot-}$/R^{2-}) Volts	k (s^{-1})[c]	$i_p/\nu^{1/2}C$ $A\ s^{1/2}\ l/V^{1/2}$ mmol
$C_6H_5NO_2$	0.44 (X=H)	(1) r	1.0				1.24	–	
o-Cl-$C_6H_4NO_2$	0.40	(1) r	0.96	1.10	(1.75) I	2.1	1.22	1.2	37.0
m-Cl-$C_6H_4NO_2$	0.30	(1) r	0.95	1.09	(1) r	1.10		$<<10^{-4}$	37.4
p-Cl-$C_6H_4NO_2$	0.36	(1) r	1.06	1.16	(1) r	1.85		–	35.6
m-Br-$C_6H_4NO_2$	0.30	(1) r	1.10	1.08	(1.12) r	1.95	1.24	10^{-2}	31.6
o-I-$C_6H_4NO_2$	0.40	(1) r	1.60	0.84	(1.30) I	1.51	1.22	–	27.0
m-I-$C_6H_4NO_2$	0.32	(1) r	0.93	1.05	(1.63) I	1.78	1.24	2.0	28.9
p-I-$C_6H_4NO_2$	0.30	(1) r	1.06	1.01	(1.54) I	1.78	1.23	2.1	24.9

[a] E_p – Cathodic peak potential; n = the number of electrons transferred per molecule from voltammetric data. I – Irreversible wave; r = reversible wave; scan rate 200 mV/s; electrode area 0.039 cm^2. [b] n_{app} = The number of electrons transferred per molecule from coulometric results. [c] For loss of halide ion by dianion.

irreversible wave at –1.01 V and a reversible third wave at –1.23 V. These waves were almost identical with the ones obtained from the previous iodoisomers with the exception of small shift in the peak potentials. Controlled potential coulometry at –0.50 V resulted in an $n_{app} = 1.06$. The voltammogram of the solution following coulometry revealed the original waves. The bright red solution was then electrolyzed at –1.05 V. The solution became dark brown and required the passage of 1.78 F/mol. As before, nitrobenzene was the final product of the reaction. The rate constant for the iodide cleavage process was 2.1 s^{-1}.

CONCLUSIONS

The electrochemical reduction of a series of halonitrobenzenes in liquid NH_3 containing 0.1 M KI at –40° leads to the CV and coulometric data summarized in Table 2. The reduction of the chloronitrobenzenes series resulted in the following observations: The *ortho* derivative was initially reduced to a stable radical anion; however, the dianion was unstable, eventually yielding nitrobenzene. The *meta* derivative forms both a stable radical anion and dianion upon reduction. In fact, the latter was stable even at the coulometric time scale with only 10 % decomposition observed after allowing the electrogenerated dianion to stand over a period of 15–20 min. The *para* isomer produced a stable radical anion on both the CV and the coulometric time scale; however, the electrolysis of the solution to produce the dianion resulted in quantitative conversion to nitrobenzene. *m*-bromonitrobenzene was the only bromo-compound studied. Cyclic voltammetry confirmed the formation of a stable radical anion and dianion. At the same time, evidence of slight decomposition was seen when the potential was scanned negative enough to allow the generation of the nitrobenzene dianion. Coulometric generation of the dianion produced nitrobenzene and bromide ion. The reduction of all three iodo derivatives was similar. The radical anions of the *meta* and *para* derivatives were stable enough to survive the coulometric experiments without apparent decomposition. On the other hand, the coulometrically generated radical anion of the *ortho* derivative indicated partial decomposition to nitrobenzene and iodide ion. The dianions for all three iodo isomers were unstable in liquid NH_3 as evident by rapid iodide cleavage.

The results of the electroreduction of halonitrobenzenes in liquid NH_3 can be compared with electrochemical studies in other nonaqueous solvents. In DMF and ACN, ESR measurements on electrogenerated radical anions of a series of halonitrobenzenes employing *in situ* methods resulted in the following observations.[22,23] The *meta*- and *para*-chloro, *meta*- and *para*-bromo nitrobenzenes all produced ESR spectra characteristic of their radical anions. The *ortho*-chloro and *ortho*-bromo derivatives showed a decreased stability of the corresponding radical anion, probably because of steric effects. ESR studies have also indicated that all the iodo isomers undergo complete dehalogenation in ACN and DMF. However, recent studies of halonitrobenzene radical anions in DMF as a function of temperature [14] suggest that appreciable stabilities of the radical anions (*e.g.* *m*- and *p*-iodonitrobenzene) would be obtained at half-lives greater than 5 min for –40 °C.

In liquid NH_3, one is able to prepare coulometrically stable radical anions from all the halonitrocompounds studied with the possible exception of the *o*-iodo derivative. The ability of halogenated compounds to resist halogen cleavage in NH_3 could possibly be attributed to the low temperature conditions (–40 °C). Low temperature electroreductions of *ortho* and *para* iodonitrobenzenes in DMF have indeed shown an enhanced stability of the radical anions; however, this increased stability was accompanied by a large anodic-cathodic peak separation (200 mV in the cyclic voltammogram of *ortho*-iodonitrobenzene at –70 °C). Although the temperature effect could be the main contributing factor for the enhanced stability of radical anions in liquid NH_3, dianions of halonitrobenzenes, which cannot be prepared in other nonaqueous solvents due to a rapid protonation rate, are stable in NH_3 and allow the investigation of the halogen cleavage process.

Finally, the experimental results show that the rate at which the halonitrobenzenes radical anions are dehalogenated during electrochemical reduction is in the order: *m*-chloro < *p*-chloro < *m*-bromo < *o*-chloro < *m*-iodo ~*p*-iodo < *o*-iodo.

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Electrochemical Studies on Organometallic Compounds. IV. Influence of the Substitution and of the Nature of the Ligands on the Products of the Electrochemical Reduction of Titanocene Mono and Dichlorides

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The 1e⁻ reduction of $(\eta^5\text{-}C_5Me_5)_2TiCl_2$ and $(\eta^5\text{-}C_5H_5)$ $(\eta^5\text{-}C_5H_4(CH_2)_2P(C_6H_5)_2)TiCl_2$ in THF is followed by a fast loss of Cl^-. This reaction is much slower for $(\eta^5\text{-}C_5H_4CO_2CH_3)_2TiCl_2$. The uptake of an electron by the reduction product is examined by linear potential sweep voltammetry in THF in the presence of various ligands L. A pseudoreversible behaviour is observed with L=dimethylphenylphosphine. In the presence of cyclohexyl isocyanide, the reduction gives a stable Ti(II) species.

Titanocene dichloride $(\eta^5\text{-}C_5H_5)_2TiCl_2$ *1* is reduced electrochemically in three monoelectronic stages;[1,2] during the first, a Cl^- ion is lost very rapidly after the uptake of the electron. This phenomenon can be easily followed by studying the cyclic voltammograms (*cf.* Fig. 1).

The reactions corresponding to the different peaks are given in Scheme 1 (X=H); L, a neutral ligand, can be added to the solution or can be a molecule of the solvent.

The relative magnitude of peaks A′ and A'_1 depends on the rate of recombination of Cl^- with the species Cp_2TiClL. If for a given sweep rate v, this rate is very large, A′ will appear alone; if it is very small, only A'_1 will be observed. On the other hand, if v increases, A'_1 grows relatively to A′, because the recombination reaction has less time to take place (*cf.* Fig. 1).

Compounds *1, 2, 3*

$$(C_5H_4X)_2TiCl_2 \overset{+e^-}{\rightleftharpoons} (C_5H_4X)_2TiCl_2^- \overset{-Cl^-,\ +L}{\rightleftharpoons} (C_5H_4X)_2TiClL \qquad A/A'$$

$$(C_5H_4X)_2TiClL \overset{-e^-}{\rightleftharpoons} (C_5H_4X)_2TiClL^+ \overset{-L,\ +Cl^-}{\rightleftharpoons} (C_5H_4X)_2TiCl_2 \qquad A'_1$$

Compound *4*

$$(C_5H_4X)_2TiCl_2 \overset{+e^-}{\rightleftharpoons} (C_5H_4X)_2TiCl_2^- \qquad A/A''$$

$$(C_5H_4X)_2TiClL \overset{-e^-}{\rightleftharpoons} (C_5H_4X)_2TiClL^+ \rightleftharpoons (C_5H_4X)_2TiCl_2 \qquad A'_1$$

Scheme 1.

0302-4369/83 $2.50

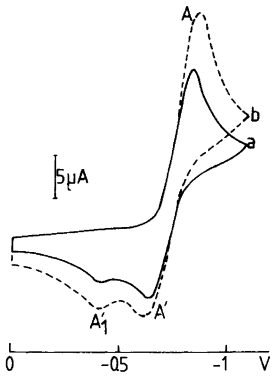


Fig. 1. Cyclic voltammograms of *1* in THF at −54 °C. Sweep rate: a, 50 mV/s; b, 100 mV/s. Starting potential: 0 V.

A priori, three factors can have an influence on the rate of recombination.

(a) The temperature. A decrease of the temperature should decrease the rate, *i.e.* A'_1 should increase. This was shown to be the case in a previous work.[2] In tetrahydrofuran at room temperature, only A′ is seen; as the temperature decreases, A'_1 appears (*cf.* Fig. 1).

(b) The ligand L. The more strongly L is bound, the slower should the recombination become. From a previous study,[2] and from the results of the present investigation, it is found that the ligands are less and less strongly bound in the sequence cyclohexyl isocyanide (CHIC), *N,N*-dimethylformamide (DMF), 2,6-dimethylphenylisocyanide (DMPIC), pyridine, dimethylphenylphosphine (DMPP) and THF.

(c) A change in the structure of the molecule. Electron-attracting substituents should increase the rate of recombination, while electron-donating ones should decrease it. This effect, which was not investigated earlier, is examined in this paper.

Table 1. Potential peaks (V) of titanocene derivatives.

	A	B	C
1	−0.8 [a]	−2.1	−2.4
	−0.85 [b]	–	–
2	−1 [a]	−2.18	–
3	−0.8 [b]	−2	−2.5
4	−0.25 [a]	–	–

[a] In THF. [b] In acetone.

The electrochemistry of the second stage of the reaction has never been systematically investigated, although it is known that the formation of a complex with nitrogen requires that the metal should be in the +2 oxidation state.[3] We report here a few preliminary results on this problem.

RESULTS AND DISCUSSION

Influence of the substituents on the first stage. We have examined three titanocene dichloride derivatives, $(\eta^5\text{-}C_5Me_5)_2TiCl_2$ *2*, $(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4(CH_2)_2P(C_6H_5)_2TiCl_2$ *3* and $(\eta^5\text{-}C_5H_4CO_2CH_3)_2\ TiCl_2$ *4*. The peak potentials of peak A (first stage), B (second stage) and C (third stage) are given in Table 1.

The peak potentials of *2* are shifted towards negative potentials, owing to the electron releasing character of the methyl groups. In THF at room temperature, peak A'_1 is well marked (Fig. 2) whereas for titanocene dichloride it did not appear under the same conditions.[2] This shows that the recombination rate is decreased, in accordance with the predictions presented above.

The peak potentials for compound *3* are practically the same as for titanocene dichloride; the substituent has neither electron-donating nor electron-releasing properties. The behaviour of this compound should thus be similar to that of *1*. In THF at room temperature, however, peak A′

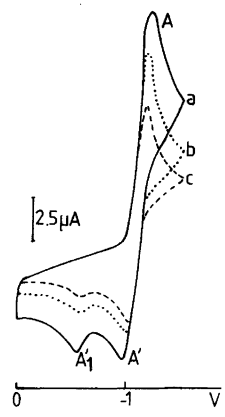


Fig. 2. Cyclic voltammograms of *2* in THF. Sweep rate: a, 500 mV/s; b, 200 mV/s; c, 100 mV/s. Starting potential: 0 V.

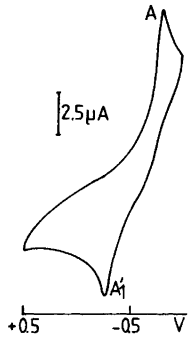


Fig. 3. Thin layer voltammogram of *3* in THF. Sweep rate: 50 mV/s. Starting potential: +0.5 V.

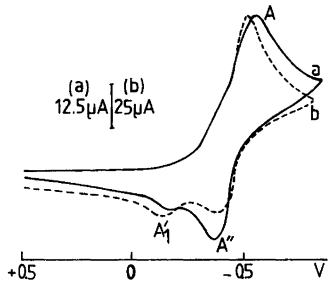
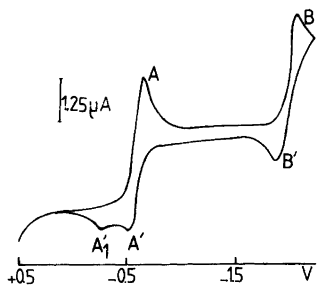


Fig. 4. Cyclic voltammograms of *4* in acetone at +35 °C. Sweep rate: a, 1 V/s; b, 200 mV/s. Starting potential: +0.5 V.



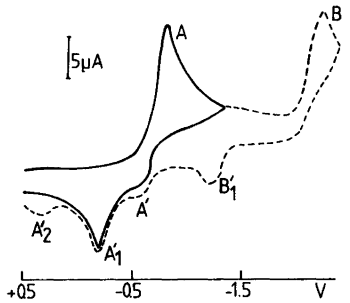


Fig. 5. (a) Cyclic voltammogram of *1* in THF in presence of DMPP. Sweep rate: 10 mV/s. Starting potential: +0.5 V. (b) Cyclic voltammogram of *1* in THF in presence of DMPP. Sweep rate: 2 V/s. Starting potential: +0.5 V.

is completely absent (Fig. 3). This can be attributed to an internal ligandation,[4] in which the phosphine ligand chelates the metal after the Cl^- ion separates.

For compound *4*, the peak potential is much more positive than for *1*, which points to a strong decrease of the electronic density on the metal. The experiments were carried out in acetone, because *4* is not soluble enough in THF.

In acetone, the voltammogram of *1* shows both peaks A′ and A'_1; an increase in the scan rate or a decrease of the temperature causes an increase of A'_1, as in THF.

Compound *4* also shows two peaks A″ and A'_1; however, A″ increases when the sweep rate increases, instead of decreasing (Fig. 4). This points to a relative stability of the anionic species $(\eta^5\text{-}C_5H_4CO_2CH_3)_2TiCl_2^-$ (*cf.* Scheme 1, $X=CO_2CH_3$); the rate at which Cl^- leaves the molecule would be much slower than for *1*, because of the decreased electronic density on the metal. At larger sweep rates, more of the anionic species would be present, whence the increase of A′.

The second reduction stage for Cp_2TiCl_2. In THF, at sweep rates larger than a few V s^{-1}, a reversible system B/B′ is obtained. At slower sweep rates, peak B′ disappears. When DMPP is added to the solution, a pseudo-reversible [2] system B/B′ is observed at slow sweep rates (Fig. 5a); peak B′ decreases and a new peak B'_1 appears at higher sweep rates (Fig. 5b).

With CHIC, peak B′ is never observed whatever the sweep rate; only B'_1 and a new peak A'_2 appear (Fig. 6). If the scan is reversed again after peak B'_1 (Fig. 7), a new peak B_1 is observed, the system B_1/B'_1 being reversible. The same peaks B'_1, A'_2 and B_1 are obtained by starting directly from the chemically prepared [5] species $(\eta^5\text{-}C_5H_5)_2TiCl\text{–}CHIC$.

These results show that the behaviour of the species $(\eta^5\text{-}C_5H_5)_2TiClL$ is in general analogous to that of *1* and can be explained on the basis of Scheme 2.

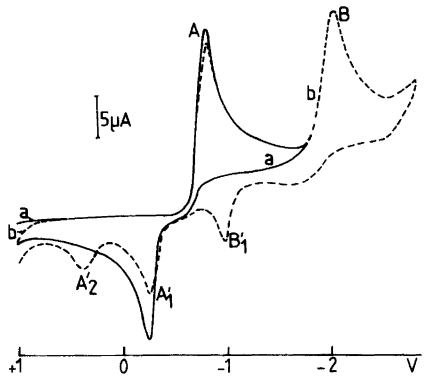


Fig. 6. Cyclic voltammograms of *1* in THF in presence of CHIC. Sweep rate: 100 mV/s. a, first sweep; b, second sweep. Starting potential: +1 V.

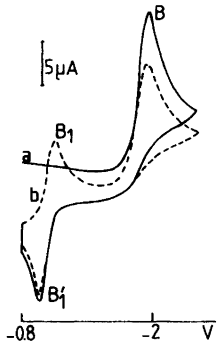


Fig. 7. Cyclic voltammograms of *1* in THF in presence of CHIC. Sweep rate: 100 mV/s. a, first sweep; b, second sweep.

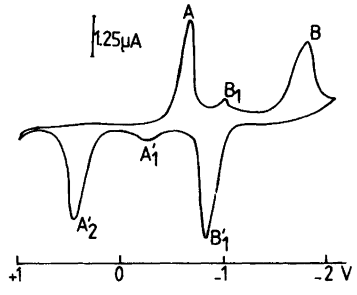


Fig. 8. Thin layer voltammogram of *1* in THF in presence of CHIC (second sweep). Sweep rate: 20 mV/s. Starting potential: +1 V.

$$Cp_2TiCl_2 \overset{+e^-,\,-Cl^-,\,+L}{\rightleftharpoons} Cp_2TiClL \qquad A/A'$$

$$Cp_2TiClL \overset{+e^-,\,-Cl^-}{\rightleftharpoons} X \qquad B/B'$$

$$X \overset{-e^-}{\rightleftharpoons} X^+ \qquad B'_1/B_1$$

$$X^+ \overset{-e^-}{\rightleftharpoons} X^{2+} \overset{+2Cl^-}{\rightleftharpoons} Cp_2TiCl_2 \qquad A'_2$$

Scheme 2.

As for the first stage, the uptake of an electron by the molecule (peak B) is followed by a fast loss of a Cl^- ion to give a titanium(II) complex, which we have designated as X. The structure of this compound, which is unstable, is now under investigation; according to our first results, X is probably a dimer in which only one molecule of CHIC per titanium atom is present. The species X^+, which is obtained by oxidation of X (peak B'_1, Scheme 2) is more stable than its analog [2] Cp_2TiClL^+, since it does not react with the Cl^- ions to regenerate Cp_2TiClL. Such a recombination, however, occurs after the oxidation of X^+ to X^{2+}, since Cp_2TiCl_2 is totally regenerated during this step (peak A'_2, followed upon a cathodic sweep by peak A in a thin-layer cell) (Fig. 8).

The stability of the Ti(II) complex depends on the ligand. The fact that in pure THF peaks B'_1 and A'_2 are not obtained points to a rapid decomposition, probably due to the fact that THF does not bind strongly enough to the molecule. In pyridine, the system B_1/B'_1 is visible only at high sweep rate or at low temperature; the behaviour is thus intermediate between that in pure THF and that in THF containing CHIC. In DMF, as in THF, the system B'/B'_1 is never observed.

All the above results were obtained under argon atmosphere. During preliminary experiments under nitrogen atmosphere, we did not find any difference in THF in the presence of strong ligands (DMPP, CHIC). Irreproducible changes in the second peak are observed in THF alone. In DMF, at low temperature on a mercury electrode, a highly reproducible modification of the second stage takes place. This shows the complexity of the reaction; it seems however that the presence of a too strongly coordinated ligand

prevents the reaction between nitrogen and the Ti(II) complex. We are currently investigating these phenomena by using differently substituted titanocene dichlorides.

EXPERIMENTAL

All manipulations were performed under argon. Tetrahydrofuran was purified by distillation from sodium benzophenone ketyl under argon.

The reference electrode was a saturated calomel electrode separated from the solution by a sintered glass disk. The auxiliary electrode was a platinum disk electrode. The supporting electrolyte was tetrabutylammonium hexafluorophosphate (0.2 M) in all cases; the salt (Fluka) was dried and deoxygenated before use.

A Tacussel UAP4 unit connected to a Tektronix oscilloscope (linear potential sweep experiments), a three electrode Tacussel Tipol polarograph, an Amel 552 potentiostat and a Tacussel IG5 integrator were used.

The complexes $(\eta^5\text{-}C_5Me_5)_2TiCl_2$ [6] *2* and $(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4(CH_2)_2P(C_6H_5)_2TiCl_2$ [4] *3* were prepared by literature methods. The carbomethoxy derivative *4* was obtained by the following procedure: A solution of $[C_5H_4CO_2CH_3]^-Na^+$ [7] (8.16 mM) in 30 ml of THF was added to $TiCl_4$ (4.08 mM) in THF at 0 °C. The solution was warmed slowly to room temperature and stirred for 2 h. A red precipitate was formed which was filtered off, then washed with 20 ml of cold THF (yield 50 %). ^1H NMR ($CDCl_3$): δ 7.17 (4 H, t, J 2.5 Hz), 6.56 (4 H, t, J 2.5 Hz), 3.90 (6 H, s). IR (KBr) $\nu c{=}o$ 1725 cm^{-1}. MS [IP 70 ev; *m/e*] 364 (M), 241 ($M-C_5H_4CO_2CH_3$).

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Electrochemical Acylation and Carboxylation of Some Activated Olefins

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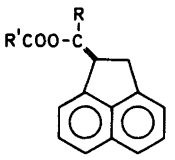
The electrochemical acylation and carboxylation of some activated olefins have been investigated. Acenaphthylene yields thus on reductive electrochemical acetylation mainly the *Z* and *E* enol acetates of 1-(1,2-dihydro-1-acenaphthylidene) ethanone, whereas carboxylation followed by methylation gives *trans*-1,2-dimethoxycarbonyl-1,2-dihydroacenaphthene. Ethyl cinnamate can be acylated and carboxylated in the 3-position, whereas benzoylacetone could be carboxylated but not acetylated. Neither carboxylation nor acylation were able to compete with the dimerization of benzylidenemalonitrile. Cyclic voltammetry showed that carboxylation generally was faster than acetylation.

The electrochemical acylation of activated olefins [1–6] has been shown to be a useful and rather general reaction. In some cases, however, *e.g.* during the reductive acylation of anthracene,[1] acenaphthylene [2] and cinnamonitrile,[2] it has been briefly reported that the enol acetate of the expected ketone was the isolated product rather than the ketone. A more detailed study of this reaction is reported below, and it is compared with the carboxylation reactions.

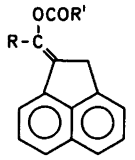
Acenaphthylene (*1*), cinnamonitrile (*2*), benzalacetone (*3*) and benzylidenemalonitrile (*4*) have been reduced in *N,N*-dimethylformamide (DMF) in the presence of acetic anhydride (*5*), 4-chlorobutyric anhydride (*6*) and carbon dioxide. The olefins were chosen as examples of a symmetrical (*1*), a mono-activated olefin (*2* and *3*) and a doubly activated olefin (*4*); *6* was included to investigate the possibility of a simultaneous acylation and ring closure.[7,8]

RESULTS

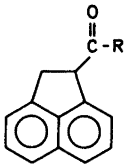
Acenaphthylene. On cyclic voltammetry *1* shows a reversible reduction at −1.65 V (aq.SCE) and an irreversible peak at $E_p = -2.45$; on addition of a tenfold excess of *5* the peak height of the first peak grows to about 1.6 times the original height and the anodic peak disappears; the second peak, if present, is hidden by the reduction of *5*. On increasing the sweep rate



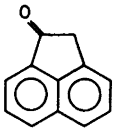
7a R = R' = CH_3
b R = R' = $(CH_2)_2CH_2Cl$
c R = $(CH_2)_2CH_2Cl$, R' = cyclopropyl



8a R = R' = CH_3
b R = R' = $(CH_2)_2CH_2Cl$
c R = $(CH_2)_2CH_2Cl$, R' = cyclopropyl



9a R = CH_3
b R = $(CH_2)_2CH_2Cl$



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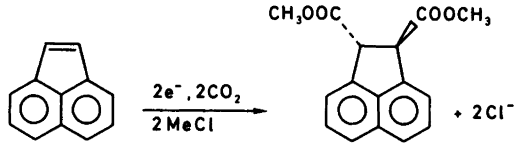


Scheme 1.

the reduction of *1* in the presence of *5* becomes reversible; the pseudo first-order rate constant of the reaction between $1^{\cdot-}$ and *5* was about 50 s^{-1}.[9]

Preparative electrochemical reduction of *1* at a mercury cathode in DMF in the presence of an excess of *5* gave predominantly the *Z* (*7a*) and *E* (*8a*) enol acetate of 1-(1,2-dihydro-1-acenaphthylidene)ethanone (*9a*) together with a little acenaphthene (*10*); minor amounts of *9a* and acenaphthylenone (*11*) were sometimes isolated, possibly formed during the work-up. No *C*-diacetylated derivatives were isolated (Table 1).

The choice between the *Z* and *E* forms was made from ^{1}H NMR Difference Nuclear Overhauser Effects and the assignment is consistent with expected chemical shifts and coupling constants (see Experimental).

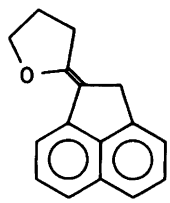
Reduction of *1* in DMF in the presence of carbon dioxide and methyl chloride yields mainly *trans* 1,2-dimethoxycarbonyl-1,2-dihydroacenaphthene (Scheme 1).

Cyclic voltammetry of *1* after addition of *6* resembles that in the presence of *5*. *6* gives an irreversible peak at −2.35 V.

Preparative reduction of *1* in the presence of an excess of *6* gave *9b* as the major product (Table 1) together with minor amounts of *7b* and *8b*. The *Z*–*E* assignment of *7b* and *8b* was done on the basis of the ^{1}H NMR spectra (see Experimental). In this case too the *Z*-isomer was formed in higher yield than the *E*-isomer. Besides these compounds, some acenaphthylenone (~8 %) was also isolated.

Reduction of a solution containing equivalent concentrations of *1* and *6* yielded no *9b*, but *7b* and *8b* together with the cyclopropane derivatives *7c* and *8c* besides some *10* and *11*. *7b* and *8b* can be transformed into *7c* and *8c* in the presence of a strong base; during the reduction, bases are generated and, in the absence of an excess of *6*, the electrogenerated base, *e.g.* $1^{\cdot-}$, may induce the ring closure. Thus if $1^{\cdot-}$ was generated in a mixture (7:3) of *7b* and *8b*, *7c* and *8c* were formed (3:2) with some *7b* still unchanged; the transformation *8b* → *8c* is thus slightly faster than the ring closure of *7b* to *7c*.

In the presence of a strong base, *e.g.* an electrogenerated base, *9b* may be cyclized to the *Z* (*12*) and *E* (*13*) isomers of 1-(2-tetrahydrofuranylidene)-1,2-dihydroacenaphthene. The *Z*/*E* assignment was made on the basis of the chemical



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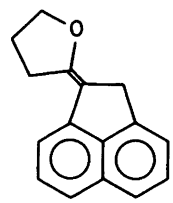

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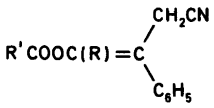
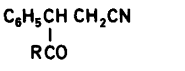
Table 1. Product distribution (isolated yield (%)) in the reductive acylation of the activated olefines *1* and *2* in the presence of *5* or *6*.

RCH=CHY	Anhydride (equiv.)	RCH_2CH_2Y and/or dimers	Ketone	Acylated enol *Z*	Acylated enol *E*	Furan derivative	Other compounds
1	*5* (10)	*10* (9)		*7a* (43)	*8a* (18)		*11* (traces)
	6 (4)	*10* (6)	*9b* (46.5)	*7b* (8.5)	*8b* (3.5)		*11* (8.5)
	6 (1)	*10* (9)		*7b* (11)	*8b* (5)		*11* (8)
				7c (3)	*8c* (4)		
2	*6* (1)	Dihydrocinnamonitrile (2)	*15b* (14)	*14b* (9)		*16* (5)	*18* (14)
		17a (6)	*15c* (7)				
		17b (10)					



14a R=R'=CH_3

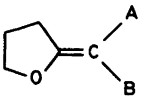
b R=$(CH_2)_2CH_2Cl$, R'=cyclopropyl



15a R=CH_3

b R=$(CH_2)_2CH_2Cl$

c R=cyclopropyl



A C_6H_5 or CH_2CN

B CH_2CN or C_6H_5

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shifts and coupling constants in the ^1H NMR spectrum (see Experimental). The transformation *9b*→*12*+*13* may be effected by electrogenerated dioxygen anion radical $O_2^{\cdot-}$ or $1^{\cdot-}$. The compounds *12* and *13* are very sensitive towards oxygen and are rapidly decomposed to *11*.

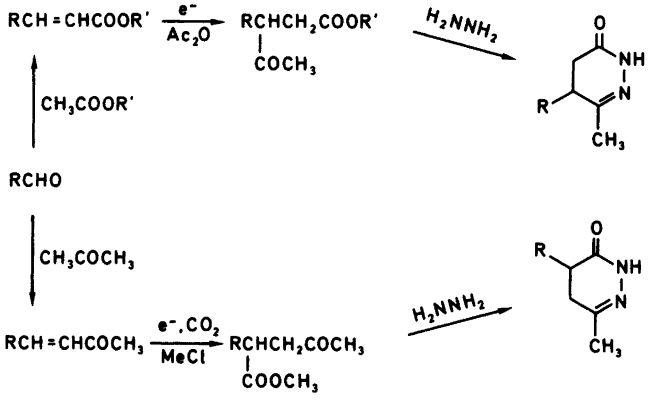
Cinnamonitrile. *2* dimerizes faster than *1* (k_{Dim}=800 M^{-1} l s^{-1});[10] CV of *2* in DMF at sweep rate v=~10 V s^{-1} gave a reversible reduction which on addition of an excess of *5* changed to an irreversible peak with increased peak height. The pseudo first-order rate constant of the reaction of $2^{\cdot-}$ with *5* was estimated from cyclic voltammetric data to be $\geq 5 \cdot 10^2$ s^{-1};[9] CV thus indicates the possibility of acetylation of $2^{\cdot-}$.

Reduction of *2* in DMF in the presence of *5* gave the enol acetate (*14a*) of 3-phenyl-4-oxovaleronitrile (*15a*) and *15a*. Similar types of products were obtained from the reduction of *2* in the presence of *6* (Table 1), but the product mixture is more complicated due to the possibility of ring closure to tetrahydrofuranylidene derivatives.

Electrochemical carboxylation of *2* followed by methylation gave methyl 3-cyano-2-phenylpropionate.

Benzalacetone. (*3*) dimerizes fast (k_{Dim}=1.35×10^5 M^{-1} l s^{-1}) [11] and reduction of *3* in the presence of *5* does not give C-acetylation in an appreciable yield, as this reaction apparently is too slow compared with the dimerization reaction. Reductive carboxylation is fast enough to compete with the dimerization, and on reductive carboxylation of *3* followed by methylation a good yield of methyl 2-phenyl-4-oxovaleriate was isolated. The acetylation of methyl cinnamate [2] gives the isomeric methyl 3-phenyl-4-oxovalerate so the two methods together give starting materials for the preparation of a number of heterocyclic compounds (Scheme 2).

Benzylidenemalonitrile (*4*) dimerizes very fast on electrochemical reduction in DMF; neither the acetylation nor the carboxylation reaction can compete with the dimerization. Reductive carboxylation followed by methylation was attempted at −35 °C, but only the ring closed dimer, *cis*-2-amino-4,5-diphenyl-1,3,3-tricyanocyclopent-1-ene,[12] plus some of the *trans* isomer were isolated.



Scheme 2.

DISCUSSION

The reduction potentials of the activated olefins *1–4* are less negative than that of the anhydride *5* and *6*; it is therefore assumed that primarily formed species is the anion radical of the activated olefin. This anion radical may react in a number of ways; it may be protonated or abstract a hydrogen atom, it may form a dimer in some way or initiate a polymerization reaction, and it may react with the anhydride.

The protonation is not a serious competing process under proper conditions, although it is difficult completely to avoid it. The rate of the dimerization reaction is dependent on the structure of the alkene; generally the rate increases with the polarization of the alkene by the activating group(s). Thus, whereas $1^{\cdot-}$ and $2^{\cdot-}$ couple with *5*, *6*, and carbon dioxide in preference to dimerization, $3^{\cdot-}$ can be carboxylated, but the dimerization is faster than the acylation; $4^{\cdot-}$ dimerizes faster than it reacts with *5* or even CO_2.

The product from the reaction between the anion radical and the anhydride is in most cases mono-C-acylated, although diacetylation has been reported at high concentrations of the anhydride.[5,6] The C-acylated product may react further with the anhydride to the O-acylated enol of the primarily formed ketone.

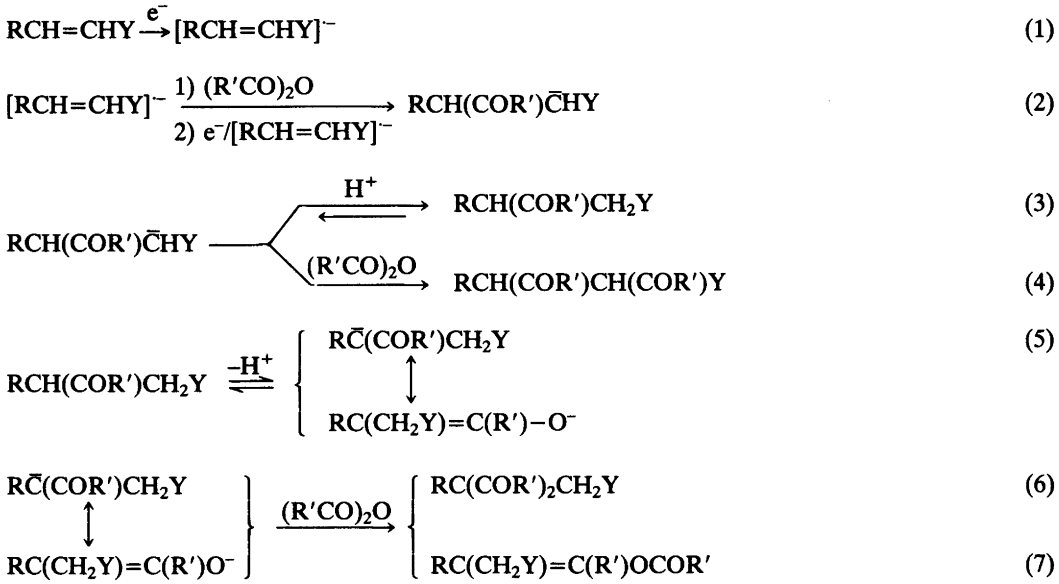
The acylation reaction is assumed to proceed as given by eqns. (1)–(7).

Reaction (4) may be important in the presence of a large excess of the anhydride; the relative importance of reactions (3) and (5) would be expected to be dependent on the relative acidity of the hydrogens at the two central carbon atoms, *i.e.* on the relative activation by Y and by R'CO (+R). Any acid-base system with a suitable pK_A, including the acylated product, may catalyze the tautomerization.

The scheme explains readily that *1* and similar symmetrically (or nearly so) activated olefins form the enol acetate on reductive acetylation. However, the product distribution must rest on a rather delicate balance involving the degree of activation and probably also experimental conditions; *2* produces comparable amounts of the enol derivatives and the ketone on reaction with *6*, whereas ethyl cinnamate gives on reaction with *5* a high yield of the ketone and no isolated yield of the enol acetate; methyl 3-phenylpropynoate yielded,[5] besides the ketone, some of the enol acetate.

The derivatives of *6* have the possibility of making a ring closure either to a cyclopropane derivative or a tetrahydrofuran derivative. Both types of ring closure require the presence of a base, which could be electrogenerated.

The tetrahydrofuran derivatives *12* and *13* are very sensitive to oxygen and are cleaved to *11*; attempts to recrystallize *12* and *13* lead to



oxidative degradation. Also hydrolysis of the enol acetate *7a* must be done in the absence of oxygen otherwise no *9a* is isolated.

EXPERIMENTAL

Reduction of 1 *in the presence of* 5. *1* (0.50 g) was reduced in DMF/0.1 M TBAI (+3.0 ml *5* at −1.7 V *vs.* SCE, n=1.8−1.9. The crude product (0.55 g) was purified on a column of silica using benzene as eluent; the following compounds were isolated (in order of elution): Acenaphthene (9 %), *7a*+*8a* (61 %) and in some experiments traces of acenaphthylenone *11* and *9a*. *7a* (43 %) and *8a* (18 %) were separated on a column of silica using acetone–hexane 1:4 as eluent.

7a (*Z*-isomer). M.p. 113−114 °C, ^{1}H NMR ($CDCl_3$): δ 2.25 (3H,s), 2.35 (3H, relatively broad s), 3.90 (2H, relatively broad s), 7.15−7.85 (6H,m). IR (KBr, cm^{-1}): 1745(s), 1680(w), 1425(m), 1365(m), 1225(s), 1185(s), 815(s), 781(s).

8a (*E*-isomer), ^{1}H NMR ($CDCl_3$): δ 2.18 (3H, relatively broad s), 2.33 (3H,s), 3.92 (2H, relatively broad s), 7.15−7.9 (6H,m). IR (film, cm^{-1}): 1740(s), 1700(w), 1370(s), 1230(s), 1177(s), 812(m), 780(s).

The *Z*/*E*-assignment was made from the following considerations: Irradiation of CH_3−C= of *7a* gave a positive NOE at C-2, whereas irradiation of CH_3CO gave a positive NOE at C-8. δ of CH_3C= in *7a* at 2.18, in *8a* at 2.35 (greater influence of the ring current in *8a*); δ of CH_3CO in *7a* 2.33, in *8a* at 2.25. J (CH_3C=/CH_2) greater in *7a* (*trans* coupling) than in *8a*.

9a. ^{1}H NMR ($CDCl_3$): δ 2.10 (3H,s), 3.4−3.7 (2H,m), 4.47 (1H,dd, J_1 7.5 Hz, J_2 4.7 Hz), 7.1−7.8 (6H,m).

Reduction of 1 *in the presence of* 6 *(1)*. *1* (0.50 g) was reduced as described above in the presence of *6* (2.5 ml), n=1.9. The crude product (0.87 g) was purified as described above. The following compounds were isolated (in order of elution): Acenaphthene (6 %), *7b* and *8b* (12 %), *9b* (46 %) and *11* (8.5 %). The isomers *7b* and *8b* were separated as described for *7a* and *8a*.

7b (*Z*-isomer). ^{1}H NMR ($CDCl_3$): δ 1.9−2.45 (4H,m), 2.65−3.00 (4H,2t), 3.62 (2H,t), 3.67 (2H,t), 4.08 (2H, rel.br.s), 7.2−7.8 (6H,m). IR (film, cm^{-1}): 3100−2800(w), 1750(s), 1675(w), 1130(s), 815(m), 778(s). MS (*m/s* (%)): 366(1), 364(4), 362(6), 258(100).

8b (*E*-isomer). ^{1}H NMR ($CDCl_3$): δ 1.9−2.5 (4H,m), 2.6−3.1 (4H,m), 3.7 (4H,2t, J 6 Hz), 3.9 (2H, rel.br.s), 7.2−7.8 (6H,m). IR (film, cm^{-1}): 3160−2850(m), 1745(s), 1675(w), 1130(s), 813(m), 775(s), MS (*m/e* (%)): 366(1), 364(4), 362(6), 258(100).

9b. ^{1}H NMR ($CDCl_3$): δ 1.9−2.3 (2H,m), 2.6−3.0 (2H, m, AB of ABM-spectrum), 3.50 (2H, t, 6 Hz), 3.62 (2H,t,6 Hz), 4.62 (1H,dd, J_1 7 Hz, J_2 5 Hz), 7.2−7.9 (6H,m). IR (film, cm^{-1}): 3100−2850(w), 1705(s), 1365(w) 778(s). MS (*m/e* (%)): 260(3), 258(9), 153(100).

In the presence of only one equivalent of *6* the reduction of *1* also forms *7c* and *8c*. They were separated on a column of silica using acetone–hexane 1:4 as eluent.

7c (*Z*-isomer). ^{1}H NMR ($CDCl_3$): δ 0.95−1.3 (5H,m), 1.8−2.3 (2H,m), 2.75 (2H,t, 6 Hz), 3.65 (2H,t, 6 Hz), 4.05 (2H,s), 7.2−7.7 (6 H,m). IR (film, cm^{-1}): 3100−2850(w), 1735(s), 1380(m), 1135(s), 812(m), 775(s).

8c (*E*-isomer). ^{1}H NMR ($CDCl_3$): δ 0.8−1.3 (5H,m), 1.5−2.3 (2H,m), 2.95 (2H,t, 6 Hz), 3.65 (2H,t, 6 Hz), 3.9 (2H,s), 7.2−7.7 (6H,m). IR (film, cm^{-1}): 3100−2850(w), 1740(s), 1380(m), 1135(s), 812(m), 775(s). MS (*m/e* (%)): 328(10), 326(29), 258(100), 152(98).

The *Z*/*E* assignment of *7c* and *8c* was based on the δ-values of CH_3−C= (*7c* 2.75, *8c* 3.95) in analogy to *7a*/*8a*; the *Z*/*E* assignment of *7b*/*8b* was made from the transformations *7b*→*7c* and *8b*→*8c* described below.

One equivalent of $1^{\cdot-}$ was generated and used as base in the presence of a 7:3 mixture of *7b* and *8b*. The reaction mixture was separated on a column of silica using diethyl ether−hexane 1:5 as eluent. Isolated were *7c*+*8c* (33 %), *7b* (13 %), *11* (7 %); *7c*:*8c* 57:43 was estimated from the integration of the ^{1}H NMR spectrum.

Cyclization of 9b. *9b* (0.30 g) was treated with $O_2^{\cdot-}$, generated by reducing air bubbling through the DMF-solution, acting as a base. The crude product (0.274 g) was separated by preparative TLC on silica with benzene as eluent. Isolated were *11*, *9b*, *12* and *13* (*Z*- and *E*-isomers of 1-(2-tetrahydrofuranylidene)-1,2-dihydroacenaphthene. *12* and *13* are slowly decomposed to *11* in the presence of O_2; they are solid compounds, but decomposed on recrystallization, so no m.p. is given.

12 (*Z*-isomer). ^{1}H NMR ($CDCl_3$): δ 1.9−2.4 (2H,m), 2.70 (2H,t, 7 Hz), 3.85 (2H,s), 4.35 (2H,t, 6 Hz), 7.1−7.8 (6H,m). IR (KBr, cm^{-1}): 3150−2900(w), 1690(m), 1610(m), 1590(m), 1190(m), 1090(s), 810(m), 775(s). MS (*m/e* (%)): 222(100), 165(87), 152(100).

13 (*E*-isomer). ^{1}H NMR ($CDCl_3$): δ 2.0−2.5 (2H,m), 2.83 (2H,t, 7 Hz), 3.98 (2H,s), 4.20 (2H,t, 6 Hz), 7.0−7.8 (6H,m). IR-spectrum (KBr, cm^{-1}): 3050−2800(w), 1680(m), 1600(w),

1175(m), 810(m), 775(s). MS (*m/e* (%)): 222(96), 165(90), 152(100).

The *Z/E* assignment of *12/13* was based on the δ-values of (CH_2–C=) signal in the hetero ring (12/13 2.70/2.83) and larger homoallylic coupling in *13* compared to *12*.

Reduction of 1 *in the presence of carbon dioxide. 1* (2 g) was reduced in DMF/TBAI at −1.7 V (SCE) at −35 °C with carbon dioxide bubbling through the catholyte. When the reduction was finished, methyl iodide (5 ml) was added. After standing overnight the solvent was removed *in vacuo*, water added and the product extracted with diethyl ether, which was dried and evaporated leaving 2.11 g. Recrystallization from methanol, gave *trans*-1,2-dicarbomethoxy-1,2-dihydroacenaphthylene m.p. 84 °C. ^{1}N NMR spectrum ($CDCl_3$): δ 3.77 (6H,s), 5.12 (2H,s), 7.2–7.8 (6H,m). The *trans* assignment was substantiated from the coupling constants of the methine protons (4.2 Hz) obtained from the satellites in the coupled ^{13}C NMR spectrum.

Reduction of 2 *in the presence of* 5. *2* (2 ml) was reduced in DMF/TBAI at −1.9 V (SCE) in the presence of *4* (10 ml). After completion of the reduction the DMF was evaporated, water and diethyl ether added, the ether layer dried and evaporated leaving a residue which mainly consisted of 3-phenyl-4-ketovaleronitrile and/or its enol ether;[2] the relative amounts differed even under apparently identical conditions. 3-Phenyl-4-ketovaleronitrile (from ethanol), m.p. 92–94 °C (94.5–95.5 °C),[13] ^{1}H NRM ($CDCl_3$): δ 2.06 (3H,s), 2.72 (1H, *J* 16.2, 7.0 Hz), 2.88 (1H, *J* 16.2, 6.8 Hz), 3.98 (1H, *J* 7.0, 6.8 Hz), 7.1–7.5 (5H,m).

Reduction of 2 *in the presence of* 6. *2* (0.5 ml, 4 mM) was reduced in DMF/TBAI in the presence of *6* (0.76 ml, 4 mM) at −1.5 to −1.8 V. After the usual work-up 0.66 g of crude product was isolated. The products were separated on a column of silica using diethyl ether–hexane 7:3 as eluent. Isolated were: 3-Phenylpropionitrile (2 %), *14b* (9 %), *15b* (14 %), *15c* (7 %), *16* (5 %), *17a* (*meso*, 6 %), *17b* (*d,l*, 10 %) and a mixture (14 %) of isomers of 1-cyano-2-amino-3-*Z/E*-tetrahydrofurfuralydene-4,5-*cis/trans*-diphenylcyclopent-1-ene (*18*).

14b. ^{1}H NMR ($CDCl_3$): δ 0.5–1.2 (5H,m), 1.95–2.4 (2H, def.q), 2.55–2.9 (2H,def.q), 3.38 (2H,s), 3.60 (2H,t, *J* 6 Hz), 7.1–7.5 (5H,m). IR (film, cm^{-1}): 3140–2850(w), 2220(w), 1755(s), 1630(m), 1110(vs), 700(s). MS (*m/e* (%)): 305(2), 303(6), 105(100).

15b. ^{1}H NMR ($CDCl_3$): δ 1.8–2.15 (2H,def.q), 2.40–2.88 (4H,m), 3.40 (2H,t, 6 Hz), 3.98 (1H,t, 7 Hz), 7.0–7.5 (5H,m). IR (film, cm^{-1}): 3100–2850(w), 2266(w), 1720(s), 702(s). MS (*m/e* (%)): 237(1), 235(4), 105(100).

15c. ^{1}H NMR ($CDCl_3$): δ 0.7–1.3 (5H,m), 2.7–3.2 (2H,m), 4.15 (1H,dd, 7 Hz, 6.5 Hz), 7.05–7.55 (5H,m). IR (film, cm^{-1}): 3100–2850(w), 2250(w), 1700(s), 1380(m), 700(s). MS (*m/e* (%)): 199(40), 77(100).

16. ^{1}H NMR ($CDCl_3$): 1.8–2.4 (2H,m), 2.6–3.1 (2H,m), .3.48 (2H,s), 4.35 (2H,t, 7 Hz), 7.1–7.5 (5H,m). IR (film, cm^{-1}): 3120–2850(w), 2200(m), 1645(m), 1175(m), 700(s). MS (*m/e* (%)): 199(90), 105(100).

17a. (*meso*-3,4-Diphenyladiponitrile), m.p. 214–216 °C (diethyl ether–hexane), ^{1}H NMR ($CDCl_3$): δ 2.3–2.5 (4H,m), 3.2–3.4 (2H,m), 7.40 (10H,s). IR (KBr, cm^{-1}): 2250(w), 1480(w), 1445(w), 1410(w), 770(m), 700(s), 625(w). MS (*m/e* (%)): 260(33), 130(100).

17b. (*d,l*-3,4-Diphenyldiponitrile), m.p. 114°C (chloroform–hexane), ^{1}H NMR ($CDCl_3$): δ 2.55–2.8 (4H,m), 3.35–3.75 (2H,m), 6.75–7.05 (4H,m), 7.15–7.40 (6H,m). IR (KBr, cm^{-1}): 2250(w), 1480(m), 1450(m), 1410(w), 780(s), 703(s), 625(m). MS (*m/e* (%)): 260(9), 130(100).

Reduction of 2 *in the presence of carbon dioxide. 2* (1 ml) was reduced in DMF/TBAI at −1.9 V (SCE) with CO_2-bubbling. After the reduction was completed, the product was methylated with methyl chloride. The solvent was evaporated, water and diethyl ether added, and the ether dried and evaporated leaving 1.33 g (A); the aqueous phase was acidified and extracted yielding 80 mg (B). The product A was nearly pure methyl 3-cyano-2-phenylpropionate, ^{1}H NMR ($CDCl_3$): δ 3.64 (3H,s), 3.6–3.9 (2H,m), 4.1–4.45 (1H,m), 7.2–7.4 (5H,br.s). MS (*m/e*): 189.

Reduction of 3 *in the presence of carbon dioxide. 3* (1 g) was reduced in DMF/TBAI at −35 °C at −1.9 V (SCE) with CO_2-bubbling, *n*=2.0. After completion of the reduction methyl iodide (5 ml) was added. The usual work-up gave 0.96 g of product, methyl 4-keto-2-phenylpentanoate, m.p. 60–65 °C; ^{1}H NMR ($CDCl_3$): δ 2.12 (3H,s), 2.65 (1H,dd, *J* 17 Hz, 5 Hz), 3.34 (1H,dd, *J* 17 Hz, 9 Hz), 4.04 (1H,dd, *J* 9 Hz, 5 Hz), 3.60 (3H,s), 7.20 (5H,s).

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Peculiar Aspects of the Anodic Oxidation of Vinylic Sulfides

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In the anodic oxidation of vinylic sulfides on platinum in acetonitrile, a non-classical sulfonium ion explains satisfactorily the transfer of the thioether group leading to an aldehyde in the presence of water, or its acetal in the presence of methanol. Some other reactions depend on the structure of the substrate, particularly the dimerization into anodically inactive forms likely to decompose during the work-up, to lead in some cases to masked ketenes having the structure of a *gem*-disulfide.

In aqueous solvents, the electrochemical oxidation of the sulfide group generally leads [1] to the sulfoxide and then to the sulfone. However, the use of non-nucleophilic solvents allows for reactions where the sole nucleophile reacting with cationic intermediates may be the sulfide group itself. Then coupling reactions [2] (to give dicationic disulfides) and electrophilic attack [3] (aromatic substitution) may occur and have demonstrated the ability to conduct electrolyses in non-aqueous systems. Recently, the anodic method was used in the deprotection of carbonyl groups (of ketones,[4] aldehydes [4] and sugars [5]) since the electrochemical oxidation of the bond is in some cases highly selective and leads under well-defined conditions to the corresponding carbonyl compounds $R^1R^2C{=}O$. Examples where anodic deprotection may be considered more efficient than classical chemical methods are available.[4,5] It is worth mentioning that the reaction can be also conducted by indirect means [6] with continuous electrochemical generation of an intermediate possessing adequate oxidizing properties in solution.

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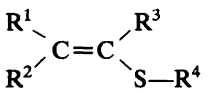
In such a field of investigation the anodic behaviour of vinylic sulfides *1*



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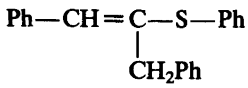
is of high interest and depends obviously on the nature of substituents R. At least four general reactions are expected, namely (i) anodic addition to the double bond, (ii) anodic cleavage of the C–S bond, (iii) classical addition to the sulfur atom and (iv) two-electron oxidation with deprotonation (when R^1 or R^3 are H) with formation of a cation, stabilized by the vicinity of the electron-rich sulfur atom. The aim of the present study is to discuss the different pathways and also to emphasize the possibility of new preparative aspects in the electrochemistry sulfur compounds.

RESULTS

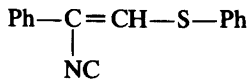
Substrates possessing the general structure *1* have been prepared and oxidized according to the procedures described in the experimental section. The vinylic sulfides studied here are:

$Ph{-}CH{=}CH{-}S{-}R^4$

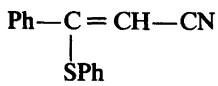
1a. $R^4 = Ph$
1b. $R^4 = n\text{-}C_3H_7$
1c. $R^4 = n\text{-}C_4H_9$
1d. $R^4 = i\text{-}C_4H_9$
1e. $R^4 = sec\text{-}C_4H_9$



1f



1g. *Z* isomer
1h. *E* isomer



1i

(a). *Cyclic voltammetry*. Compounds *1a–1i* exhibit an irreversible two-electron step at a platinum anode in superdry acetonitrile (in the presence of activated alumina). The electrolyte was Bu_4NBF_4. With compounds *1b, 1c, 1d* and *1e*, another poorly defined two-electron step was also observed at a rather positive potential. The data concerning the first step are reported in Table 1. It has to be emphasized that the peak currents are larger when R^3 is different from H (for example for *1f* and *1i*).

When the electrolysis solution contains a noticeable concentration of nucleophile such as water or methanol, a second step is observed at a potential a little more anodic than the main one with compounds *1a–1f*. Its peak potential and its current are bound to the nucleophile concentration in the bulk (Fig. 1). An increase of the main wave is observed with *1g/h* and *1i* (Fig. 2). Whatever the electrolyte and the nature of the solvent may be, the presence of a base (lutidine in excess or Na_2CO_3) does not affect at all the current and the potential of the first and main peak.

Table 1. Cyclic voltammetry of vinylic sulfides *1*. $C=3.10^{-3}$ M l^{-1}; 0.1 V s^{-1}; reference electrode: Ag/Ag^+ 0.01 M CH_3CN; Bu_4NBF_4 0.1 M in acetonitrile in the presence of activated alumina–platinum disk.

Compounds	First peak E_{p_v}	$I_{p_{\mu A}}$
1b	0.87	8.5
1c	0.88	10.8
1d	0.88	11
1e	0.88	12
1a	0.97	14
1f	1.11	26
1g	1.31	10.8
1h	1.38	13
1i	1.60	15.3

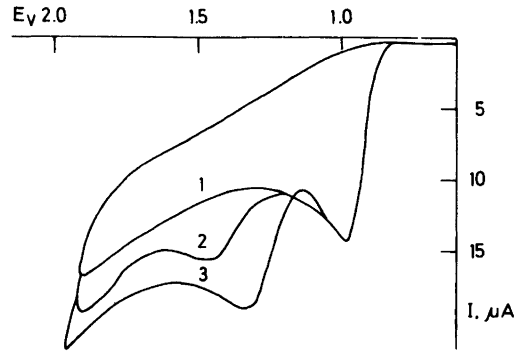
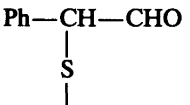


Fig. 1. Cyclic voltammetry of Ph–CH=CH–S–Ph; 3.10^{-3} M l^{-1} in CH_3CN–Bu_4NBF_4 0.1 M l^{-1}; platinum disk –Ag/Ag+ 0.01 M–0.1 V s^{-1}.
1. In dry acetonitrile (activated aluminal).
2. In acetonitrile plus water (3.10^{-3} M l^{-1}).
3. In acetonitrile plus methanol (3.10^{-3} M l^{-1}).

(b). *Macroscale electrolyses*. Generally the results depend on the nature of the substituents on the ethylenic double bond, According to the nature of R^1, R^2. R^3 and R^4, the main results are listed below.

(1). Migration of the sulfide group. Compounds *1a–1e* lead to the aldehydes *5*,



5

with a yield of the order of 50 %. Most of those aldehydes are not very stable and this fact may partly explain why the selectivity of the reaction is moderate. Such a formation of aldehydes of this series from *1* had been previously reported.[7] An interpretation is given here. Thus, a condition to observe such a reaction is that R^1 is a hydrogen. The other substituents and a basic medium may favor an ECE process (the chemical step here is the fast deprotonation of the radical cation) and allow the formation of a short-life intermediate having the structure of a vinylic cation probably stabilized by the vicinity of the

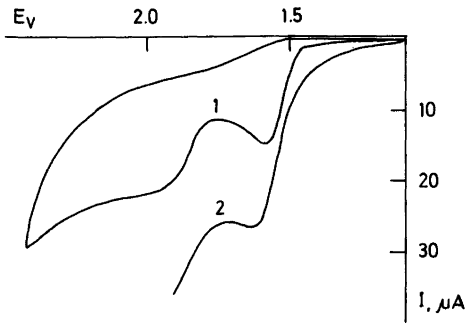
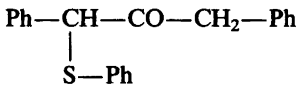


Fig. 2. Cyclic voltammetry of NC–CH=C(Ph)–S–Ph; 3.10^{-3} M l^{-1} in CH_3CN–Bu_4NBF_4 0.1 M l^{-1}; platinum disk –Ag/Ag+ 0.01 M–0.1 V s^{-1}.
1. In dry acetonitrile (activated alumina).
2. In acetonitrile plus methanol (6.10^{-3} M l^{-1}).

sulfur atom. Then a non-classical sulfonium ion [3,8] *3* may be suggested, reacting with nucleophiles according to two chemical routes *α* and *β*. (Scheme 1).

Where $R^2=C_6H_5$, benzoic acid *7* was isolated (yield between 10 and 30 %) and this involves an easy oxidation of the keto sulfide *4* at the fixed potential necessary to perform the anodic oxidation of *1*. Similar fragmentation is probably feasible from *5* but we failed in isolating and identifying most of the other oxidation products having obviously rather low molecular weights. However, organic disulfides R^4–S–S–R^4 *6* were characterized. They may be obtained either from a final anodic fragmentation of *4* and *5* or from the cleavage of the radical cation of *1* when its deprotonation rate is not large enough. Actually, it was possible to demonstrate that the formation of the disulfide *6* could be *nearly* quantitative in the total absence of a basic agent (such as Na_2CO_3) in the electrolysis solution. As *4* and *5* are obviously candidates for further oxidation (leading to cleavage reactions) their yield appears to be strongly dependent on the water concentration; the higher the stability of the intermediate *3* in the bulk, the lower the occurrence of the fragmentation reactions.

In the same way the ketone *8* is also obtained from the vinylic sulfide *1f*



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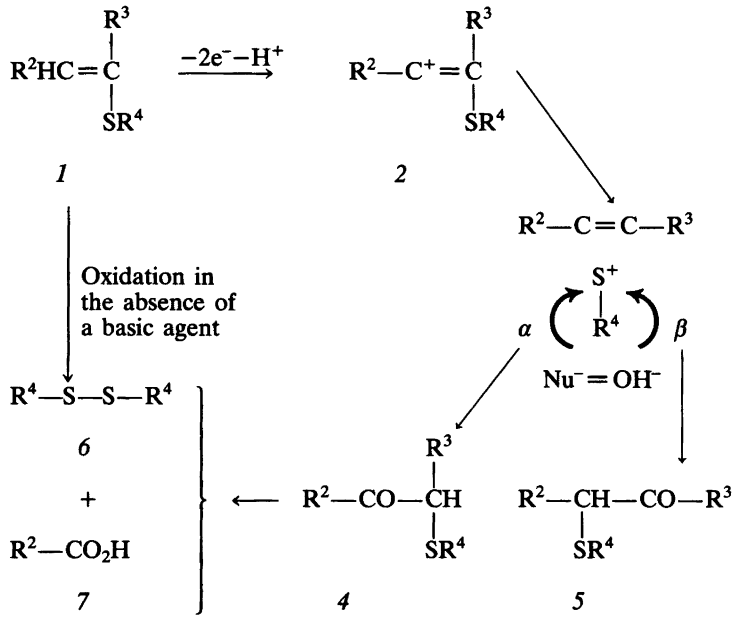
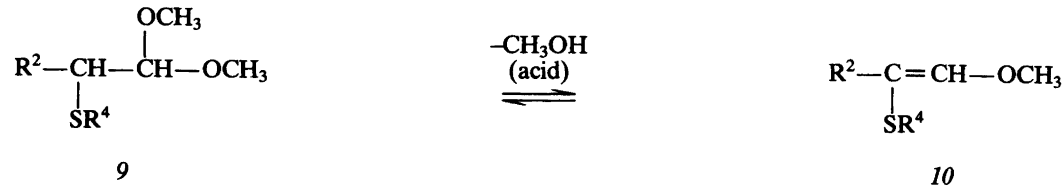


Scheme 1.

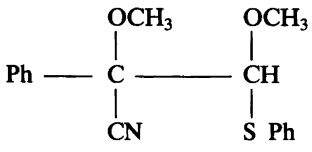


Scheme 2.

(2). Oxidation in the presence of methanol. With sulfides of the type *1a–1e*, the oxidation in the absence of water (when the electrolyses are performed in a glove box) in dry methanol–acetonitrile mixture (30–70 % v/v) leads to the acetal *9* of the aldehyde *5* previously isolated in the course of electrolyses conducted in the presence of moisture. The acetal *9* may be transformed reversibly into the enol ether *10* obviously formed during the attack of the intermediate *3* by the nucleophile MeO^-. A similar reaction occurs in the presence of butanol.

The yields of *9* are higher than those observed for *5* (this experimental evidence could be due to the protection of the aldehyde) and may reach 70 %. However, small amounts (up to 10 %) of benzoic acid ($R^2=C_6H_5$) are also obtained. Nevertheless, it is worth mentioning that with *1f* under such experimental conditions, the rearranged ketone *8* is the main compound (yield: 25 %) even in the presence of methanol.

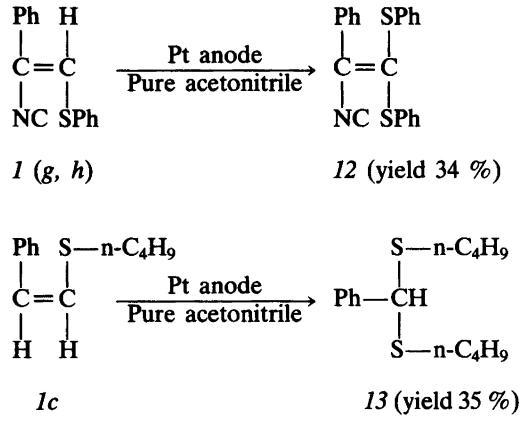
(3). Anodic addition to the ethylenic double bond. When the ethylenic double bond is strongly activated by an electron-withdrawing group such as the cyano substituent, the results of the anodic oxidation are dramatically changed. Then, in the presence of methanol, for example with *1g*, a vicinal dimethoxy thioether possessing the structure *11* appears to be the main electrolysis product



11

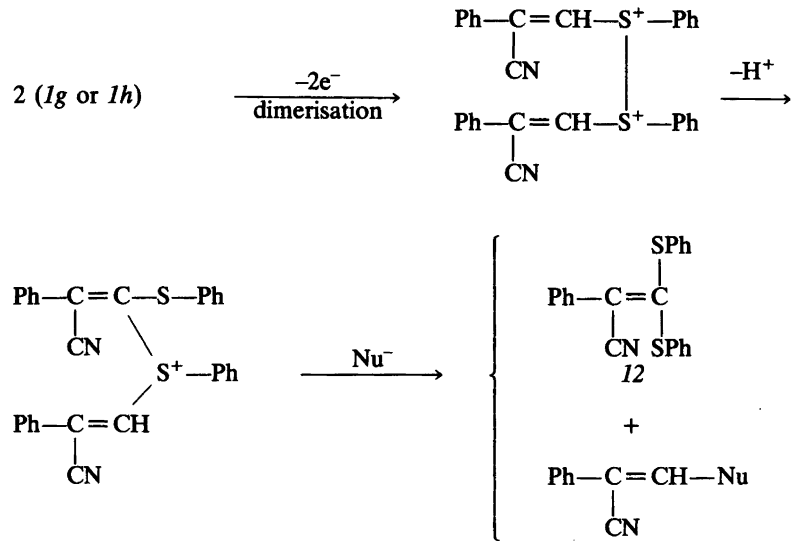
and is isolated with a yield of 43 %. The absence of a potential leaving proton in the β position of the thioether group and the withdrawing effect of the cyano group are responsible for the strong electrophilic character of the radical cation generated by a first electron transfer; these evidences consequently suggest an ECE process where the chemical reaction is a nucleophilic attack.

(4). Other reactions. The formation of electrophilic groups or atoms in the molecules already possessing the thioether substituent may lead to other kinds of reactions. Thus, after the work-up, some products of the anodic oxidation of the series *1* have the structure of *gem*-disulfides; Scheme 3.



Scheme 3.

Compound *12* is particularly interesting as it corresponds to the masked structure of a ketene. In the absence of nucleophile and of any possibility of fast deprotonation, oxidized forms of *1g* and *1h* may lead to dimers possessing a structure of disulfonium $-S^+-S^+-$ which can react with nucleophiles mainly during the work-up. Such dimeric structures are known and their formation has been demonstrated for both monosulfides [2] and cyclic macrodisulfides.[9] Thus, the *gem*-disulfide *12* could result from the reaction of such a dimer according to the following process:



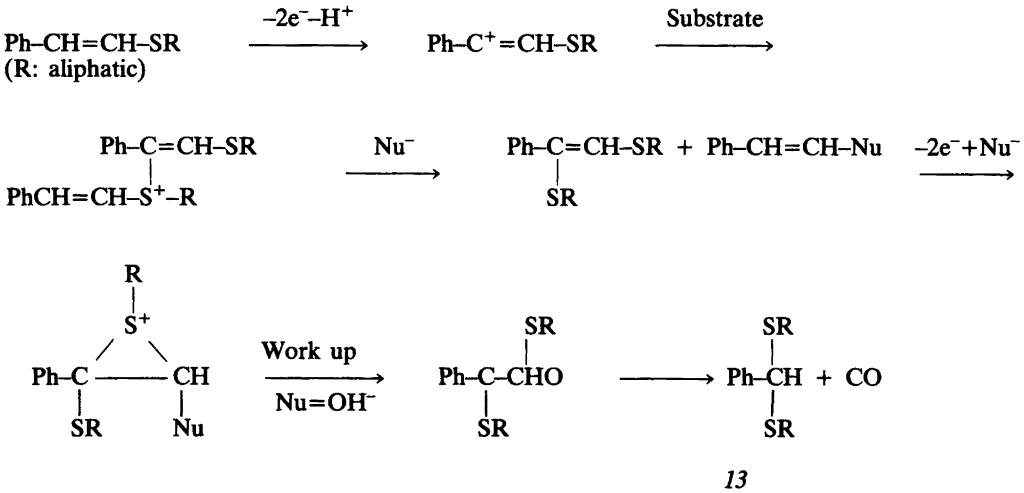
Scheme 4.

However, the formation of *13* observed only in pure acetonitrile which could also proceed through a sulfonium dimer is more problematic to explain. Nevertheless, an oxidation mechanism may be proposed taking into account the nucleophilic effect of the non-oxidized thiogroup (under such conditions, the formation of the sulfonium cation would not be intramolecular as mentioned previously for *1a*) and the occurrence of an easy decarbonylation [10] during the work-up.

CONCLUSION

The present work describes the different chemical pathways observed in the oxidation of vinylic sulfides. It is obvious that the reactions are dramatically controlled by both the structure of the substrate and the nature of the medium. Most of the possible anodic reactions observed in general for the different classes of thioethers are seen for only one series of unsaturated sulfides.

Under conditions where the deprotonation of



Scheme 5.

Table 2. Main products from macroscale electrolyses.

Substrate	In presence of water E(V)	Product	Yield %	IR ν_{CO}	NMR ppm	MS	In presence of methanol E(V)	Product	Yield %	IR ν_{C-O-C}	NMR ppm	MS
1a	1.0	*5*	35	1715	4.75 (CH) 9.5 (CHO)	228	1.0	*9*[a]	65	1070–1115	3.30 and 3.45 (OCH_3) 4.35–4.7 (CH–CH)	274
1b	0.9	*5*	62	1700	9.1 (CHO)		0.9	*9*	70	1090–1120	3.25–3.45 (OCH_3) 4.0 –4.8 (CH–CH)	
1c	0.9	*5* *13*	26 35	1705	9.1 (CHO)	268	0.9	*9*	60	1070–1115	3.25–3.45 (OCH_3) 4.06–4.63 (CH–CH)	
1d	0.9	*5*	57	1710	9.1 (CHO) 2.45 (CH)		0.9	*9*	65	1080–1115	3.34–3.45 (OCH_3) 4.06–4.65 (CH–CH)	
1e	0.9	*5*	48	1715	9.4 (CHO)		0.9	*9*	45	1070–1120	3.24–3.48 (OCH_3) 4.06–4.63 (CH–CH)	
1b	1.10	*8*	30	1710	3.3 (CH_2) 4.68 (CH)		1.0	*8*	25	ν_{CO}=1710		318
1g/1h	1.25	*12*	34	ν_{CN} 2210	only aromatic protons	345	1.25	*11*	43	1080–1110 ν_{CN} 2250	3.32–3.55 (OCH_3) 4.4 (CH)	

[a] In presence of butanol the acetal Ph–CH(S–Ph)–CH(O–n-C_4H_9)$_2$ is obtained (50 %).

the radical cation is fast, the occurrence of a non-classical sulfonioum ion may explain satisfactorily the moving of the thioether group. Such a concept was already given [8] in some nucleophilic displacement reactions concerning some classes of vinylic thioethers for which R^1 was the leaving group. The present results underline the potential of H^+ as a leaving group in anodic oxidations on organic sulfide substrates, and the similarity with SN reactions in organic chemistry where the existence of other types of leaving groups are necessary.

On the other hand, when deprotonation reactions are inhibited, anodic additions and cationic adducts formations may occur. Thus, the ability of the thioether group to behave as the main nucleophile allows probably the observation of new reactions. They are explained by the transient formation of unusual dimeric forms (anodically inactive) likely to decompose during the work-up into masked ketenes possessing the structure of *gem*-disulfides (obtained in a good yield if we consider that two molecules of substrate are necessary to build up one of protected ketene).

EXPERIMENTAL

^1H NMR spectra were recorded on a Perkin-Elmer-HITACHI R 24 A Spectrometer. IR spectra were taken with a PERKIN-ELMER 257 Spectrophotometer. Mass spectra were obtained on a MAT 311 VARIAN (Rennes). Elemental analysis were performed by Rhône-Poulenc and were in agreement with calculated values.

Substrates. Vinyl sulfides Ph–CH=CH–S–R (*1a–1e*) were prepared as follows: 5.10^{-2} M of appropriately dried sodium thiolate was dissolved in 30 ml of 1-methyl-2-pyrrolidone; then 5.10^{-2} M of β-bromostyrene was added and the mixture was heated for 2 h at 135 °C. The residue was diluted with 100 ml of ether and washed with water (3×100 ml). Yield=95 %. The substrates were sufficiently pure to be used for electrolysis. The separation of *Z*/*E* isomers was possible by chromatography on a silica gel column (Merck type 60) (elution with cyclohexane–ethyl acetate 98:2).

We used an already described procedure for the preparation of *1f*,[11] *1g*, *1h* and *1i*.[12]

Electrochemical experiments. Cyclic voltammetry experiments were performed with the use of a SOLEA-TACUSSEL potentiostat, equipped with a TP-PRT Plug-in and a SEFRAM TGM 100 XY recorder. Working electrode: Solea-Tacussel. Platinum disk electrode Pt 30, diameter of active platinum surface: 1 mm. Reference electrode: Ag/Ag^+ 0.01 M in CH_3CN.

Acetonitrile was purified as already described.[13] An H-cell was used for macroelectrolyses with a platinum anode (area: 16 cm^2), and a steel rod cathode. Substrate concentration: 0.1 M l^{-1}; supporting electrolyte: $LiClO_4$ or NaClO 0.2 M l^{-1}.

After electrolysis the resulting solution was evaporated, diluted with 100 ml water and extracted with 100 ml ether. The organic layer was washed with 100 ml water three times and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed on a silica gel (Merck type 60) column. Results of electrolysis are collected in Table 2.

By-products, such as benzoic acid and diphenyldisulfide, were characterized from oxidation of β-thiophenylstyrene, *1a*, but the yields were lower in the presence of a basic agent (Na_2CO_3 or lutidine).

Experiments in methanol–acetonitrile mixture 4:6 were conducted in a glove box to avoid any trace of water.

The oxidation of phenyl-3-thiophenyl-3-propenonitrile *1i* is impossible owing to strong inhibition phenomena.

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Electrochemical Reduction of Substituted 15,16-Dihydropyrenes in Dimethylformamide

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The electrochemical reduction of a series of 15,16-disubstituted 15,16-dihydropyrenes and some benzannelated derivatives was studied by differential pulse polarography in dimethylformamide. The large spacing between the first and second reduction waves, compared to polycyclic benzenoid hydrocarbons, is probably due to (a) steric inhibition of ion-pairing to the electrolyte cation by the 15,16-substituents and (b) the conversion of the Huckel 14-electron ($4n+2$) starting material to a 16-electron dianion. The first reduction potentials of the compounds correlate fairly well with the Huckel LUMO energy, and with an equation previously derived by Streitwieser for benzenoid hydrocarbons.

Some of the most significant insights which organic electrochemists have attained into the principles underlying the cathodic behavior of organic compounds have come from studies of the electrochemical behavior of aromatic hydrocarbons in aprotic media.[1] Such studies provide information concerning the thermodynamics and kinetics of the injection of electrons into unfilled orbitals of the hydrocarbon to form successively the corresponding radical anion at potential E_1, (eqn. 1), and dianion at potential E_2, (eqn. 2), without complications introduced by either coupled chemical reactions or the presence of electro-active substituents. Almost all of the

$$ArH + e^- \rightleftarrows ArH^{\cdot-}\ E_1 \quad (1)$$

$$ArH^{\cdot-} + e^- \rightleftarrows ArH^{-2}\ E_2 \quad (2)$$

hydrocarbons which have been investigated in this manner have been polycyclic benzenoid substances, since they are available in diversity. For this reason, however, one may reasonably inquire whether in fact any of the electrochemical behavior exhibited by such substances derives specifically from their benzenoid nature. Are there any aspects of such behavior which are not shared by nonbenzenoid hydrocarbons? A brief study of the electrochemical behavior of *trans*-15,16-dimethyl-15,16-dihydropyrene (*1a*) which we carried out several years ago [2] first forced us to address this question. As will be elaborated in the Discussion section, we observed that ΔE, the spacing between the first and second reduction potentials in aprotic media, is significantly larger for *1a* than for a variety of polycyclic benzenoid hydrocarbons. We interpreted the difference as due to steric hindrance to ion-pairing between the dianion of *1a* and the cation of the supporting electrolyte, caused by the methyl groups at the 15,16-positions. However, our subsequent observation [3] that ΔE is actually *smaller* for cyclooctatetraene (COT) than for benzenoid hydrocarbons suggested that something more might be involved.

As a consequence of considerable recent synthetic activity in this area, a number of substi-

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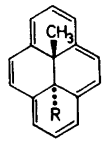
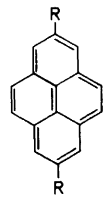


1a, R = CH_3
b, R = C_6H_5

tuted 15,16-dihydropyrenes are now available.[4-6] We wish to report a study of the electrochemical behavior of *1–8*, which has permitted us to derive further insights into this ring system and into the question posed above. The electrochemical technique employed was differential pulse polarography (DPP)[7] at a dropping mercury electrode, because (a) the high signal-to-background ratio characteristic of this technique enabled us to make measurements upon the limited amounts of samples available, and (b) the sharply peaked signals permitted accurate measurements of reduction potential.



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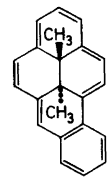
b, R = *t*-Bu



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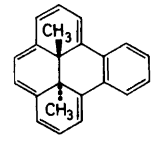

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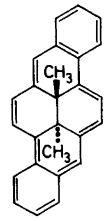

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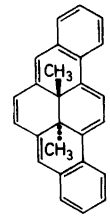

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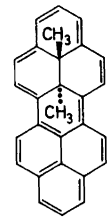

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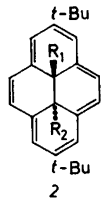
RESULTS

Precision of DPP measurements. It was necessary to establish at the outset that data of sufficiently high precision could be obtained by the DPP technique, which is widely used in analytical work but only infrequently in mechanistic studies. A series of 4–8 polarograms were recorded on an expanded voltage scale (1 volt=30 cm) and at low potential scan rate (2 mV/s) and short polarographic drop time (1 s) to minimize instrumental artifacts;[8] the DPP peak potentials could be corrected where desired to normal DC polarographic half-wave potentials ($E_{1/2}$) by the method described in the Experimental section. The standard deviation of a set of DPP peak-to-peak measurements was typically 0.8 to 2mV; for example, for *2d*, ΔE was measured as 762, 764, 763, 763, 764 and 764 mV, corresponding to a mean value of 763 mV and standard deviation of 1mV. The ΔE values are therefore considered to be reliable to ±1–2 mV.

Series 1 and 2. Compounds *1a–b* and *2a–g* all exhibit the same electrochemical behavior: two DPP polarographic peaks are observed in dimethylformamide (DMF) containing tetrabutylammonium perchlorate. The values of E_1, E_2 and ΔE measured for these substances are presented in Table 1, together with data for pyrene (*3a*) and 2,7-di-*t*-butylpyrene (*3b*). A representative voltammogram is shown in Fig. 1.



2

2a, R_1=R_2=Me
2b R_1=Me; R_2=Et
2c, R_1=Me; R_2=n-Pr
2d, R_1=R_2=Et
2e, R_1=Et; R_2=n-Pr
2f, R_1=R_2=n-Pr
2g, R_1=R_2=n-Bu

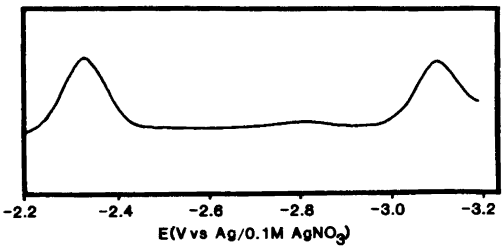


Fig. 1. Derivative pulse polarogram of 2,7-di-*t*-butyl-15,16-dibutyl-15,16-dihydropyrene (*2g*) in dimethylformamide containing 0.1 M Bu_4NClO_4; 2 mV/s, 1 s drop time.

Table 1. Reduction potentials of substituted 15,16-dihydropyrenes and pyrenes.[a]

Compound	$-E_1$/V	$-E_2$/V	ΔE/V
1a	2.22	3.01	0.69
1b	2.150	2.893	0.743
2a	2.344	3.080	0.736
2b	2.357	3.104	0.747
2c	2.348	3.100	0.752
2d	2.331	3.094	0.763
2e	2.333	3.098	0.765
2f	2.332	3.103	0.771
2g	2.334	3.108	0.774
3a	2.521	3.104	0.583
3b	2.580	3.173	0.593

[a] Potentials are differential pulse polarographic peak potentials in dimethylformamide/0.1 M Bu_4NClO_4, relative to Ag/0.1 M $AgNO_3$.

Benzo series. The benzodihydropyrenes *4–8* were found to exhibit diverse cathodic behavior, characterized in general by more than two voltammetric waves. The first wave in each case is presumably associated with formation of the radical anion; we have not chosen to speculate upon the processes responsible for the waves at more negative potential, and the very small available amounts of sample (1–3 mg) precluded preparative experiments which might have provided information on this point. The [a]-ring benzannelated compound was examined by conventional DC polarography and exhibited three

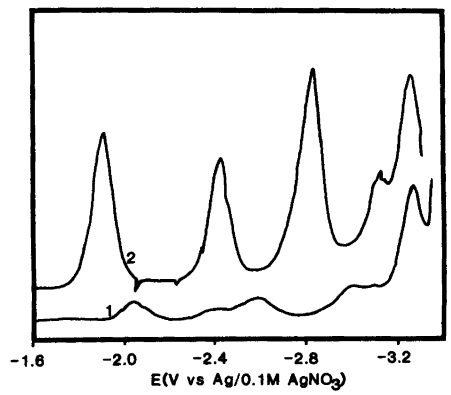


Fig. 2. Derivative pulse polarograms of benzodihydropyrenes *5* and *8* in dimethylformamide containing 0.1 M Bu_4NClO_4; curve 1, compound *5*; curve 2, compound *8*.

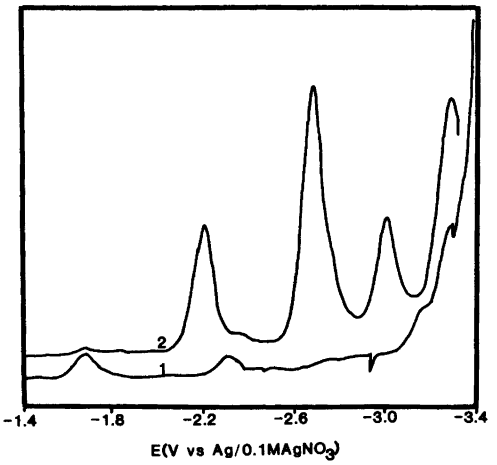


Fig. 3. Derivative pulse polarograms of benzodihydropyrenes *6* and *7* in dimethylformamide containing 0.1 M Bu_4NClO_4; curve 1, compound *6*; curve 2, compound *7*.

waves at –2.08, –2.62 and 3.1 V. The other substances were examined by differential pulse polarography. (Figs. 2 and 3). The first voltammetric wave for each substance was found to be as follows: *5*, –2.04 V; *6*, –1.68 V; *7*, –2.220 V; *8*, –1.91 V.

DISCUSSION

By subtraction of eqn. (2) from eqn. (1) (Introduction) one obtains eqn. (3), from which it may be noted that the ΔE, the spacing between E_1 and E_2, provides a measure of the position of the disproportionation equilibrium illustrated in eqn. (3).

$$ArH + ArH^{-2} \rightleftarrows 2ArH^{-} \qquad (3)$$

$$\Delta G = -F(E_1 - E_2) = -F\Delta E$$

It is important to note that theoretical calculations,[3] which predict values of ΔE for eqn. (3) of upwards of 5V, are in severe disagreement with experiment. Typical values of ΔE lie in the range 0.5–0.6 V for a wide variety of hydrocarbon structures; it is generally believed that the dianion on the left side of the equilibrium of eqn. (3) is more extensively solvated and ion-paired to the supporting electrolyte cation than is the

radical anion, thus driving the equilibrium much more to the left than the calculations, which do not take this effect into account, would indicate. The fact that ΔE lies within the narrow range 0.5 to 0.6 V for a variety of aromatic structures suggests that solvation effects on eqn. (3) are roughly similar for most benzenoid aromatic structures. Though surprising, this seems to be in fact a rather good approximation. Indeed, there seem to be only minor differences between the electrochemical reduction potentials of such substances in solvents as remarkably diverse as aqueous dioxane, 2-methoxyethanol, and DMF.[9,10]

We noted previously [2] that ΔE is larger for *1a* in dimethylsulfoxide (0.68 V) than for the general run of benzenoid aromatic hydrocarbons; *e.g.*, 0.55 V for pyrene (*3a*). We argued that the methyl groups in *1a* sterically inhibit approach of a tetraalkylammonium ion to either face of the π-system. However, we subsequently observed [3] that ΔE is unusally small (<0.2 V) for COT, even though the degree of ion-pairing to the planar COT dianion should be sterically similar to species such as pyrene dianion. It was clear, therefore, that the steric argument might not be sufficient to explain the data. The results obtained in the present study provide useful information on this problem. Generally speaking, the data in Table 1 confirm beyond question the existence of a steric effect upon association of 15,16-dihydropyrene dianions with tetrabutylammonium ion, inasmuch as ΔE increases in a regular way as the R_1 and R_2 groups increase in size. More specific conclusions are possible, however. Note that one face of the π-system becomes increasingly hindered as one proceeds through both the series *1a,b* and *2a-c*. Since ΔE also increases concomitantly, we can conclude, contrary to what one might have guessed, that such systems do not respond to increased hindrance on one face of the π-system simply by correspondingly greater ion-pairing and solvation on the other face, although this presumably does occur to some extent. A more significant conclusion may be reached by examining the symmetrically substituted series, *2a,d,f,g*; ΔE increases by 27 mV in going from *2a* to *2d*, but subsequent increases are small, *i.e.*, 8 mV from *2d* to *2f*, and only 3 mV from *2f* to *2g*. Examination of space-filling models leads to the same conclusion, that the largest increase in effective group size in this system comes in going from *2a* to *2d*. This is not what one would expect from some other measures of effective group size, *e.g.*, conformational A-values,[11] Taft steric constants,[12] or effects of group size upon the rates of S_N2 displacements,[13] but these generally describe the steric constraints upon attack on an alkyl chain from a direction *normal* to the (average) axis of the chain, whereas ion-pairing to anions derived from *2a-f* involves approach along an axis *parallel* to the axis defined by C-15 (or C-16) and the carbon directly bound to it.

What one would really like to know, however, is what ΔE would be in the absence of the two methyl groups of *2a*. Would it be 0.59 V, the value for *3b*, or would it be larger? We think the latter is more likely, because even if the methyl groups were removed the tetrabutylammonium ion could not approach much closer than with *2a* anyway because of the intrinsic bulk of the π-system itself (recall that the van der Waals' "thickness" of substances such as benzene is *ca.* 3.7 Å).[14] If we accept the latter argument, we are left with the conclusion that the large values of ΔE observed for *1* and *2* are not primarily steric in origin, but that a second effect is involved. We believe that this effect is electronic. Addition of two electrons to the diatropic [15] Huckel $4n+2$ dihydropyrene system converts it to a paratropic [15] $4n$ system, the relative instability of which will drive eqn. (3) to the right; conversely, reduction of COT to its dianion converts a 4n system to a $4n+2$ species, the stability of which would drive eqn. (3) to the left. In general, ΔE should therefore be small for Huckel $4n$ systems and large for $4n+2$ systems. Data in the literature support this hypothesis. Thus, a number of nearly planar bridged [14] annulenes exhibit ΔE values in the range 0.6–0.7 V,[16] while [16]annulene,[17] a doubly-bridged [16]annulene,[18] and two bridged [12]annulenes [19] all have ΔE in the range 0.2–0.3 V, and, as already noted, COT or [8]annulene exhibits a ΔE value of less than 0.2 V.[2] The only apparent exception to this generalization in the literature is the ΔE of 0.65 V for an isomer of [12]annulene,[19] but this substance *and* its dianion (unlike COT) are both non-planar and could be expected to behave anomalously. It may be inappropriate to include COT in this list because of the large geometrical change involved in reduction of the tub-shaped neutral molecule to the nearly-planar radical anion, but it is notable

that a small value of ΔE is observed for all of the other [4*n*]annulenes, despite the variety of geometrical situations represented among them.

The electrochemical data on benzo derivatives *4–8* is instructive. It may initially seem surprising that while annelation of a single benzene ring onto the nucleus of *1a* lowers the reduction potential (*4* and *5*), a second benzannelation either lowers the reduction potential (*6*) or increases it (*7*) depending upon the position of annelation. However, the data are better understood by examining the degree of correlation between the polarographic half-wave potentials and m_{n+1}, the coefficient of the lowest unoccupied molecular orbital (LUMO) computed by the Huckel M. O. method (Table 2). The experimental half-wave potentials can be compared with values computed by the empirical correlation found by Streitwieser [10] for a series of hydrocarbons in DMF containing tetrabutylammonium iodide (eqn. (4))

$$E_{1/2}=$$

$$(2.407\pm0.182)M_{n+1}-(0.396\pm0.093)-1.010 \quad (4)$$

where $E_{1/2}$ is the polarographic half-wave potential relative to Ag/0.1 M $AgNO_3$ and the last term is the correction factor necessary to correct potentials measured relative to this reference to the mercury pool reference used by Streitwieser. As Table 2 shows, there is rather good agreement between the calculated and observed reduction potentials. In every case the dihydropyrene is easier to reduce than predicted by the empirical relationship, but the variations are close to or within the error limits of the Streitwieser correlation, hence the effect may not be real. It is interesting to note that there is an especially large deviation with the dibenzo compound *6*, for which other evidence [20] indicates the possibility of a low-lying triplet state; on the other hand, the parent substance of the series, *1a*, deviates equally from the Streitwieser line. An alternate way to view the data is to construct a Streitwieser-type correlation line for the data of Table 2 (eqn. (5)).

Table 2. Correlation between first reduction potential and Huckel LUMO energy.

Compound	$-m_{n+1}{}^{a}/\beta$	$-E_{1/2}$/V	
		Experimental [b]	Calculated [c]
6	0.226	1.70	1.95±0.14
8	0.285	1.93	2.09±0.15
5	0.325	2.06	2.19±0.15
4	0.325	2.08	2.19±0.15
7	0.357	2.22	2.27±0.15
1a	0.445	2.23	2.48±0.16

[a] Energy of Huckel LUMO. [b] Differential pulse polarographic peak potentials were corrected to DC half-wave potentials by the addition of 0.015 V (see Experimental). [c] Calculated by the equation $E_{1/2}=(2.407\pm0.182)m_{n+1}-(0.396\pm0.093)-1.010$; note that m_{n+1} is a negative number (see Ref. 10).

$$E_{1/2}=$$

$$(2.488\pm0.302)M_{n+1}-(0.208\pm0.0336)-1.010 \quad (5)$$

Examination of eqn. (5), in which the intercept is written in two parts to facilitate comparison with eqn. (4), shows that the line has the same slope (within experimental error) as the Streitwieser line, but different intercept. This could perhaps be related to a somewhat different degree of solvation of the radical anions of these substances compared to polycyclic benzenoid hydrocarbons.

Finally, the similarity of the ΔE values for *1a* and *2a* and for *3a* and *3b* demonstrate the minimal effect of groups at positions 2 and 7 upon ion-pairing, in contrast with the systematic changes associated with substituents at positions 15 and 16. The space-filling models show that substituents at the latter positions should indeed play a much greater role in shielding the π-system, even when the groups at 2 and 7 are *t*-butyl.

CONCLUSIONS

The two-electron reduction of dihydropyrenes in DMF affords dianions which are destabilized relative to those from benzenoid aromatic hydrocarbons. The effect is thought to arise (a) because the 15, 16 substituents create steric hindrance to solvation and ion-pairing of the corresponding dianion, and (b) from the fact that dihydropyrene dianons, as Huckel 4*n* systems, are electronically destabilized compared to those from benzenoid aromatic species. It is concluded that ΔE will

generally be large (>0.6 V) for reduction of $4n+2$ annulenes, and small (<0.4 V) for $4n$ systems. It may be, therefore, that the ΔE value can be used to estimate the diatropic or paratropic nature of a new dianion.

Finally, the sensitivity of the voltammetry to small steric differences deserves comment. The total change in ΔE across the series *2a–g* corresponds to an overall change in ΔG for eqn. (4) of less than 1 kcal, yet each steric increment, even those as minor as the change from *2d* to *2e*, is faithfully mirrored in the DPP voltammetric data.

EXPERIMENTAL

Dimethylformamide was distilled from calcium hydride at aspirator pressure. Tetrabutylammonium perchlorate (G. Frederick Smith Chem. Co.) was dried overnight in an Abderhalden apparatus at 56 °C. Differential pulse polarography (DPP) was carried out using a PAR Model 170 Electrochemistry System with Model 172 drop timer. DPP peak potentials were found to be positive of polarographic half-wave potentials. The appropriate correction factor was found to be 0.015 V by measuring the difference between the DPP peak potential of pyrene on an expanded scale and the polarographic half-wave potential, defined as the point where log $i/(i_d-i)=0$ and located by an appropriate computer program. $E_{p,DDP}$ and $E_{1/2}$ were found to be –2.521 and –2.536 V, respectively. Potentials were measured relative to a reference electrode consisting of a silver wire immersed in a 0.1 M solution of silver nitrate in acetonitrile, contained in a length of heavy-walled Teflon tubing closed at one end by a short length of porous Vycor rod.

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Radiotracer and Electrochemical Study of the Adsorption and Electrocatalytic Oxidation of Glycerol at a Platinized Platinum Electrode

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Adsorption and electrocatalytic oxidation of glycerol at a platinized platinum electrode have been studied in acidic medium (1 mol dm^{-3} $HClO_4$). Adsorption phenomena were studied by a radiotracer method using ^{14}C-labelled glycerol. Competitive adsorption of glycerol with chloride ions was studied using ^{36}Cl-labelled HCl. Results of potentiostatic polarization studies were examined in the light of information obtained from radiotracer adsorption measurements. An attempt was made to explain the role of strongly chemisorbed organic species and chemisorbed oxygen in the polarization behaviour observed. It was assumed that the shape of the polarization curve is determined by two main factors: reactions on free metal sites and processes taking place on the top of the chemisorbed oxygen.

The electrocatalytic oxidation of aliphatic diols and some polyols at platinized and smooth platinum electrodes has been the subject of several studies. (See, for instance, Refs. 1–5 and references therein.) Despite the many-sided approach to the experimental material accumulated during the last decade, some controversy exists between the different authors concerning the mechanistic interpretation of the phenomena observed. Some authors are of the opinion that the oxidation process takes place exclusively *via* strongly chemisorbed species. Another group of authors assumes that weakly adsorbed species are involved in the main process and the formation and oxidation of the strongly chemisorbed species should be considered as side reactions. Unfortunately, it is very problematic to obtain direct information on the adsorption processes by pure electrochemical methods. However, the radiotracer method offers a unique possibility of gaining direct information on the adsorption processes of organic species.[6] In accordance with the considerations outlined above, the aim of this paper is to present some results obtained from a study of the adsorption and electrooxidation of glycerol by the simultaneous application of radiotracer and electrochemical methods.

EXPERIMENTAL

The radiotracer method used for the adsorption studies, preparation of the electrode and its pretreatment prior to the adsorption measurements are described elsewhere.[7] Glycerol labelled with ^{14}C and HCl labelled with ^{36}Cl of 7.4×10^{6} Bq $mmol^{-1}$ specific activity were used. Some polarization measurements with non-labelled glycerol were carried out using the experimental technique and apparatus described in Ref. 8. All measurements were carried out in the presence of 1 mol dm^{-3} $HClO_4$ supporting electrolyte. Potentials quoted in this paper refer to the hydrogen electrode immersed in this supporting electrolyte (rhe scale). The roughness factors of the platinized platinum electrodes used were about 300–400.

RESULTS

A typical polarization curve of glycerol obtained from a point-by-point potentiostatic measurement going in the direction of the higher

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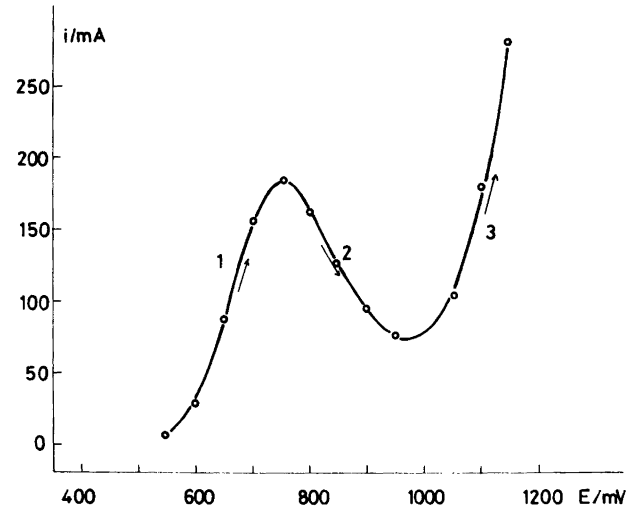

Fig. 1. Potentiostatic polarization curve of glycerol: $c=10^{-1}$ mol dm^{-3}.

potentials is shown in Fig. 1. Similar curves may be obtained in the case of smooth platinum electrode by potentiodynamic technique. In the potential range 500–1200 mV three distinct sections may be observed on the polarization curve. (Sections 1, 2 and 3 on the curve in Fig. 1.) It should be mentioned here that the polarization behaviour of glycerol was studied in the concentration range where the oxidation rate at a fixed potential did not change significantly with the concentration of glycerol. ($c>1\times10^{-2}$ mol dm^{-3}). The attainment of a stationary or quasi-stationary state requires 10–20 min depending on the potential. The transient phenomena observed following the potential shifts to more positive values are different at the different sections of the polarization curve. These observations attest that the rate of the electrooxidation process is governed by some kind of secondary phenomena which are slower than the charge transfer processes involved in the overall transformation. According to the literature [9] the main factors which should be taken into consideration for the explanation of the occurrence of the three sections on the polarization curve are as follows:

1. Inhibition of the oxidation process by strongly chemisorbed organic species. Oxidation of these species results in an increase of the oxidation rate as a result of formation of free metal sites (first section).

2. Inhibition of the oxidation process by oxygen adsorption (second section).

3. Oxidation of the organic species on the oxide layer formed on the surface of platinum (third section).

The occurrence of the reaction on the oxidized surface may be shown by means of separate experiments studying the oxidation on preoxidized electrodes. In this case the polarization curve shown in Fig. 2 is taken starting from high

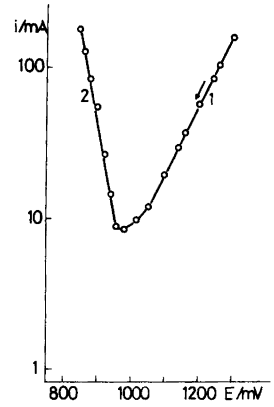

Fig. 2. Polarization curve of glycerol at a preoxidized electrode (1400 mV, 15 min). $c_{\text{glycerol}}=10^{-1}$ mol dm^{-3}.

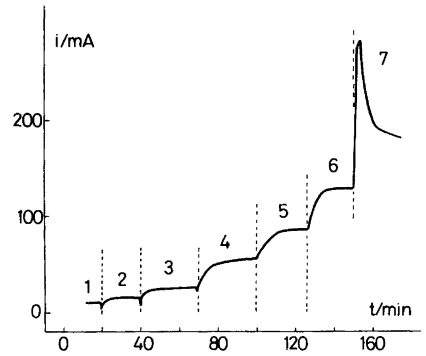

Fig. 3. Current transients in the case of a preoxidized electrode. Potential: (1) 960; (2) 940; (3) 920; (4) 900; (5) 880; (6) 860; (7) 800 mV.

potentials. This curve may be conceived as if it were composed of two linear lg *i–E* plots. However, it is of interest to show how section 2 in Fig. 2 is obtained. This is demonstrated by Fig. 3.

It may be seen from the curve presented in Fig. 3 that the switching of the potential to a lower value is followed by the slow increase of the current (sections 1–6). It can be shown by blank experiments carried out in the absence of glycerol that these phenomena occur only at potentials where the reduction rate of the oxide layer is low and the amount of charge consumed during the reduction does not attain a significant value. However, switching the potential to a value where both the reduction rate and the extent of the reduction are considerable the initial increase of the current is followed by its rapid decrease (section 7).

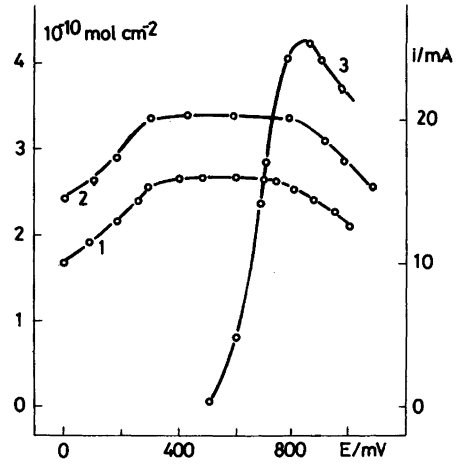

Fig. 4. Potential dependence of the adsorption of glycerol at concentrations 2×10^{-3} (1) and 2×10^{-2} (2) mol dm^{-3}. Polarization curve at $c=2\times10^{-2}$ mol dm^{-3} (3).

It may be assumed that the slow increase of the anodic current (oxidation rate) should be attributed to the slow formation of free metal sites by the slow reduction of the oxide layer. At each potential a steady (or equilibrium) state may be attained with respect to the number of free metal sites. The rapid decrease of the current at potentials E<800 mV presumably may be explained by the increasing role of the inhibition caused by organic chemisorbed species formed on the free metal sites.

All these considerations outlined above may be confirmed by the radiotracer adsorption measurements.

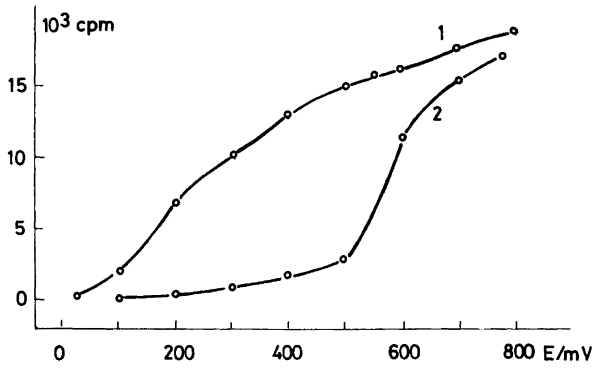

Fig. 5. Potential dependence of the adsorption of chloride ions ($c_{HCl}=4\times10^{-3}$ mol dm^{-3}) in the absence (1) and in the presence of glycerol (2) $c_{glycerol}=10^{-1}$ mol dm^{-3}. Radiation intensity which is proportional to the amount adsorbed is expressed in counts/min (cpm).

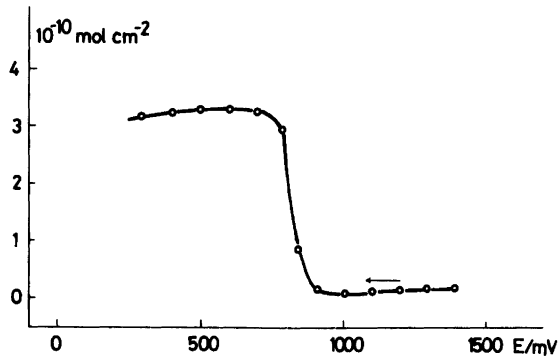

Fig. 6. Potential dependence of the adsorption of glycerol on a preoxidized electrode (E=1400, t=15 min) $c=2\times10^{-2}$ mol dm^{-3}.

In Fig. 4 the potential dependence of the adsorption of glycerol is shown at two different concentrations and the polarization curve taken at the higher concentration is presented as well. Species adsorbed in the potential range 200–600 mV are strongly attached to the surface as no exchange of the adsorbed labelled species with non-labelled ones added to the solution phase was observed in a separate experiment. However, it should be emphasized that there is no possibility of distinguishing between the adsorption of glycerol and that of its oxidation products. At least some of the oxidation products remain labelled with ^{14}C and the experimental method used here detects all adsorbed particles present in labelled form. Thus the potential dependence presented in Fig. 4, strictly taken, cannot be considered as the potential dependence of the adsorption of glycerol, at least at the potentials where the oxidation of glycerol takes place. However, there is a possibility to show by an indirect tracer method described in Ref. 10 that with increasing potentials the strongly chemisorbed species are eliminated from the surface. Using ^{36}Cl-labelled chloride ions and non-labelled glycerol the effect of the latter on the adsorption of the former may be studied. Fig. 5 shows the potential dependence of the adsorption of chloride ions in the presence and absence of glycerol. Glycerol inhibits the adsorption of Cl^--ions in a wide potential range (100–500 mV). This phenomenon can be ascribed to the strong chemisorption of glycerol. Increase of Cl^- adsorption may be observed only at potentials where the oxidation of glycerol starts. This means that the oxidation process is preceded by the elimination of strongly chemisorbed species which can displace specifically adsorbed anions. This behaviour is similar to that observed in the case of other simple alcohols, and ethylene glycol.[11]

The radiotracer method permits the study of adsorption phenomena also on a preoxidized electrode. Results of this study are shown by Fig. 6. Practically no adsorption of glycerol occurs on the oxidized surface. Increase of the adsorption may be observed only at the potentials where the increase of the oxidation current was found in the case of the experiments presented in Fig. 3. Fig. 7 shows that the increase of the oxidation current

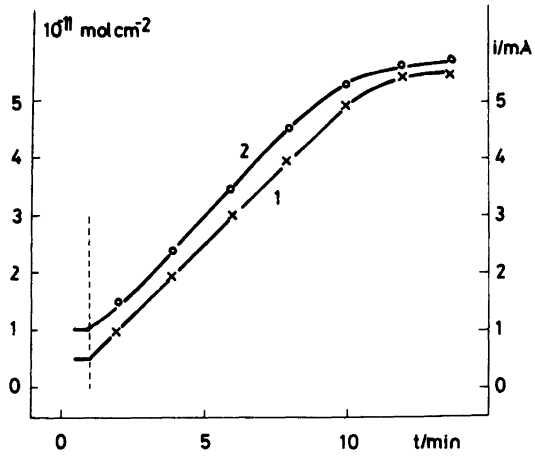

Fig. 7. Simultaneous change of the adsorption of glycerol (1) and the oxidation rate (2) following a potential switch from 900 mV to 850 mV in the case of a preoxidized electrode, $c_{glycerol}=2\times10^{-2}$ mol dm^{-3}.

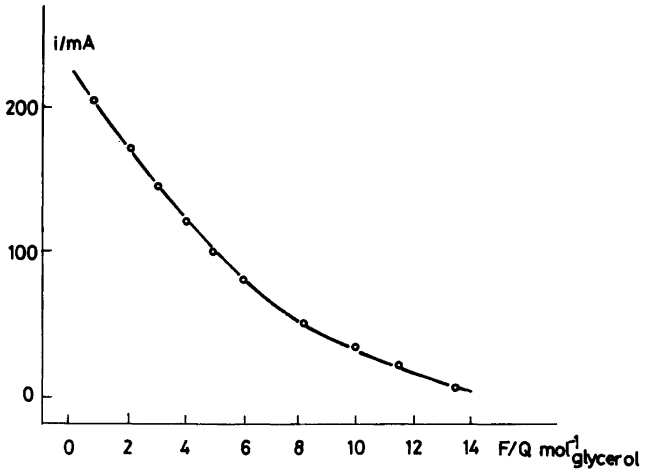

Fig. 8. Current – as a function of the charge passed through the system in the course of the exhaustive oxidation of 1 mmol glycerol at E=700 mV. Charge consumed in the oxidation is referred to 1 mol glycerol and expressed in F units.

at a given potential is connected with the simultaneous increase of the coverage with respect to glycerol. At potentials where the reduction rate of the oxide layer becomes significant the increase of the adsorption initially results in the increase of the oxidation rate but this stage is followed by the rapid decrease of current despite the continuous increase of the adsorption. Experiments carried out with preoxidized electrodes confirm our initial assumption that the increase of the oxidation rate in the potential range 800–900 mV should be attributed to the increase of free metal sites formed by the partial reduction of the oxide layer. Glycerol adsorbs on these sites and the oxidation rate is proportional to the coverage with respect to the adsorbed species. The observation made in the case of a considerable reduction of the oxide layer requires further explanations. This problem will be discussed later.

Experiments aiming at the exhaustive oxidation of a given amount of glycerol show that its complete transformation into CO_2 may be achieved even at low potentials. This is shown in Fig. 8. (For the complete oxidation of glycerol 14 F are required.)

However, there is no doubt that the oxidation process takes place in a stepwise manner. The first step in this reaction sequence is the formation of glyceraldehyde and to a lesser extent that of dioxyacetone. At low potentials and high glycerol concentrations the accumulation of these intermediates in the solution phase may be observed.[12,13] (The presence of α-oxy-carbonyl compounds in the solution phase can be easily detected by the Fehling reaction.)

DISCUSSION

It may be concluded from the simultaneous application of radiotracer adsorption and electrochemical polarization measurements that, in accordance with previous views, in a wide potential range two different reactions occur with glycerol at a platinum electrode. The first reaction is the oxidation process on bare metal surface sites. This was clearly demonstrated by the observations made in the course of the reduction of the oxide layer. A significant oxidation current may be attained at very low glycerol coverages when the predominant part of the surface is covered by chemisorbed oxygen. On the other hand, following the elimination of the oxide layer there is a significant decrease of the oxidation rate despite the increase of the coverage with respect to glycerol. This phenomenon may be explained on the basis of information available from the literature concerning the behaviour of modified platinum electrodes.[14] It has been shown that the catalytic activity of a platinum electrode may be increased by the

presence of strongly adsorbed species (for instance, metal adatoms).

It is assumed that the increase of catalytic activity is due to the prevention of the self inhibition of the oxidation process by the organic species. The inhibition is caused by side reactions leading to the strong chemisorption of the organic species studied. However, for the formation of these strongly chemisorbed species there is a need for several neighbouring surface sites. In the presence of preadsorbed species leaving only isolated free surface sites available for the reaction studied there will be insufficient neighbouring surface sites for the further formation of strongly chemisorbed species. It may be assumed that similar phenomena may play a role in the case of the stepwise reduction of the oxide layer. At the beginning of the reduction of the oxide layer the free metal sites are isolated from one another. Thus the formation of strongly chemisorbed species is inhibited. As the reduction of the oxide layer becomes more pronounced free surface sites will be formed around an adsorbed organic species. Thus more and more strongly chemisorbed species are formed and the reaction rate should decrease as the number of the strongly chemisorbed species increases.

The strong chemisorption of the organic species is accompanied by a significant change of the composition and structure of the molecule (dehydrogenation, rupture of C–C bonds *etc.*). These fragments cannot be involved in the main reaction sequence resulting in well-defined intermediates which can be detected in the solution phase. Presumably the oxidation of strongly chemisorbed species leads directly to CO_2 without the participation of any desorbable intermediate. On increasing the potential of the electrode the oxidation of strongly chemisorbed species occurs and free sites available for the main oxidation process are produced. At a given potential both processes, the formation of strongly chemisorbed species on the free sites and oxidation of these species, occur simultaneously. Finally, a steady state should be attained with respect to the number of sites freed from strongly chemisorbed species. Thus it may be expected that the rate of the main oxidation process will be proportional to the number of these sites.

It is known from the literature [1–5] that in the oxidation of alcohols the first step is a dehydrogenation reaction. Thus in the case at the oxidation of glycerol the reactions leading to glyceraldehyde may be formulated as follows:

$$HOCH_2CH(OH)CH_2OH \rightleftharpoons [HOCH_2CH(OH)CH_2OH]_a \quad (1)$$

$$[HOCH_2CH(OH)CH_2OH]_a \rightarrow [HOCH_2CH(OH)CHOH]_a + H_a \quad (2)$$

$$H_a \rightarrow H^+ + e \quad (3)$$

$$H_2O \rightleftharpoons OH_a + H^+ + e \quad (4)$$

$$[HOCH_2CH(OH)CHOH]_a + OH_a \rightarrow \rightarrow [OHCH_2CH(OH)CH(OH)_2]_a \rightarrow HOCH_2CH(OH)CHO + H_2O \quad (5)$$

These processes are accompanied by the formation of strongly chemisorbed species which originate either from the weakly adsorbed species figuring in eqn. (1) or from the adsorbed radical figuring in eqn. (2). On the other hand, the oxidation of these strongly chemisorbed species should also be taken into consideration. The rate of reaction 2 presumably does not depend on the potential as this step is a chemical process without charge transfer. Only the coverage with respect to the weakly adsorbed species may be some function of the potential.

Similar argumentation may refer to the formation of strongly chemisorbed species as, according to our initial assumption, these species are formed from the weakly adsorbed molecules.

In contrast to this the oxidation of strongly chemisorbed species depends significantly on the potential. Thus the coverage with respect to strongly chemisorbed species; consequently, the number of sites available for the weak adsorption will depend on the potential. At the same time the rate of the main oxidation process depends on the coverage with respect to the weakly adsorbed species. The applicability of this model has been shown in the case of the interpretation of the phenomena observed in the course of the oxidation of ethylene glycol and its derivatives.[15]

The occurrence of the maximum on the polarization curve presented in Fig. 1 should be explained by the adsorption of oxygen. With increasing potentials the extent of the oxygen adsorption increases and the number of the sites available for the oxidation process decreases. As a result of this phenomenon, a maximum and thereafter the decrease of the oxidation rate should be observed. However, there is an additional phenomenon, the oxidation of the organic species on the oxide layer, which should be taken into consideration.

The nature of the reaction occurring at the oxide layer is not clear. According to the results obtained by means of tracer adsorption studies there is no significant adsorption of organic species on preoxidized electrodes. Thus there is no possibility of treating the problem in terms of heterogeneous kinetics involving reactions with adsorbed species. However, it is possible that the coverage with respect to the reacting species is so low that the adsorption cannot be followed by the method used in this work. On the other hand, the assumption of some direct reaction of the surface oxide or absorbed oxygen with organic molecules cannot be excluded completely. Disregarding the problems connected with the mechanism of this process, it is an experimental fact that the rate of the oxidation of the oxidized surface increases with increasing potentials.

Thus the initial inhibition caused by the formation of oxide layer should be followed by the increase of the oxidation rate as the potential is shifted to more positive values.

Summarizing our considerations it can be stated that the general tendencies observed in the electrocatalytic oxidation of glycerol are very similar to those reported in different alcohols, diols and their oxidative derivatives. Unfortunately, at present only a qualitative explanation of the phenomena observed could be given on the basis of the available experimental material.

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Electron-transfer Fluorescence Quenching of Radical Ions *

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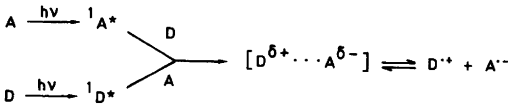
The radical anions of anthraquinone and 9,10-dicyanoanthracene and the radical cation of thianthrene fluoresce in oxygen-free fluid solution at room temperature. The fluorescence of the radical anions is quenched by added electron acceptors, while the fluorescence of the cation is quenched by added electron donors. The fluorescence quenching is treated in terms of an electron-transfer mechanism. The results agree with Marcus' theory and Weller's equation *only* if very large solvent and/or bond reorganization energies (20–30 kcal/mol) are introduced.

Since the pioneering work of Weller and coworkers,[1] fluorescence quenching *via* electron-transfer mechanisms has been a subject of great interest among photochemists and photophysicists.[2–5] Most work in this field has been centered around electron-transfer to or from the singlet excited state of fluorescing aromatic molecules in the presence of electron donors (D) or acceptors (A), respectively. Thus, the reaction is accompanied by fluorescence quenching of the light absorbing molecule.[1]

The produced radical ions may back-transfer the electron to reform A and D or may go on producing new products. In the presence of oxygen, photooxygenations may occur.[2,6]

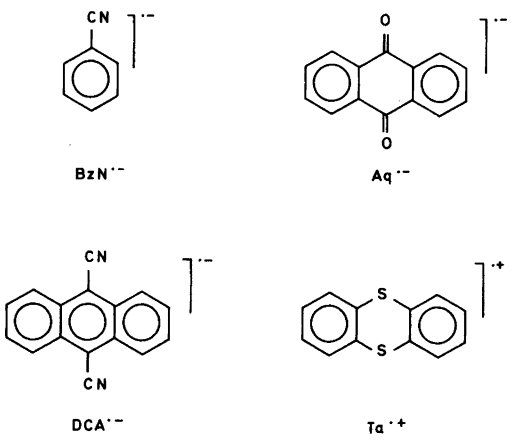


We have previously studied electron-transfer from photoexcited ion radicals [7] and have recently been interested in using ion radicals as light absorbing species in photogalvanic cells. For this purpose we have investigated the fluorescence quenching of molecules that are themselves radical ions which were produced electrochemically.

$$D \xrightarrow{e^-} D^{\cdot-} \xrightarrow{h\nu} (D^{\cdot-})^* \xrightarrow{A} [D\cdots A]^{\cdot-} \longrightarrow D + A^{\cdot-}$$

$$A \xrightarrow{-e^-} A^{\cdot+} \xrightarrow{h\nu} (A^{\cdot+})^* \xrightarrow{D} [A\cdots D]^{\cdot+} \longrightarrow A + D^{\cdot+}$$

The radical ions chosen for this study are the anthraquinone radical anion ($Aq^{\cdot-}$), the 9,10-dicyanoanthracene radical anion ($DCA^{\cdot-}$), and the thianthrene radical cation ($Ta^{\cdot+}$). The radical anion of benzonitrile ($BzN^{\cdot-}$) has been studied briefly.



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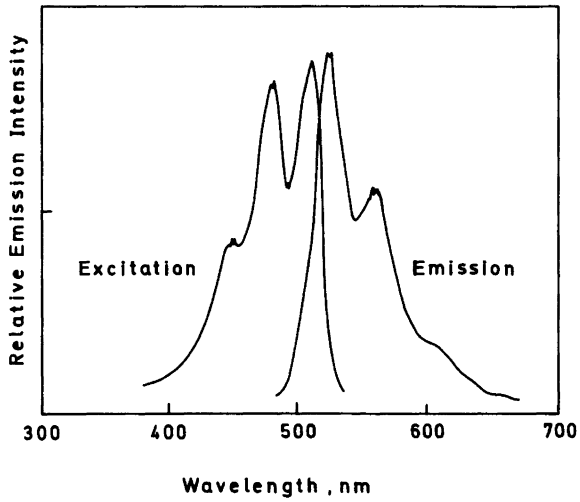

Fig. 1. Corrected fluorescence excitation and emission spectra of $DCA^{\cdot-}$ 10^{-5} M in oxygen-free MeCN at room temperature.

RESULTS

Fluorescence data. The radical ions investigated in this study all exhibited strong fluorescence in oxygen-free solutions at room temperature. Table 1 contains the emission maxima of several radical ions in various solvents. Fig. 1 shows, as an example, the corrected fluorescence emission and excitation spectra of $DCA^{\cdot-}$ in MeCN. The emission spectrum is quite similar in structure to the fluorescence spectrum of DCA, but red-shifted about 100 nm. For all the compounds studied, the fluorescence excitation spectra were in good agreement with the corresponding absorption spectra. The excitation energies ($\Delta E_{o,o}$, Table 2) could be estimated from the wavelength of the overlap between the fluorescence excitation and emission spectra.

The fluorescence lifetimes (τ, Table 2) were determined by single photon counting [8] (see Experimental). The lifetime of $Aq^{\cdot-}$ in DMF was measured in three different laboratories which resulted in excellent mutual agreement.

Fluorescence quenching and electrochemical considerations. The emission of the radical anions and cations in oxygen-free solutions were quenched by a wide variety of electron acceptors and donors, respectively (Tables 3–5). In each case, the quenching followed the well-known Stern-Volmer equation,[9]

$$I_o/I_Q = 1+k_q \tau[Q] \quad (1)$$

where I_o and I_Q are the relative fluorescence intensities in the absence and presence of quencher (Q), k_q is the bimolecular quenching rate constant and τ is the fluorescence lifetime. For

Table 1. Maxima in nm of corrected fluorescence spectra of radical ions.[a]

Solvent	$Aq^{\cdot-}$	$DCA^{\cdot-}$	$BzN^{\cdot-}$	$Ta^{\cdot+}$
DMF[b]	575	525, 560	505	[d]
MeCN	[d]	530, 560	[d]	[d]
Toluene	470	[d]	[d]	[d]
CH_2Cl_2[c]	[d]	[d]	[d]	580

[a] In fluid solutions at room temperature. [b] Dimethylformamide. [c] Solvent mixture of methylene chloride, trifluoroacetic anhydride and trifluoroacetic acid (45:5:1, v/v). [d] Not investigated.

Table 2. Fluorescence data of radical ions[a].

Fluorescer	Solvent	$\Delta E_{o,o}$[b] eV	τ[c] ns
$Aq^{\cdot-}$	DMF	2.21	13.7
$DCA^{\cdot-}$	DMF	2.38	13.5
$BzN^{\cdot-}$	DMF	2.56	[d]
$Ta^{\cdot+}$	CH_2Cl_2[e]	2.18	4.7

[a] In oxygen-free solution at room temperature. [b] Excitation energy. [c] Fluorescence lifetime. [d] Not determined. [e] See footnote *c* of Table 1.

Table 3. Fluorescence quenching of $Aq^{\cdot-}$.[a]

Quencher	k_q[b] 10^9 $M^{-1}s^{-1}$	$E^{\circ}_{A/A^{\cdot-}}$[c] V *vs.* SCE	ΔG[d] kcal/mol
1 Acridine	13.9	−1.63[e]	−33.4
2 Benzophenone	12.0	−1.77[e]	−30.2
3 1-Naphthonitrile	11.0	−1.90[e]	−27.2
4 2-Chloroquinoline	13.6	−1.92[f]	−26.8
5 3-Cyanopyridine	4.89	−2.03[e]	−24.2
6 1-Bromonaphthalene	2.70	−2.13[f]	−21.9
7 1-Chloronaphthalene	1.61	−2.19[f]	−20.5
8 Benzonitrile	0.708	−2.23[e]	−18.2
9 3-Bromopyridine	0.562	−2.29[f]	−17.3
10 3-Chloropyridine	0.095	−2.39[f]	−15.9
11 2-Chloropyridine	0.102	−2.40[f]	−15.7
12 Bromobenzene	0.036	−2.44[f]	−14.8

[a] In oxygen-free DMF at room temperature. [b] Slope of Stern-Volmer plot (eqn. (1)) divided by lifetime (Table 2). [c] Reduction potential. [d] Calculated from eqn. (2). [e] This study; determined by cyclic voltammetry, see Experimental. [f] Determined by kinetic analysis of homogeneous redox catalysis of electronic reductions. Data from Andrieux, C. P., Blocman, C., Dumas-Bouchiat, J.-M. and Savéant, J. M. *J. Am. Chem. Soc. 101* (1979) 3431; Andrieux, C. P., Blocman, C., Dumas-Bouchiat, J.-M., M'Halla, F. and Savéant, J. M. *Ibid. 102* (1980) 3806.

Table 4. Fluorescence quenching of $DCA^{\cdot-}$.[a]

Quencher	k_q[b] 10^9 $M^{-1}s^{-1}$	$E^{\circ}_{A/A^{\cdot-}}$[c] V *vs.* SCE	ΔG[d] kcal/mol
1 Acridine	15.8	−1.63[e]	−38.1
2 Benzophenone	11.0	−1.77[e]	−34.8
3 1-Naphthonitrile	9.48	−1.90[e]	−31.8
4 2-Chloroquinoline	4.59	−1.92[f]	−31.4
5 3-Cyanopyridine	4.44	−2.03[e]	−28.8
6 1-Bromonaphthalene	0.370	−2.13[f]	−26.5
7 1-Chloronaphthalene	0.059	−2.19[f]	−25.1
8 Benzonitrile	0.022	−2.23[e]	−24.2
9 3-Bromopyridine	0.022	−2.29[f]	−22.8
10 2-Bromopyridine	0.0023	−2.30[f]	−22.6

[a-f] See footnotes *a–f* of Table 3.

Table 5. Fluorescence quenching of $Ta^{\cdot+}$.[a]

Quencher	k_q[b] 10^9 $M^{-1}s^{-1}$	$E^{\circ}_{D^{\cdot+}/D}$[c] V *vs.* SCE	ΔG[d] kcal/mol
1 Pyrene	7.23	−1.36[e]	−46.8
2 Acenaphthene	6.11	−1.52[f]	−43.1
3 Hexamethylbenzene	5.32	−1.61[e]	−41.0
4 Anisole	1.38	−1.76[f]	−37.6
5 Naphthalene	0.957	−1.84[e]	−35.7
6 Thiophene	0.140	−2.03[f]	−31.4
7 Toluene	0.149	−2.37[e]	−23.5

[a] In oxygen-free CH_2Cl_2/trifluoroacetic anhydride/trifluoroacetic acid (45:5:1) at room temperature. [b] Slope of Stern-Volmer plot (eqn. (1)) divided by lifetime (Table 2). [c] Voltammetric half-wave oxidation potential. [d] Calculated from eqn. (3). [e] Data from Eberson, L. *Adv. Phys. Org. Chem. 18* (1982) 79, Table 9. [f] Data from Eberson, L. *Private communication.*

each fluorescer-quencher pair, five suitable concentrations of Q were used. Data treatment according to eqn. (1) gave correlation coefficients generally >0.999. All quenching runs were performed twice and in all cases the data could be reproduced.

The slopes ($k_q\,\tau$) and the lifetimes (Table 2) allowed for determination of the quenching rate constants, k_q (Tables 3–5).

The free energy change (ΔG) involved in an electron-transfer process from an excited donor radical anion to a neutral acceptor is given by eqn. (2),[1,10]

$$\Delta G(\text{kcal/mol}) = 23.06(E^\circ_{D/D^{\cdot-}} - E^\circ_{A/A^{\cdot-}} - \Delta E_{o,o}) \quad (2)$$

where $E^\circ_{D/D^{\cdot-}}$ is the reversible electrode potential of the fluorescer couple $D/D^{\cdot-}$, $E^\circ_{A/A^{\cdot-}}$ the reversible electrode potential of the acceptor couple, and $\Delta E_{o,o}$ is the excitation energy defined above. By cyclic voltammetry (CV) we found $E^\circ_{Aq/Aq^{\cdot-}} = -0.87$ V *vs.* SCE and $E^\circ_{DCA/DCA^{\cdot-}} = -0.89$ V *vs.* SCE; both redox systems show reversible behavior in CV. Electrode potentials for the neutral acceptors are given in Tables 3 and 4.

Similarly, ΔG for quenching of an excited acceptor radical cation $(A^{\cdot+})^*$ by neutral donors is given by eqn. (3),[1,10]

$$\Delta G(\text{kcal/mol}) = 23.06(-E^\circ_{A^{\cdot+}/A} + E^\circ_{D^{\cdot+}/D} - \Delta E_{o,o}) \quad (3)$$

where $E^\circ_{A^{\cdot+}/A}$ is the reversible electrode potential of the fluorescer couple $A^{\cdot+}/A$ and $E^\circ_{D^{\cdot+}/D}$ that of the donor couple. CV gave $E^\circ_{Ta^{\cdot+}/Ta} = 1.21$ V *vs.* SCE. Electrode potentials for the neutral donors are given in Table 5.

Values of k_q measured by fluorescence quenching (eqn. (1)) and ΔG values calculated from eqn. (2) or (3) (Tables 3–5) have been used to construct Weller plots [1] for the fluorescence quenching of radical ions, see Figs. 2–4.

Miscellaneous. In order to test for exciplex formation as a possible mechanism in the fluorescence quenching, we have looked for exciplex emission from $Aq^{\cdot-}$ quenched by BzN in non-polar as well as polar solvents. Aq dissolved in toluene was reduced by sodium amalgam and enough BzN was added under nitrogen to quench >90 % of the $Aq^{\cdot-}$ fluorescence. The emission spectrum was scanned at highest spectrofluorimeter sensitivity in the region 500–800 nm. No exciplex emission, however, could be detected. A similar experiment using DMF as solvent gave likewise a negative result. A combined electron- and energy-transfer process,

$$(Aq^{\cdot-})^* + BzN \rightarrow Aq + (BzN^{\cdot-})^*$$

which would be expected to give rise to emission from $BzN^{\cdot-}$ (see Table 1) is unlikely, since the process is endothermic. No such emission could be detected.

DISCUSSION

Figs. 2–4 show that our data on fluorescence quenching of radical ions do not fit Weller's curve for electron-transfer fluorescence quenching.[1]

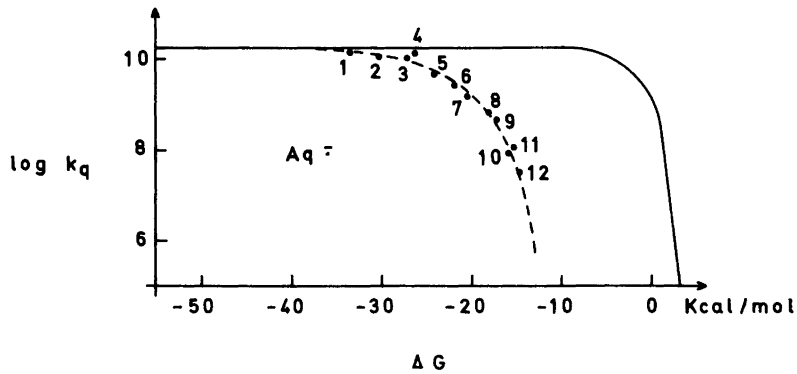

Fig. 2. Fluorescence quenching plot of $Aq^{\cdot-}$ in DMF. Quencher numbers refer to Table 3. Solid line calculated by Rehm and Weller for an electron-transfer quenching mechanism.[1]

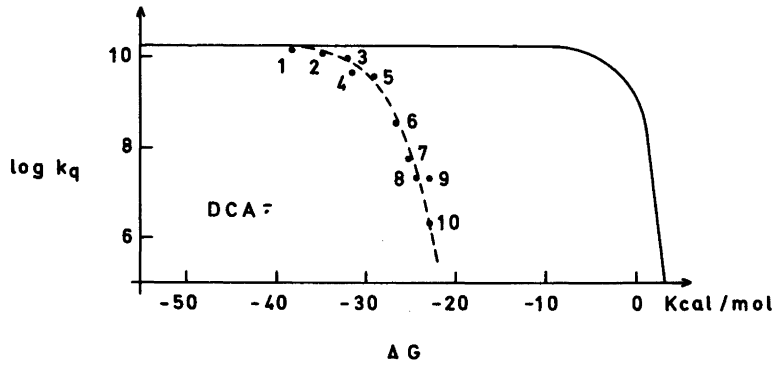

Fig. 3. Fluorescence quenching plot of DCA^- in DMF. Quencher numbers refer to Table 4. Solid line: See Fig. 2.

Although our curves are similar in shape to Weller's curve, they are shifted some 20–30 kcal/mol, far outside the range of reasonable experimental error. Numerous experimental works on electron-transfer fluorescence quenching of neutral molecules,[1–6] including our own, have verified Weller's curve. It is therefore surprising to us to see this large discrepancy.

There might be several ways to explain our data on radical ions. Perhaps fluorescence quenching of these ions does not follow an electron-transfer mechanism. An *energy*-transfer mechanism, however, is not likely (see above), and the fact that our curves *in shape* are similar to Weller's curve certainly indicates some type of charge-transfer mechanism.

An electron-transfer mechanism involving an exciplex may be considered. The intermediacy of an exciplex formed *via* a nonrelaxed charge-transfer state has recently been suggested by Kramer *et al.* in the fluorescence quenching of the cationic dye oxonine.[5a] Exciplexes, however, are generally formed in cases where a stabilization of the excited fluorescer is obtained.[11] Thus, formation of an exciplex from an excited fluorescer and a quencher is an exothermic process. Short-lived exciplexes could therefore be involved in systems where the observed k_q is diffusion-controlled. In order to explain our present results by formation of exciplexes, these would have to be formed also in cases where k_q is several orders of magnitude less than diffusion-controlled. Why would the excited radical-ion/quencher pair pass through a higher lying energy state (endothermic exciplex formation) when simple electron-transfer according to eqn. (2) or (3) is highly exothermic? Attempts to detect emission from an exciplex failed (see above).

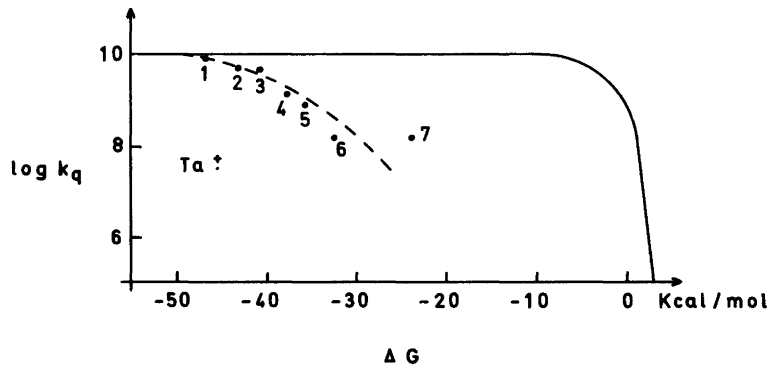

Fig. 4. Fluorescence quenching plot of Ta^{+} in methylene chloride–trifluoroacetic anhydride–trifluoroacetic acid (45:5:1). Quencher numbers refer to Table 5. Solid line: See Fig. 2.

As a possible explanation of our results one might argue that our fluorescers being radical ions are surrounded by a highly organized solvent shell, thus preventing close contact to a quencher molecule. Weller's curve is based upon an empirical equation (eqn, (4))[1]

$$k_q = \frac{2\times10^{10}}{1+0.25[\exp(\Delta G^*/RT)+\exp(\Delta G/RT)]}\ M^{-1}s^{-1} \quad (4)$$

where ΔG is given by eqn. (2) or (3) and ΔG^*, the activation free enthalpy, was assumed to be a monotonous function of ΔG, eqn. (5):

$$\Delta G^* = [(\Delta G/2)^2+(\Delta G_o^*)^2]^{1/2}+\Delta G/2 \quad (5)$$

Here, ΔG_o^*, the activation free enthalpy at $\Delta G=0$, was found experimentally to be 2.4 kcal/mol.[1] Based on an outer-sphere, adiabatic electron-transfer theory Marcus [12,13] has derived an equation similar to eqn. (4), but with ΔG^*

$$\Delta G^* = \Delta G_o^* [1+\Delta G/4\Delta G_o^*]^2 \quad (6)$$

With $\Delta G_o^*=2.4$ kcal/mol both eqns. (5) and (6) together with eqn. (4) are in good agreement with experimental results for electron-transfer fluorescence quenching of neutral molecules when $\Delta G>-15$ kcal/mol. For more exothermic reactions, however, the Marcus relation (eqn. (6)) predicts a drastic decrease in k_q (Marcus' inverted region [1,12,13]). Thus, neither Weller's nor Marcus' equations agree with our experimental data for fluorescence quenching of radical ions, at least with the assumption $\Delta G_o^*=2.4$ kcal/mol.

The main contribution to ΔG_o^* is a solvent reorganization energy due to electron removal from or addition to the involved molecules, although bond reorganization energies may play some role as well.[12] Eberson has recently argued that the widely accepted value of $\Delta G_o^*=2.4$ kcal/mol need not and indeed should not be valid for all electron-transfer reactions.[12,14] Thus, a value of $\Delta G_o^*=10$ kcal/mol was found for one-electron reductions of diacyl peroxides.[14a]

As mentioned above, radical ions might be expected to require large solvent reorganization energies in electron transfer reactions, thus resulting in large ΔG_o^* values. The fact that each radical ion (Figs. 2–4) gives a separate Weller/Marcus plot even though a variety of quenchers are used, suggests that the solvent reorganization energy of the fluorescing radical ion is more important than that of the quencher. In order to make our data fit Weller's or Marcus' equations, ΔG_o^* values of the order of 20–30 kcal/mol are required.

Clearly, much more experimental work is required to test for very large ΔG_o^* values. We are presently looking at the contributions to ΔG_o^* from bond reorganizations and solvent reorganizations from both the radical ions and the quenchers.

EXPERIMENTAL

Spectra. All emission spectra were recorded on an Aminco SPF-500 Corrected Spectrofluorimeter. Scan-rate: 50 nm/min. Band widths: 2 nm. Oxygen-free solutions were transferred to quartz-cells with four polished sides. Absorption spectra using the same cells were recorded on a Varian Cary Model 219 Spectrophotometer.

Materials. DMF was distilled under vacuum and stored over Molecular Sieves 4 A. MeCN was spectrograde (Merck, Uvasol) and used as received. Aq and DCA were recrystallized from ethanol and toluene, respectively, prior to use. Other chemicals were generally highest purity available and used as received.

Lifetimes. Fluorescence lifetimes of $Aq^{\cdot-}$, $DCA^{\cdot-}$ and $Ta^{\cdot+}$ were determined by single photon counting.[8] Oxygen-free solutions 10^{-5} M in the radical ions were placed in sealed tubes. The lifetime of $Aq^{\cdot-}$ was measured in three different laboratories at New York University (Professor D. I. Schuster), University of California, Los Angeles (Professor V. N. Schumaker) and Max-Planck-Institut, Mülheim, BRD (Professor K. Schaffner). The lifetimes of $DCA^{\cdot-}$ and $Ta^{\cdot+}$ were determined in the Mülheim laboratory.

Formation of radical ions was carried out by electrolysis at constant potentials in H-cells.[15] The reference electrode was an Ag/AgI (0.1 M tetrabutylammonium iodide, TBAI) electrode. During reduction an Hg-electrode and a graphite stick were used as cathode and anode, respectively, while oxidation was carried out with Pt-electrodes. The potentiostat (from Tage Juul, Copenhagen, 100 V and 300 V) was adjusted to a potential at the limiting current of the compound (10^{-5} M) to be reduced or oxidized. The solutions were purged with N_2 for 15 min prior to and during the reduction or oxidation.

Redox potentials were obtained by cyclic voltammetry at a Pt-electrode (oxidations) or Hg-drop (reductions). The reference electrode was an Ag/AgI (0.1 M TBAI) electrode (–0.56 V relative to the standard calomel electrode, SCE). The solvent was dried over anhydrous Alumina and purged with N_2 for 20 min prior to use. The supporting electrolyte was TBAI or tetrabutylammonium fluoroborate (TBAFB) at 0.1 M. The potentials were measured at scan rates 40, 60, 80 and 100 v/s. Potentials in this study (see text and Tables 3 to 5) were all reversible. The obtained reduction potential for DCA was in good agreement with a literature value of –0.82 V *vs.* SCE.[16]

Fluorescence quenching procedure. Formation of the fluorescing radical ions and all sample manipulations were carried out in a glove-box which has been flushed overnight with oxygen-free N_2. Solutions of the radical ion were pipetted into each of five volumetric flasks (10 ml). Appropriate amounts of a quencher stock solution were added using pipettes and the mixtures were diluted to the 10-ml mark. The flasks were shaken and their contents transferred to five Quartz cuvettes which were then stoppered and removed from the glove-box. The concentration of the fluorescer was $\sim 10^{-6}$ M giving rise to optical densities ~0.05. The relative emission intensities were determined at the wave-length of maximum emission and the data were analyzed according to eqn. (1) using a least-squares program. In order to check for possible ground-state complexation or reactions the absorption spectrum and the fluorescence excitation and emission spectra were recorded for the solution containing the highest concentration of quencher. These spectra were in all cases analogous to those without added quencher.

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Short Communication

The Second Order Coupling Mechanism of 4-Methoxybiphenyl Cation Radical

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We recently observed that the overall reaction order $R_{A/B}$ during the dimer-forming reactions of 4-methoxybiphenyl (MB) cation radical changed from 3 at low substrate concentration (C_A) to 2 at C_A greater than about 2 mM.[1] We suggested that the reaction involves competing mechanisms, cation radical–substrate coupling (1–2) and cation radical dimerization (3) with rate laws (4) and (5), respectively. Our data were re-examined by Savéant [2] and found to be consistent with a single mechanism (1–2) with a change in rate-determining step with increasing C_A so that the rate law changed from (4) to (6). We neglected to consider this possibility although we were well aware that the reaction order analysis [3] does not distinguish between the two second order rate laws, (4) and (6). The theoretical data necessary to distinguish between the latter is available for the derivative cyclic voltammetry (DCV) analysis used.[4] The slopes,

$$MB^{\cdot+} + MB \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} MB^+ - MB^{\cdot} \quad (1)$$

$$MB^+ - MB^{\cdot} + MB^{\cdot+} \xrightarrow{k_2} MB^+ - MB^+ + MB \quad (2)$$

$$2\,MB^{\cdot+} \xrightarrow{k_3} MB^+ - MB^+ \quad (3)$$

$$\text{Rate} = k_2 K_1 [MB^+]^2 [MB] \quad (4)$$

$$\text{Rate} = k_3 [MB^+]^2 \quad (5)$$

$$\text{Rate} = k_1 [MB^+][MB] \quad (6)$$

$d \ln R_I'/d \ln \nu^{-1}$ where R_I' is the derivative peak current ratio and ν the voltage sweep rate, are significantly different for processes obeying rate laws (5) and (6). Under the conditions of our measurements [1] the theoretical values of the slopes are −0.307 (rate law (5)) and −0.373 (rate law (6)).

The data in Table 1 were obtained in the same experiments as the data reported in Table 1 of Ref. 1. The value of $d \ln R_I'/d \ln \nu^{-1}$ varied from −0.485 to −0.127 while C_A varied from 8.0 to 0.125 mM. The data of most interest are those at C_A greater than 2 mM. The theoretical value for rate law (6) was only slightly greater than the observed slope at C_A equal to 4.0 mM but significantly smaller than the value observed at C_A equal to 8.0 mM. It is not unexpected that experimental voltammetric data deviate from theoretical values when substrate concentrations are much larger than about 1 mM. We can conclude that the data are more consistent with rate law (6) than with rate law (5) at the high end of the C_A range. Thus, it seems likely that the suggestion [2] that only mechanism (1–2) is in-

Table 1. Derivative cyclic voltammetry analysis of the coupling reactions of 4-methoxybiphenyl cation radical in acetonitrile.[a]

C_A/mM [b]	$-d \ln R_I'/d \ln \nu^{-1}$ [c]
8.0	0.485
4.0	0.352
2.0	0.267
1.0	0.242
0.5	0.215
0.25	0.175
0.125	0.127

[a] Logarithmic analysis according to Ref. 4. [b] Substrate concentration. [c] Slope defined in the text.

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volved in the dimer forming reactions of $MB^{\cdot+}$ is correct. However, since some problems were encountered with electrode filming and attributed to adsorption,[1] the data are not ideal.

Linear sweep voltammetry (LSV) analysis would be the least ambiguous way to distinguish between rate laws (5) and (6). This follows from the fact that the reaction orders in substrate (A) and primary intermediate (B) are separable by LSV.[5] However, because of the electrode filming problems, electrode potential measurements were not reliable and it was not possible to make the LSV analysis.

An Arrhenius activation energy of 10.6 kcal/mol was observed at high C_A and this was attributed to the activation energy for reaction (3). In view of the discussion in the previous paragraphs it appears more likely that the observed activation energy reflects that for forward reaction (1). The transition states for reaction (2) and forward reaction (1) differ only by charge and thus many of the factors contributing to the entropy of activation for the two processes will be of similar magnitude. This suggests that the activation energy for reaction (3) could be even greater than 11 kcal/mol if this reaction does not compete with (1) under the reaction conditions.

Our primary interest at the outset of this study [1] was to determine whether or not the cation radical–substrate coupling mechanism which we had observed in a related study on the coupling reactions of 4,4'-dimethoxystilbene cation radical [6] was of general importance in contradication to the belief that ion radical dimerization is the most likely reaction pathway for the dimer-forming reactions of anion radicals [7] as well as cation radicals.[8] The importance of ion radical–substrate coupling has now been amply demonstrated for both cation radicals [1,6] and anion radicals.[9–11]

Although we agree with Savéant [2] that our data are most consistent with rate-determining forward reaction (1) at the higher concentrations, we challenge his statements that this represents a failure in the reaction order approach.[3] *In fact, the further calculations on this system* [2] *used the reaction orders that we derived using the reaction order approach.* The data, especially at the higher concentrations (Table 1) *do not* fit the theoretical working curve [5] for reaction (1). The data cannot be expected to fit any theoretical working curve that does not take into account the electrode filming which was reported as a severe problem.[1] Taking the latter into account would appear to be a very complex theoretical problem which would require very much more experimental data as well. As far as we are aware, Savéant [2] has done no experiments on this system. The criticism of the reaction order approach in this case is surely not justified since without it the rate data could not have been obtained. It was because of such problems as we encountered in this case that the reaction order analysis was developed in the first place.[3]

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Studies on the Occurrence of Hydrogen Transfer. 65.* The Significance of Solvents and Solvent Mixtures in the Reductive Cleavage of Compounds of the Type $ArSO_2Y$ (Y=OR, Ar', Cl) with Alkali Amalgams

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The dependence of the yields of the reductive fission of hexyl benzenesulfonate and mesitylphenyl sulfone with alkali-metal amalgams (primarily lithium amalgam) was studied under standard conditions in toluene using a series of eight aprotic and six protic solvents as co-solvents. Yields about 5 % were measured in toluene, but addition of equimolar amounts (w.r.t. ester or sulfone) of the alcohols methanol, ethanol, isopropyl alcohol or tert-butyl alcohol as co-solvent raised the yields to 80–100 % (Fig. 1).

In toluene, portion-wise addition of substoichiometric amounts (w.r.t. ester) of the co-solvents DMF, THF, dioxane and isopropyl alcohol resulted in the values recorded in Fig. 2. The observed effects are attributed to the different solubilities of Li^+-solutions (of unknown composition) in toluene.

A reaction mechanism for the reductive fission of $ArSO_2Y$ (Y=OR, Ar', Cl) with alkali-metal amalgams is proposed.

In the 64th communication of this series we described a surprisingly high influence of the reaction medium on the cleavage of some arylsulfonyl chlorides and -hexyl esters and diarylsulfones ($ArSO_2Y$; Y=Cl, OC_6H_{13}, Ar') with alkali amalgams (M/Hg; M=Li, Na, K), yielding arylsulfinates $ArSO_2M$ and MY. In toluene the reaction (1) is nearly totally suppressed using lithium amalgam. However, the addition of an amount of isopropyl alcohol equimolar to $ArSO_2Y$ leads to a yield of more than 90 %.

In this context the influence of the following solvents on the yield of $ArSO_2M$ and YH was investigated with and without admixture of isopropyl alcohol in amounts equivalent to $ArSO_2Y$: diethyl ether, tetrahydrofuran (THF), acetonitrile (ACN), dioxane and *N,N*-dimethylformamide (DMF). The behaviour of diethylether, THF and ACN was similar to toluene. Higher yields were observed using dioxane. Nevertheless DMF is the best solvent overall; the "normal" reductive cleavage (1) and also the "anomalous" reductive cleavage (2) of sterically hindered $ArSO_2Y$ proceed with yields greater than 90 %.

The aim of this paper was to obtain a better understanding of the factors influencing the behaviour of good and bad solvents and co-

$$\underset{\text{Amalgam}}{ArSO_2Y + [2\ M^{1+}/Hg/+2e]} \longrightarrow ArSO_2M + YM + [Hg] \qquad (1)$$
$$\downarrow HX$$
$$YH + MX$$

Scheme 1. Y=Cl, OR, AR'.

* Part 64, see Ref. 1.
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$$\underset{\text{Amalgam}}{ArSO_2Y+[2\ M^+/Hg/+2e]} \longrightarrow \begin{array}{ll}[Ar|]^{(-)}+[|SO_2Y]^- & +2\ M^+ +[Hg] \\ \downarrow H^+ & \downarrow H^+ \\ ArH & [HSO_2OR] \longrightarrow HOR+SO_2\end{array} \qquad (2)$$

Scheme 2. Y=OR, Ar[1].

solvents in the reductive cleavage of $ArSO_2Y$ by alkali amalgams.

The influence of the reaction media and the co-solvents on the reductive cleavage of arylsulfonyl hexyl ester and diarylsulfones with alkali amalgams. Hexyl benzenesulfonate is cleaved to benzenesulfinate and hexanol according to Scheme 1 under standard conditions [1] using the following solvents without (and with) isopropyl alcohol as a co-solvent (the yields of hexanol and sulfinates are, in general, equal). Toluene 5(90); diethyl ether 7(91); THF 86(98); dioxane 97(98); ACN 65(89); ACN 65(89); DMF 95(98). The following yields were obtained in the following solvents without (and with) isopropyl alcohol starting with hexyl-4-methoxybenzenesulfonate which possesses a more negative reduction potential:[4] Toluene 5(6); diethyl ether 5(5); THF 9(67); dioxane 41(88); ACN 5(7); DMF 90(98).

With the sterically hindered hexyl mesityl sulfonate and hexyl 2,4,6-tri-isopropylbenzene sulfonate, the reaction media influence the yields of the products obtained with lithium amalgam according to Scheme 1; on occasion the partition of the reactions (1) and (2) can be altered in favour of reaction (2).

Hexylmesitylsulfonate gave (in %): (Hex~ hexanol; Sulf~sulfinate; Mes~mesitylene; TiPB~1,3,5-triisopropylbenzene).

Toluene. (~5 (Hex), trace (Sulf), 5 (Mes);

Diethyl ether. 6 (Hex)~5 (Mes); *THF* 65 (Hex), 11 (Sulf), 26 (Mes);

Dioxane. 85 (Hex), 69 (Sulf), 10 (Mes); *ACN*: 7 (Hex), ~5 (Mes);

DMF. 99 (Hex), trace(Sulf), 85 (Mes).

Only in the case of dioxane was a shift favoring reaction (2) observed on addition of an equimolar amount of isopropyl alcohol 88 (Hex), 21 (Sulf), 61 (Mes).

For 2,4,6-tri-isopropylbenzenesulfonylhexyl ester the yields of the cleavage (2) are zero in toluene, diethyl ether and THF, and low in dioxane (7 % Hex; with the equimolar amount of isopropyl alcohol: 59 (Hex), 54 (TiPB)); in DMF however, the yield is practically quantitative!

Starting from sodium or potassium amalgam and hexyl benzyl sulfonate, the yields of the cleavage products are somewhat lower, following the more positive reduction potentials [1] of the sodium and potassium amalgams. The same strong influence of solvent and co-solvent exists for the reductive cleavage of benzene mesityl sulfone and dimesityl sulfone.

The influence of the pK_a-values of the co-solvents on the reductive cleavage of benzenesulfonyl hexyl ester and benzene mesityl sulfone with lithium amalgam according to (1). The reductive cleavage with lithium amalgam under standard conditions was investigated for a series in toluene, using hexyl benzenesulfonate and 14 solvents of different pK_a-values in the molar relation 1:1. The results are compiled in Fig. 1. (A similar dependence is found for the electroreductive cleavage of phenyl mesityl sulfone.

Co-solvents containing alcoholic functions and a pK_a-value of 14–18 (but not diethylmalonate or cyclopentadiene) strongly favour the reductive cleavage; it would be incorrect, however, to assume that the pK_a-value alone is responsible for this effect; thus also in the aprotic solvents THF, dioxane and especially DMF, practically quantitative yields are obtained. In our opinion the increase in yield is caused by undefined processes at the interface of the amalgams. The

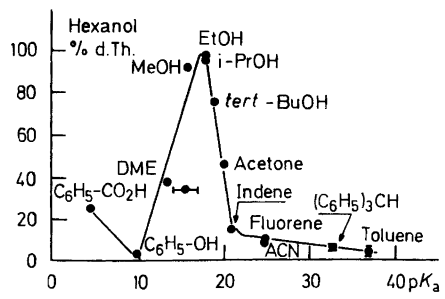


Fig. 1. Dependence of the reductive cleavage of $C_6H_5SO_2OC_6H_{13}$(n) with Li/Hg in toluene from the pK_a-values of the added proton donors. Plot of the pK_a-values *vs.* the yield of hexanol in %. MDE=malonicaciddiethylester.

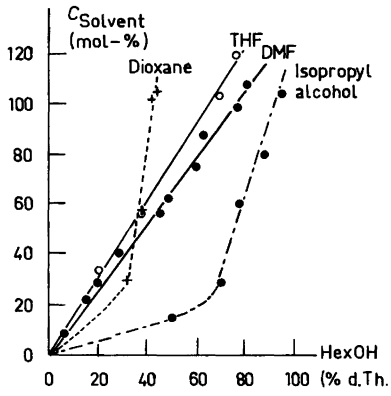


Fig. 2. Dependence of the reductive cleavage of $C_6H_5SO_2OC_6H_{13}$(n) (2.5 mmol) with Li/Hg (0.67 % w.w. Li=7.5 g =6 mmol Li) in toluene (5 ml) on the added amount of DMF, THF, dioxane and isopropyl alcohol; temperature 23 °C ±1; reaction time: 2 h. Co-solvents: added amount of DMF, THF, dioxane and isopropyl alcohol in mol % in relation to the mol number of the ester (2.5 mmol=100 %).

transfer of electrons from the amalgam to substrate will be favoured (a) by solvation of the substrate and (b) by increasing the solubility of the reaction products, which have to be removed from the surface of the amalgam. We assume that the effect (b) is more important than (a).

The influence of other co-solvents on the standardized reductive cleavage of benzenesulfonyl hexyl ester with lithium amalgam. Fig. 2 shows the results obtained for the reductive cleavage of n-hexyl-benzenesulfonate in the standardized experiment in toluene after stepwise addition of increasing amounts of co-solvents such as THF, DMF, dioxane and isopropyl alcohol (max. substrate co-solvent 1:1).

For the co-solvents THF and DMF a roughly linear relationship is observed between the formation of hexanol and the amount of added co-solvents. (20 mol percent co-solvent leads to the formation of 15–20 % hexanol; the yields of hexanol are 60–80 % if the relation substrate–co-solvent is 1:1. However the yield continues to increase on super stoichiometric addition of the co-solvent.)

Bearing in mind that the yields of the reductive cleavage of both arylsulfonylesters and diaryl sulfones depend in a parallel fashion on the nature and amount of the added co-solvent, we conclude that the solubility (in toluene) of the lithium salts ($ArSO_2Li$ and LiY) formed at the surface of the amalgam is the predominant factor and not the solvation of the starting material. On this basis we assume that the aryl-sulfinate $ArSO_2Li$ and the co-solvent form a 1:1 adduct, which is soluble in toluene. In the case of dioxane as co-solvent, linearity ($ArSO_2Li$–dioxane 1:1) was observed only to the limit of 30 % dioxane; thereafter, increasing amounts of dioxane hindered the reductive cleavage corresponding to a solvation complex of a hypothetical composition: $ArSO_2Li$–dioxane $(1:2)_n$ which presumably has a low solubility in toluene and therefore will be accumulated at the surface of the amalgam.

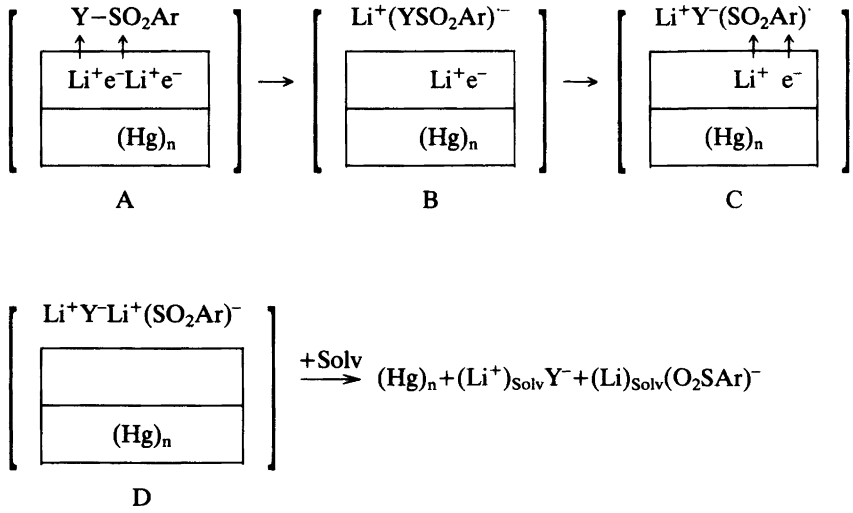
There are likewise two linear areas for isopropyl alcohol as a co-solvent. Addition of 10 mol percent of isopropyl alcohol leads to the formation of 40 mol percent of lithiumhexanolate (and $ArSO_2Li$). We assume that $ArSO_2Li$, lithiumhexanolate and isopropyl alcohol form a solvation complex with a high solubility in toluene. The steeper part of the curve seems to correspond to a second solvation complex of a different composition but also with a sufficient solubility in toluene.

For the interpretation of the solvation complex formation, we refer to the solvation concept development by Gutmann.[3] In this view the lithium ion is the electrophile, the oxygen function in the co-solvents, the nucleophile.

If the assumption is correct that the surface of the amalgam has to be freed by solution of the lithium salts formed, then isopropyl alcohol is the most efficient co-solvent. It is, however, too early to speculate on the composition of the solvation complex which seems to be a dynamic species.

Mechanistic considerations on the reductive cleavage at the interface of amalgams. The reductive processes at the surface of alkali amalgams on the one hand, and the normal electroreduction using a mercury cathode with supporting electrolytes and an electric field on the other, show many common features but also differences.

Common features. It is characteristic for both procedures that the electrons leave the mercury surface under over-voltage conditions and are transferred to the depolarizer (double bonds,[4] sulfonyl derivatives [4] and onium salts [4]). The potential of the mercury surface is constant,



Scheme 3. Solv=Solvents; Y=Cl,OC_6H_{13},Ar'.

similar to a potentiostatic electroreduction, with the exception of an insignificant endphase. Metal amalgams can substitute for an expensive potentiostat; but they have the disadvantage that the potential can only be varied in a limited manner by exchanging the metal in the amalgam.

Differences. (a) Parallel to the transfer of electrons to the depolarizor, an equimolar amount of cation leaves the surface of the amalgam and neutralizes the initial radical anion. This reaction is blocked if the solubility of the products formed at the mercury surface is low, as is observed for toluene as a solvent. By the addition of an equimolar amount of a co-solvent a quantitative yield can be obtained.

(b) In the electroreduction, a variable electric field is present between cathode and anode, in contrast to the amalgams. The electrochemical transfer of electrons requires the cooperation of a supporting electrolyte, which lowers the energy barrier between the electrode and the substrate. There exists a binding interaction between supporting electrolyte and radical anion (or the follow-up products), as could be checked experimentally by the application of optically active supporting electrolytes.[4]

(c) The reduction with amalgams can be performed in solvents with very low conductivity. An example is the reduction of carboxylic acid chlorides by alkali amalgams, which will be reported later and in another context.

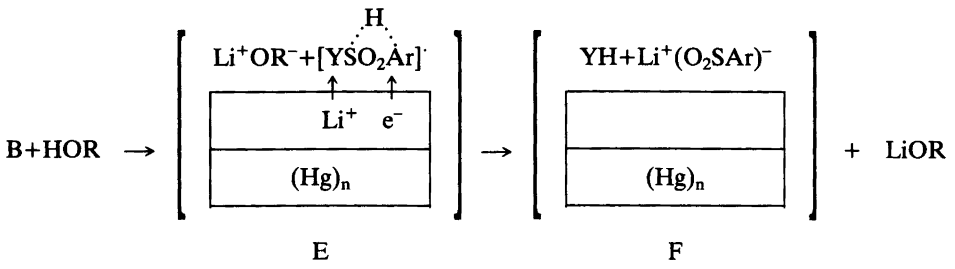
Mechanistic considerations of the reduction with amalgams in the example of the reductive cleavage of arylsulfonylesters and diarylsulfones. In analogy to our conclusions on the Clemmensen-reduction as a four electron process with zinc amalgam in mineral acids,[5] we propose the following reaction mechanism for the reductive cleavage with lithium amalgam (Li/Hg) (Scheme 3).

In toluene as reaction medium, the reaction products Li^+Y^- and $Li^+(O_2SAr)^-$ are transferred into solution by co-solvent with a high coordination activity, such as THF and DMF. Under these conditions the reduction yield will be quantitative.

In our opinion the protic co-solvents with pK_a-values of 14–18 (methanol, ethanol, isopropyl alcohol, see Fig. 1) interact (like THF and DMF) using their oxygen function as nucleophilic centre with Li^+ as an electrophile.

It is possible, however, that the radical anion B is protonated by these protic co-solvents favouring the transfer of the second electron on the one hand and removing the Li^+ from the mercury surface by solvation on the other hand.

It is well known that arylsulfonylchlorides are reduced quantitatively in an *acidic* medium forming the thiol. Using our procedure [2] (toluene as solvent and an equimolar amount of isopropyl alcohol or DMF) only lithiumsulfinate and LiCl are formed under standard conditions. Isopropyl



$$F+Solv \longrightarrow YH+(Li^+)_{Solv}(O_2SAr)^-+(Li^+)_{Solv}OR$$

Scheme 4. Solv=Solvents; Y=OC_6H_{13}, Ar'.

alcohol and DMF remove the cleavage products from the mercury surface by solvation. With DMF as solvent or co-solvent the reduction of *p*-toluenesulfonylchloride consumes more than 2 mols of lithium (available as Li/Hg) and simultaneously DMF is partially destroyed.

In summary. (a) The course of the reduction will be determined by the potentials of the amalgams and the substrates.

(b) Both the potential of the amalgam [6] and the reactivity of the substrate can be influenced by the solvent, which removes the chemisorbed cleavage products from the mercury surface by solvation.

(c) Using aprotic solvents in the amalgam reduction, a sequence of decreasing reductive power is observed following the rule of Gutmann [3] for the decreasing donor quality of the solvents: DMF>THF≥dioxane>ACN>diethylether>toluene.

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The Picolyl Group, an Electroactive Protection Group for Alcohols *

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The quality of the picolyl group as electroactive protecting group is being investigated. 4- (*1*) and 2-picolyl ethers (*2*) are formed in 63 to 83 % yield by a *Williamson* synthesis from butanol, decanol and the picolyl chlorides. With 1,4-pentanediol the chemoselectivity for the protection of the primary to the secondary hydroxyl group is 4.3 to 4.6:1. In *N,N*-dimethylformamide–1 % methanol *1* and *2* are cleaved at −2.5 to −2.7 V (s.c.e.) ** to yield 45–92 % butanol or decanol. In the acidic electrolyte, 0.5 M HBF_4-methanol, deprotection occurs already at −1.35 to −1.4 V in 70 % yield. By coelectrolysis of *1,2* and *p*-cyanobenzyl 2-ethylhexyl ether (*6*) in the acidic electrolyte exclusively *1,2* can be cleaved, while in neutral medium only *6* is selectively deprotected.

In organic synthesis the hydroxyl group is conveniently protected as an ether against elimination, substitution or oxidation. The ether is frequently a better protecting group than the ester because it is more stable towards acids and bases. Often benzyl ethers are used, which can be cleaved by hydrogenation, chemical or cathodic reduction.[2] The electrochemical cleavage at controlled potential is an attractive method for the selective deprotection of diols with different protecting groups. This has been demonstrated in the synthesis of a 1,3-dialkoxy lipid with the tritylone (=9,10-dihydro-10-oxo-9-phenyl-9-anthracenyl) and the *p*-cyanobenzyl residue as protecting groups.[3] In order to extend the number of electroactive protecting groups for potential selective deprotection we looked more closely at the picolyl group, which has already been used for the protection of carboxylic acids,[4] alcohols [5] and thiols.[6] As electron attracting groups facilitate the cathodic cleavage,[7] it should be reduced more easily than the benzyl group.

RESULTS

In the *Williamson* synthesis of picolyl ethers, two equivalents of an alkoxide are reacted with picolyl chloride hydrochloride,[8] as the free picolyl chloride is unstable. This reaction mode, however, is unsuitable for protective use, as at most 50 % of the alcohol are converted to the ether (eqn. (1)). To consume only one equivalent of alcohol, the picolyl chlorides were generated from their hydrochlorides just before use [9] and reacted with the stoichiometric amount of sodium alkoxide in dioxane/HMPA (Table 1). The phase transfer catalyzed [10] etherification that has been successfully applied in methylation [11,12] and benzylation [12,13] failed in this case. The two phase system, decanol in CH_2Cl_2/Bu_4NI and 2-picolyl chloride hydrochloride in 50 % aqueous NaOH, produced after 10 h of intense stirring only 25 % *2b*. An excess of alkylation reagent as described in Refs. 12 and 13 was not applied because of the instability of the picolyl chlorides against base.

The reduction potentials of the picolyl ethers were determined by differential pulse polarography [14] in 0.1 M Bu_4NClO_4–*N,N*-dimethylformamide (DMF). With −2.6, −2.73 V

* See Ref. 1.
** All potentials unless otherwise stated *vs.* s.c.e.

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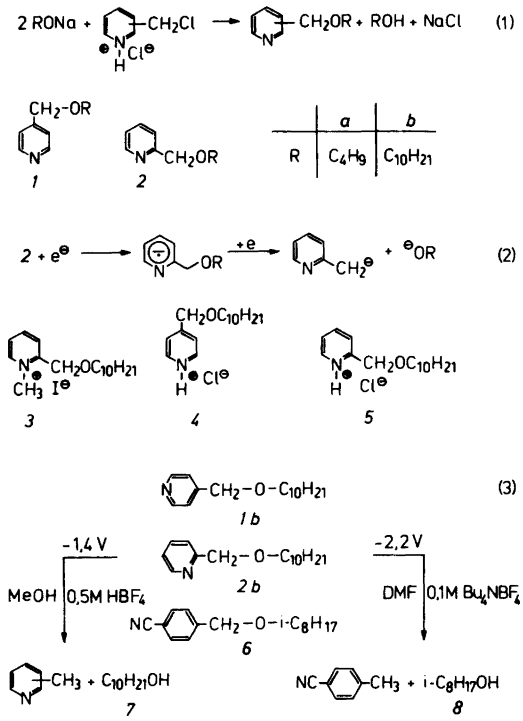


Table 1. Preparation of picolyl ethers.

Alcohol	Picolyl chloride	Picolyl ether	Yield (%)
Butanol	4–	*1a*	83
Butanol [a]	2–	*2a*	63
Decanol	4–	*1b*	70
Decanol	2–	*2b*	67

[a] Etherification with 2.5 equiv. of alcohol.

for *2a* and −2.54 V for *1a* they are fairly negative and range between the benzyl, −3.1 V,[15] and the *p*-cyanobenzyl group, −2.2 V.[3] The preparative cathodic reduction at −2.7 V in 0.1 M Bu_4NClO_4–DMF cleaved *2a* to 38 % and *1a* to 60 % butanol. As reaction path the cleavage of *1* or *2* to an alkoxide and a picolyl anion *via* a picolyl anion radical is assumed (eqn. (2)).[15] The moderate yields in butanol could be due to a decomposition of *1* or *2* by the strongly basic picolyl anion. To suppress such a possible side reaction, methanol was added as proton donor. Indeed in 0.1 M Bu_4NClO_4–DMF–1 % metha-

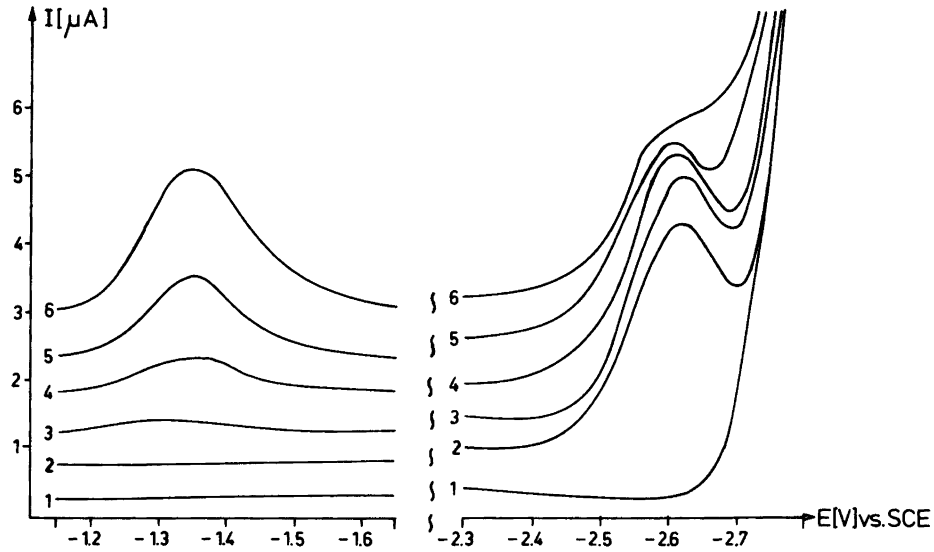


Fig. 1. Polarography of *1b* in 0.1 M Bu_4NClO_4-acetonitrile. (1) electrolyte; (2) (1)+10^{-3} M *1b*; (3) (2)+0.5×10^{-3} M HBF_4; (4) (2)+1.0×10^{-3} M HBF_4; (5) (2)+2.0×10^{-3} M HBF_4; (6) (2)+3.0×10^{-3} M HBF_4.

nol the butanol yield rose for *1a* to 92 % and for *2a* to 45 %. *1b* afforded under the same conditions 77 % decanol, however, *2b* being more difficult to reduce yielded only 13 %, possibly due to a simultaneous decomposition of the electrolyte. Protecting groups with very negative cleavage potentials are inconvenient as they are restricted to substrates with unreducible functional groups. Therefore we tried to shift the reduction potential of the picolyl group to more anodic values.

The potential for the electron transfer to a substrate can be decreased by the use of a mediator [16] (*outer activation*).[17] As an example, butyl benzyl ether ($E_{1/2}$: −3.1 V) can be cleaved at −2.67 V with biphenyl as electrocatalyst.[18] *1a*, however, was not reducible with anthracene (−1.96 V) as mediator; in cyclic voltammetry the

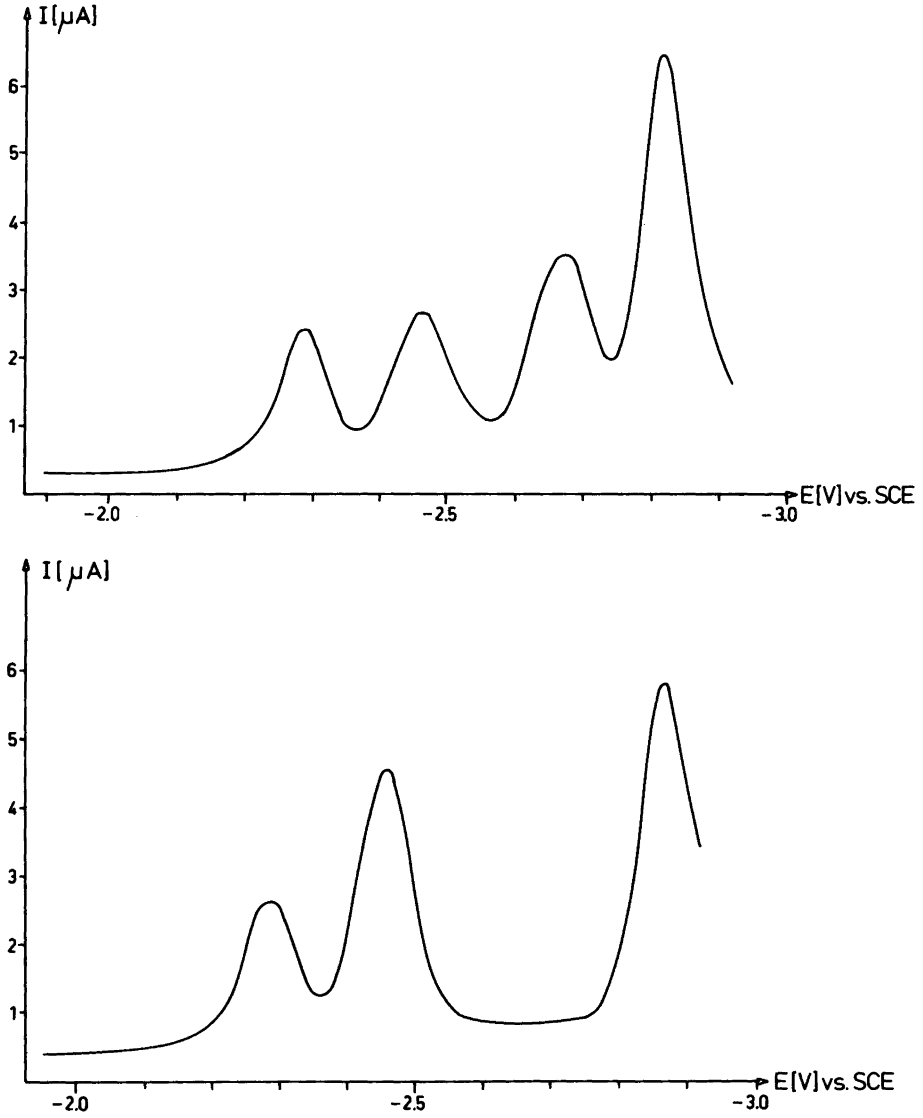


Fig. 2. Differential pulse polarograms of *1b*, *2b* and *6* (10^{-3} M) in 0.1 M Bu_4NBF_4−DMF; (a) *2b* and *6*; (b) *1b* and *6*.

$i_{p,a}$ and $i_{p,c}$ of antracene did not change on addition of *1a*.[16] Other mediators such as phenanthrene (−2.45 V), naphthalene (−2.49 V) and biphenyl (−2.67 V) were excluded because of their high reduction potentials. The reduction potential of a substrate can also be lowered by *inner activation*,[17] *e.g.* by extension of its conjugated system [$E_{1/2}$ (cinnamyl ether): −2.5 V, $E_{1/2}$ (benzyl ether): −3.1 V], or by electron attracting groups [$E_{1/2}$ (*p*-nitrobenzyl ether): −1.3 V]. In *1* and *2* the electron attracting ability of the nitrogen can be increased by quaternization. While pyridine is reduced at −2.61 V [19] the pyridinium salts have reduction potentials of −1.31 V [20] or −1.08 V.[21] The methiodide *3* ($E_{1/2}$: −1.22 V), prepared in 62 % yield from *2b*, is indeed reduced 1.45 V more anodically than *2b*. The preparative electrolysis in 0.1 M Bu_4NClO_4–methanol at −1.35 V, however, failed due to the formation of precipitates, the yield in decanol was only 5 %. The hydrochlorides *4* and *5*, prepared from *1b* and *2b* with dry HCl in ether, are reduced at $E_{1/2}$: −1.29 V (*4*) and −1.35 V (*5*); however, the preparative electrolysis of *5* in 0.1 M Bu_4NBF_4–acetonitrile at −1.35 V afforded no decanol.

With an acidic electrolyte it should be possible to protonate *1* and *2* and to reduce them at the potential of their hydrochlorides. In the polarography of *1b* as with pyridine [20,22] an increasing acidity of the electrolyte indeed decreased the current at −2.6 V and caused a new reduction peak to appear at −1.35 V (Fig. 1). The preparative electrolysis of *1b* or *2b* at −1.4 or −1.35 V in 0.5 M HBF_4–methanol afforded 72 or 70 % decanol. A control experiment secured that *1b* and *2b* are stable in the acidic electrolyte.

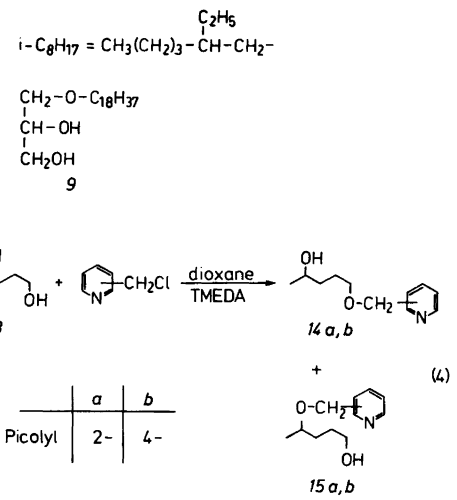
After the conditions had been worked out to prepare and to cleave the picolyl ethers in good yields, we studied how selectively the picolyl group can be cleaved in the presence of a second electroactive group and how chemoselectively picolyl ethers can be formed from diols. In a competition experiment the picolyl ethers (*1b*, *2b*) and the *p*-cyanobenzyl ether (*6*) were coelectrolyzed. In a neutral electrolyte at −2.2 V only *6* should be cleaved, while reduction at −1.35 V in an acidic medium should cleave *1b* and *2b* but not *6*. *6* was prepared from sodium 2-ethyl-1-hexoxide and *p*-cyanobenzyl bromide in 86 % yield and is reduced at $E_{1/2}$: −2.3, −2.47 V in 0.1 M Bu_4NBF_4–DMF. As polarography revealed, the reduction of *6* did not influence that of *2b* (Fig. 2a) but that of *1b*, here the first peak of *1b* is shifted anodically and merges with the second one of *6* (Fig. 2b). Possibly *6* acts partly as a mediator for the reduction of *1b*. The results of the preparative coelectrolysis of *1b* and *6*, and *2b* and *6* (eqn. (3)) are summarized in Table 2.

Table 2. Potential selective cleavage of the picolyl- or the *p*-cyanobenzyl group.

Ether	Yield of alcohol
−1.4 V, 0.5 M HBF_4–MeOH;	
1b+*6* (1:1)	70 % *7*
	0 % *8*
2b+*6* (1:1)	83 % *7*
	0 % *8*
−2.2 V, 0.1 M Bu_4NBF_4–DMF;	
1b+*6* (1:1)	10 % *7*
	74 % *8*
2b+*6* (1:1)	0 % *7*
	57 % *8*

Table 2 demonstrates that in acidic medium the selective cleavage of the picolyl group besides the *p*-cyanobenzyl group is indeed possible. In a neutral electrolyte the *p*-cyanobenzyl ether can be deprotected selectively in the presence of a 2-picolyl ether, while with the 4-picolyl ether the selectivity is not quite so good. The latter is in accordance with the smaller difference in the reduction potentials for *6* and *1b*: ΔE=0.18 compared to that of *6* and *2b*: ΔE=0.39 V.

The chemoselectivity in the reaction with a primary or secondary hydroxyl group was tested with *9* [23] as substrate. After reaction of *9* with NaH or Na and subsequent treatment with 2-picolyl chloride in dioxane/HMPA 55 % of the 1-*O*-octadecyl-2,3-*O*-di(2-picolyl)-glycerol (*10*) and only 25 % of the wanted monoprotected product as a mixture of 10 % 1-*O*-octadecyl-2-*O*-(2-picolyl)-glycerol (*11*) and 15 % 1-*O*-octadecyl-3-*O*-(2-picolyl)-glycerol (*12*) were obtained. As the selectivity for the benzylation of 1,3-butanediol is described as good,[24] we also increased the distance between the two hydroxy groups. Indeed 1,4-pentanediol (*13*) prepared from ethyl levulinate by reduction with $LiAlH_4$ according to Ref. 25 yielded 67 % 1-(2-picolyloxy)-pentan-4-ol (*14a*) and 16 % 4-(2-picolyl-



oxy)-pentan-1-ol (*15a*) after reaction with sodium in dioxane/TMEDA and treatment with 2-picolyl chloride (eqn. (4)). In the same way, treatment with 4-picolyl chloride yielded 54 % 1-(4-picolyloxy)-pentan-4-ol (*14b*) and 12 % 4-(4-picolyloxy)-pentan-1-ol (*15b*). The chemoselectivity for the primary to the secondary hydroxyl group is under these conditions 4.3–4.6:1.

The picolyl group thus turned out to be a quite useful electroactive protecting group. Picolyl ethers can be prepared in good yields, whereby a primary hydroxyl group can chemoselectively be protected. The ethers are stable towards acids and bases and can be cleaved potential selectively either at −1.4 V in an acidic electrolyte or at −2.6 V in neutral medium.

EXPERIMENTAL

IR spectra were recorded on the Perkin-Elmer instruments 298 and 421. ^{1}H NMR spectra were obtained with a Varian HA 100 and a Bruker WM 300 spectrometer, using TMS as an internal standard. Mass spectra were produced by the Varian MAT 111, SM 1 and CH 7 spectrometers.

Gas and liquid chromatography. For gas chromatography a Varian 1440 instrument coupled to the Spectra Physics integrator Minigrator Autolab was used together with the following glass columns: Column A: Ø 2 mm, 1.7 m, 10 % FFAP on chromosorb WAW DMCS; column B: same as A but 4 % FFAP; column C: same as A but 4 % SE 30; column D: same as A but 4 % OV 225. Nitrogen was used as carrier gas. Gas chromatographic yields were determined according to Ref. 26 with hydrocarbons from Merck as standard. For liquid chromatography silica gel (0.063–0.2 mm) Merck was used. Medium pressure liquid chromatography was performed in a Pharmacia chromatography tube, combined with a pump Type SC 1 with pulsation damper Duramat from C+G, Heidelberg, and an Isco fraction collector Modell 238. HPLC was carried out with a Lewa-pump, type HU 1 and a steel-column, length 50 cm, internal diameter 1.6 cm from Knaur with silica gel 7 μm (Merck). Analytical TLC was done on TLC-aluminium foils 60 F_{254} from Merck and polyethylene foils Polygram SiL G/UV_{254} from Macherey-Nagel.

Electrochemistry. Cyclic voltammogramms were recorded by a Wenking 68 Fr 0.5 potentiostat, combined with a Wavetek function generator 133 and a Hewlett-Packard XY-recorder Typ 7045 A (voltage scan: 40 mV/s) in a Methrom EA 876 vessel with a glassy carbon (Ø 5 mm) cathode and a platinum-foil (Ø 5 mm) anode. Polarogramms were obtained with a Bruker polarograph 310 with a Methrom drop controller E 354 S and a vessel EA 876. Reference electrodes were the cadmium/amalgam (Marple) [27] and the saturated calomel electrode. Preparative electrolyses were done with a Wenking potentiostat (3 A, 60 V) and a dc-integrator (construction: Dr. H. Luftmann, Univ. Münster). The temperature was regulated with a Lauda TK 30 D thermostat to 20 °C. An undivided cylindrical cell A (150 ml, water jacket) with three electrodes: mercury pool (Ø 5 cm), platinum foil (Ø 2 cm), Marple or Ag/Ag^+-reference electrode, and a divided cell B (as A, but with a G4 glass frit) were used. All electrolyses were done under nitrogen.

Solvents were distilled and if necessary dried.[28] DMF was stirred for two days over P_2O_5, distilled at 55 °C/20 Torr under nitrogen, and stored over molecular sieves (4 A) in the refrigerator. Methanol was used in *p.a.* quality (Merck). Diisopropyl ether was purified by column filtration on basic aluminium oxide activity I (Woelm). Tetrabutylammonium perchlorate (Bu_4NClO_4) was prepared from Bu_4NHSO_4 and sodium perchlorate, doubly crystallized from water and vacuum dried at 100 °C. Similarly Bu_4NBF_4 was obtained from $NaBF_4$.

4- and 2-picolyl butyl ether (1a *and* 2a). 0.46 g (20 mmol) sodium are dissolved at 80 °C under nitrogen in 10 ml butanol, after 1 h the excess butanol is distilled off at 15 Torr and 30 ml dioxane–HMPA (1:1) are added to the white solid. 3.28 g (20 mmol) 4-picolyl chloride hydrochloride are dissolved in 40 ml water, 8 N potassium hydroxide is added to the solution

until the colour changed to rose. The ether extract (3×30 ml, dried on $MgSO_4$) is added at 40 °C within 15 min to the alkoxide, then stirring is continued at room temperature overnight. For work-up most of the solvent is distilled off, 40 ml water are added, extracted with ether (5×20 ml), dried ($MgSO_4$) and distilled at 50 °C/0.01 Torr to yield 2.74 g (83 %) *1a*.

1a: IR (film): 3080–2860, 1600–1580, 1110, 800 cm^{-1}. ^1H NMR (CCl_4): δ 1.0 (3H, t), 1.6 (4H, m), 3.5 (2H, t), 4.5 (2H, s), 7.2 (2H, d), 8.6 (2H, d). MS [*m/e* (% rel. int.)]: 165 (1, M), 122 (5), 108 (10), 93 (100), 92 (99). Anal. $C_{10}H_{15}NO$: C, H, N.

2a: B.p. 49 °C, 0.1 Torr. IR (film): 3080–2860, 1600–1500, 770 cm^{-1}. ^1H NMR (CCl_4): δ 1.0 (3H, s), 1.6 (4H, m), 3.6 (2H, t), 4.6 (2H, s), 7.1–8.5 (4H, m). MS [*m/e* (% rel. int.)]: 122 (2), 108 (10), 93 (100), 92 (35). Anal. $C_{10}H_{15}NO$: C, H, N.

*4- and 2-picolyl decyl ether (*1b *and* 2b*)*. To 1.8 g (75 mmol) sodium hydride in 100 ml dioxane 8.14 g (50 mmol) decanol in dioxane are added at 80 °C under nitrogen and with stirring, stirring is continued for 3 h and then at room temperature overnight. 7.5 g (59 mmol) 4-picolyl chloride, prepared before use as above, in dioxane are added dropwise to the alkoxide. Thereafter 15 ml HMPA are added and stirred overnight at room temperature. For work-up 100 ml water are added, extracted with ether (4×50 ml), dried ($MgSO_4$), distilled at 0.01 Torr and filtered through silica gel (ethyl acetate) to yield 8.9 g (70 %) *1b*. Analogous 8.35 g (67 %) *2b* are obtained.

1b: B.p. 139–141 °C/0.01 Torr. IR (film): 3050–2840, 1600–1500, 1100, 780 cm^{-1}. ^1H NMR (CCl_4): δ 0.9 (3H, t), 1.3 (14H, m), 1.6 (2H, q), 3.3 (2H, t), 4.3 (2H, s), 7.0 (2H, d), 8.5 (2H, d). MS [*m/e* (% rel. int.)] 249 (3, M), 234, 220, 206, 102, 178, 164 (all 1), 150 (95), 108 (100), 93 (50). Anal. $C_{16}H_{27}NO$: C, H, N.

2b: IR (film): 3050–2800, 1560–1580, 1120, 750 cm^{-1}. ^1H NMR (CCl_4): δ 0.9 (3H, t), 1.3 (14H, m), 1.6 (2H, q), 3.4 (2H, t), 4.5 (2H, s), 7.0–8.4 (4H, m). MS [*m/e* (% rel. int.)]: 108 (18), 93 (100). Anal. $C_{16}H_{27}NO$: C, H, N.

Cathodic cleavage of *1* and *2* in a neutral electrolyte

General conditions. 2.5 to 5 mmol *1, 2* are dissolved in the electrolyte and electrolyzed at the polarographically determined reduction potentials with initial currents of 100 to 150 mA in cell A until 3 to 4 F/mol had been consumed. Work-up (I) for butanol: The electrolyte is distilled at a maximum temperature of 40 °C under reduced pressure and cooling with liquid nitrogen to separate DMF and butanol from the supporting electrolyte. Work-up (II) for decanol: Water is added to the electrolyte, then extracted with ether (1×100 ml, 3×50 ml), the ether extracts are washed with 2 N H_2SO_4, water and thereafter dried ($MgSO_4$).

Electrolysis of 1a. 0.83 g (5 mmol) *1a* in 50 ml 0.1 M Bu_4NClO_4–DMF are electrolyzed until 1127 As are consumed. After work-up (I) the amount of butanol was quantitatively determined by GLC on column A (100 °C/iso.).

Electrolysis of 1b. 1.25 g (5 mmol) *1b* in 80 ml 0.1 M Bu_4NClO_4–DMF–5 % methanol are electrolyzed until 1200 As were consumed. After work-up (II) decanol is quantitatively determined by GLC on column C (140 °C, iso.).

Cathodic behaviour of *3*, *4* and *5*

Preparation of *3*. 0.63 g (2.5 mmol) *2b* are stirred for 3 days with 0.71 g (5 mmol) methyl iodide in 5 ml diisopropyl ether. Crystals, precipitating during the reaction, were collected and new methyl iodide added. 0.61 g (62 %) *N*-methyl-2-decyloxymethylpyridinium iodide (*3*) were thus obtained.

3: Yellow plates. M.p. (ethyl acetate) 92 °C. IR (KBr) 2920–2850, 1625–1455, 1120, 770 cm^{-1}. ^1H NMR (acetone-d_6): δ 0.9 (3H, t), 1.3 (14H, m), 1.7 (2H, q), 3.7 (2H, t), 4.6 (3H, s), 5.2 (2H, s), 8.1–9.5 (4H, m). MS [*m/e* (% rel. int.)]: 264 (1), 249 (1), 142 (54), 127 (43), 122 (54), 108 (49), 93 (100). Anal. $C_{17}H_{30}NOI$: C, H, N.

Preparative electrolysis of 3. 0.587 g (1.5 mmol) *3* in 50 ml 0.1 M Bu_4NClO_4-methanol are electrolyzed in cell B at 0 °C and −1.22 V. After short electrolysis a yellow-grey precipitate deposited and the initial current of 100 mA dropped to 0 mA, which terminated the electrolysis.

Preparation of 4 *and* 5. When dry HCl gas is bubbled into a solution of 0.63 g (2.5 mmol) *1b* or *2b* in 20 ml ether, *4* and *5* precipitate as yellow crystals.

4: Yield: 93 %. M.p. 85 °C (subl.). IR (KBr): 3070, 2850–2920, 1630–1500, 1110, 785 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t), 1.1–1.5 (14H, m), 1.7 (2H, q), 3.7 (2H, t), 4.8 (2H, s), 8.0 (2H, d), 8.8 (2H, d). MS [*m/e* (% rel. int.)]: 250 (69), 249 (100), 108 (94). Anal. $C_{16}H_{28}NOCl$: C, H, N.

5: Yield: 97 %. M.p. 65 °C (subl.). IR (KBr): 3020, 2920–2850, 2500, 1600, 1160, 770 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t), 1.1–1.5 (14H, m), 1.7 (2H, q), 3.7 (2H, t), 5.1 (2H, s), 7.8–8.8 (4H, m). MS [*m/e* (% rel. int.)]: 250 (39), 108 (100).

Anal. $C_{16}H_{28}NOCl$: C, H, N.

Preparative electrolysis of 5. 0.571 g (2 mmol) *5* in 60 ml 0.1 M Bu_4NBF_4-acetonitrile are electrolyzed in cell A at −1.8 V (Ag/Ag^+) with 10 mA for 30 h until 470 As had been consumed. In the electrolyte neither decanol, nor *5* or *2b* could be detected by GLC.

Cathodic cleavage of *1*, *2* in an acidic electrolyte

Preparative electrolysis of 2b. 1.25 g (5 mmol) *2b* dissolved in 100 ml 0.5 M HBF_4-methanol are electrolyzed in cell A at −1.4 V until *2b* could be detected no longer by TLC (CH_2Cl_2: CH_3CO_2Et, 20:1) or GLC (column C, 50 °C, 8 °C/min). The electrolyte is then diluted with water, extracted with ether (3×75 ml), the ether extracts are neutralized ($NaHCO_3$) and dried ($MgSO_4$). Decanol is quantitatively determined (yield: 70 %) by GLC (column C, 120 °C, iso) and isolated (64 %) by bulb-to-bulb distillation. Similarly from *1b* 72 % decanol were found by GLC and 65 % isolated by bulb-to-bulb distillation.

Chemical stability of 2b *in the acidic electrolyte.* 0.25 g (1 mmol) *2b* are stirred for 2 days in 0.5 M HBF_4-methanol at room temperature. After the usual work-up only *2b* could be detected by GLC and TLC.

Selective cathodic cleavage of *1b*, *2b* and *6*

Preparation of 6. To 0.8 g (33 mmol) NaH in 20 ml dioxane are added dropwise at 80 °C under stirring 3.2 g (25 mmol) 2-ethyl-1-hexanol in 10 ml dioxane. After a further 3 h at 80 °C, 4.75 g (25 mmol) *p*-cyanobenzyl bromide in 20 ml dioxane are added, stirring is continued for 1 h at 80 °C and then for 12 h at room temperature. For work-up 50 ml water are added, the organic layer is separated and the aqueous layer extracted with ether (3×30 ml). The combined organic layers are dried ($MgSO_4$), the ether evaporated and the residue purified by column chromatography (silica gel, CH_2Cl_2) to 5.24 g (86 %) *p*-cyanobenzyl 2-ethylhexyl ether (*6*).

6: IR (film): 3010, 2220, 1600–1500, 1100, 820 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (6H, t), 1.5–1.1 (9H, m), 3.1 (2H, d), 4.1 (2H, s), 6.9 (2H, d), 7.1 (2H, d). MS [*m/e* (% rel. int.)]: 245 (1, M), 117 (100). Anal. $C_{16}H_{23}NO$: C, H, N.

Preparative electrolysis

6 and 2b *in a neutral electrolyte.* 3 mmol *6* and 3 mmol *2b* dissolved in 60 ml 0.1 M Bu_4NBF_4–DMF are electrolyzed in cell B at −1.38 V (Marple) with an initial current of 100 mA. After 615 As had been consumed, 50 ml water were added then extracted with ether (1×50 ml, 5×30 ml) and the ether extracts dried ($MgSO_4$). 2-Ethyl-1-hexanol, *p*-tolunitrile and *2b* are quantitatively determined by GLC (column B, 70 °C, 8 °C/min). Decanol could not be detected. Similarly *6* and *1b* were coelectrolyzed.

6 and 2b *in an acidic electrolyte.* 3 mmol *2b* and 3 mmol *6* dissolved in 60 ml 0.5 M HBF_4-methanol are electrolyzed in cell B at −1.35 V (Ag/Ag^+) until by GLC and TLC *2b* was no longer detectable. For work-up water was added, then extracted with ether, the ether extracts washed with $NaHCO_3$, water and dried ($MgSO_4$). Decanol and *6* were quantitatively determined by GLC (column B, 8 °C/min). 2-Ethyl-1-hexanol and *p*-tolunitrile could not be detected. Similarly *6* and *1b* were coelectrolyzed.

Protection of 9.[23] To 0.13 g (5.41 mmol) sodium hydride in 30 ml dioxane, 1.86 g (5.41 mmol) *9* are added at 80 °C under nitrogen and with stirring, which is continued for 2 h. Then 0.69 g (4.75 mmol) 2-picolyl chloride in dioxane are added dropwise to the alkoxide.

With 10 ml HMPA stirring is continued overnight at room temperature. For work-up 50 ml water are added, extracted with ether (5×30 ml), washed with saturated NaCl-solution (2×30 ml), dried ($MgSO_4$) and evaporated. Separation with HPLC (Si 60, p=45 bar, CH_2Cl_2–ethyl acetate–acetone 3:3:1) yielded 1.33 g (55 %) 1-*O*-octadecyl-2,3-*O*-di-(2-picolyl)-glycerol (*10*); 0.3 g (15 %) 1-*O*-octadecyl-3-*O*-(2-picolyl)-glycerol (*11*) and 0.2 g (10 %) 1-*O*-octadecyl-2-*O*-(2-picolyl)-glycerol (*12*).

10: IR (film): 3100, 3000–2800, 1580, 1520, 1100, 730 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.8 (3H, t), 1.3 (30H, m), 1.6 (2H, m), 3.5 (2H, t), 3.6–3.9 (5H, m), 4.6 (2H, s), 4.9 (2H, s), 7.1–8.5 (8H, m). MS [*m/e* (% rel. int.)]: 526 (2, M), 434 (43), 418 (6), 404 (16), 243 (15), 93 (100). Anal. $C_{33}H_{54}N_2O_3$: C, H, N.

11: IR (film): 3400, 3100, 2820–2900, 1580, 1460, 1100, 760 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t), 1.1–1.3 (30H, m), 1.6 (2H, m), 3.4 (2H, t), 3.5–3.8 (6H, m), 4.8 (2H, s), 7.2–8.5 (4H, m). MS [*m/e* (% rel. int.)]: 436 (0.5, M), 184 (0.8), 152 (35), 93 (100). Anal. $C_{27}H_{49}NO_3$: C, H, N.

12: IR (film): 3500–3100, 2850–3000, 1600, 1100, 750 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.7 (3H, t), 1.1–1.3 (30H, m), 1.6 (2H, m), 3.4 (2H, t), 3.5–3.8 (6H, m), 4.7–5.0 (2H, m), 7.2–8.6 (4H, m). MS [*m/e* (% rel. int.)]: 436 (1, M), 184 (0.7), 152 (18), 93 (100). Anal. $C_{27}H_{49}NO_3$: C, H, N.

Selective protection of 13 *with 2-picolyl chlo-*

ride: 1.0 g (9.6 mmol) *13* [25] and 0.221 g (9.6 mmol) sodium are heated under reflux in 15 ml dioxane under nitrogen and with stirring until the reaction was complete. After cooling, 1.22 g (9.6 mmol) 2-picolyl chloride and 1 ml TMEDA are added and stirring is continued overnight. For work-up 30 ml water are added, the aqueous layer is extracted with ether (3×30 ml), the ether layers are washed with saturated NaCl-solution (2×15 ml) and dried ($MgSO_4$). The yields and product-ratios were determined by GLC (column D): 1.27 g (67 %) 1-(2-picolyloxy)-pentan-4-ol (*14a*), 0.28 g (16 %) 4-(2-picolyloxy)-pentan-1-ol (*15a*). The reaction mixture was separated by HPLC (Si 60, 7 μm, p=45 bar, CH_2Cl_2/MeOH 9:1).

14a: IR (film): 3380, 2800–2960, 1740, 1360–1425, 1100, 750 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.1 (3H, d), 1.5–1.8 (4H, m), 3.2 (1H, s), 3.6 (2H, t), 3.8 (1H, sext.), 4.6 (2H, s), 7.1–8.6 (4H, m). MS [*m/e* (% rel. int.)]: 196 (<1, M), 122 (3), 108 (30), 93 (100). Anal. $C_{11}H_{17}NO_2$: C, H, N.

15a: IR (film): 3380, 2800–2960, 1740, 1560–1425, 1100, 750 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.2 (3H, d), 1.5–1.8 (4H, m), 3.2 (1H, s), 3.6 (2H, t), 3.8 (1H, sext.), 4.6 (2H, m), 7.1–8.6 (4H, m). MS [*m/e* (% rel. int.)]: 196 (<1, M), 165 (1), 131 (15), 108 (31) 92 (100). Anal. $C_{11}H_{17}NO_2$: C, H, N.

Selective protection of 13 *with 4-picolyl chloride.* The amounts and procedures are the same as above. The yields were 1.00 g (54 %) 1-(4-picolyloxy)-pentan-4-ol (*14b*) and 0.21 g (12 %) 4-(4-picolyloxy)-pentan-1-ol (*15b*).

14b: IR (film): 3400, 2850–2900, 1630, 1570–1430, 1120, 820 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.2 (3H, d), 1.5–1.9 (4H, m), 3.2 (1H, s), 3.4 (2H, t), 3.9 (1H, sext.), 4.5 (2H, s), 7.2 (2H, s), 8.6 (2H, s). MS [*m/e* (% rel. int.)]: 196 (<1, M), 110 (15), 108 (40), 92 (100). Anal. $C_{11}H_{17}NO_2$: C, H, N.

15b: IR (film): 3400, 2850–2900, 1630–1430, 1120, 820 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.2 (3H, d), 1.5–1.9 (4H, m), 3.2 (1H, s), 3.4 (2H, t), 3.9 (1H, sext.), 4.5 (2H, m), 7.2 (2H, s), 8.6 (2H, s). MS [*m/e* (% rel. int.)]: 196 (<1, M), 165 (<1), 110 (10), 108 (30), 92 (100). Anal. $C_{11}H_{17}NO_2$: C, H, N.

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Electrochemical Reactions. Part 26.* Radicals Derived by Reduction of *N*-Alkylpyridinium Salts and Homologous *N,N'*-Polymethylenebispyridinium Salts. Cleavage of the Carbon–Nitrogen Bond

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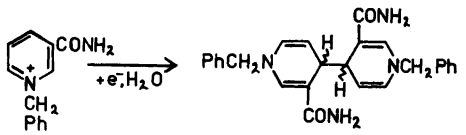
The stability of the radical zwitterions derived by electron addition to *N*-alkyl-2,4,6-triphenyl- and 4-phenylpyridinium salts has been examined. Rapid carbon–nitrogen bond cleavage occurs for benzyl and allyl substituents only when the 2,6-diphenyl substituents are present. The *N*-propyl group is not lost. Homologous *N,N'*-polymethylenebis(2,4,6-triphenylpyridinium) salts show two reversible one electron waves, distinct for the ethylene derivative and merging as the carbon chain lengthens so that ΔE° reaches the theoretical value of (RT/F) ln 4.

The reduction of 1-alkylpyridinium salts in aqueous media either electrochemically or by means of dissolving metals, gives a radical which dimerizes through the 4-position.[1] The resulting mixture of *meso*- and (±)-isomers has in some cases been separated and the individual isomers characterised. We could anticipate a competing reaction of cleavage of the carbon–nitrogen bond with loss of the alkyl group as a radical, but so far as we are aware this second reaction has not been observed and, for example, reduction of 1-benzylpyridinium [2] and 1-benzyl-3-carbamoylpyridinium [3] (*1*) in aqueous media leads to dimer formation even though benzyl is a group likely to be lost as the corresponding radical.

Katritzky [4] has recently documented several useful reactions of 1-alkylpyridinium salts where



1

the alkyl group is transferred to an attacking nucleophile. It has been suggested that some of these reactions could proceed by electron transfer from the nucleophile to the pyridinium ion followed by alkyl–nitrogen bond cleavage with combination of radical species to form the observed product.[5] As a contribution to this problem, we have looked for evidence of carbon–nitrogen bond cleavage on reduction of pyridinium salts.

1-Methylpyridinium salts with electron-withdrawing substituents such as 4-carboxy, 4-ethoxycarbonyl or 4-cyano form radical-zwitterions on electrochemical reduction in aprotic solvents, stable on the timescale of cyclic voltammetry, by addition of one electron to the lowest π-antibonding orbital.[6] From a survey of the influence of substituents on the stability of pyridinium radical-zwitterions, it should be possible to detect a reaction to yield the alkyl radical and the pyridine, if such a reaction exists. The phenyl rings in 1-alkyl-2,4,6-triphenylpyridinium salts also stabilise the one electron addition product and such salts were also favoured by Katritzky and coworkers for the substitution process, so we

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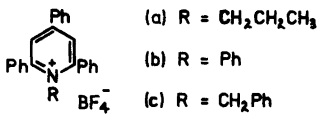
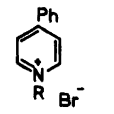


(a) R = $CH_2CH_2CH_3$
(b) R = Ph
(c) R = CH_2Ph
(d) R = $CH_2CH{=}CH_2$

2



(a) R = $CH_2CH_2CH_3$
(b) R = CH_2Ph

3

began with an examination of this series of compounds. Russian workers have examined the polarography of such salts.[7]

Compound *2a* is a typical *N*-alkyl derivative which shows two well-separated one electron waves on cyclic voltammetry in dimethyl formamide (see Table 1). The first wave is reversible at slow sweep rates and is due to formation of the radical-zwitterion. The second wave is irreversible under our conditions. Compound *2b* shows two overlapping one electron waves and only the first cathodic peak shows a corresponding anodic peak. The potential of the first electron wave is almost the same for compounds *2a* and *2b* but the second electron wave is moved to more positive potentials on replacing the *N*-alkyl by an *N*-phenyl group.

In contrast, neither the 1-benzyl compound *2c* nor the 1-allyl derivative *2d* show reversible behaviour on cyclic voltammetry under the same conditions as before or at sweep rates up to 0.5 V s^{-1}. An irreversible wave corresponding in height to a one electron process is seen at a potential similar to that of the reversible one electron wave of *2a* and other irreversible reactions occur at more negative potentials.

The simplest explanation for these observations is that radical-zwitterions derived from the *N*-benzyl and *N*-allyl compounds *2c* and *2d* undergo rapid homolytic carbon–nitrogen bond cleavage. Benzyl and allyl are good leaving groups in this process because the departing alkyl radical is stabilised by resonance. The other primary product is 2,4,6-triphenylpyridine.

A further question now arises. Does the combined steric crowding of the 2,6-diphenyl substituents contribute towards carbon–nitrogen bond cleavage? In order to answer this question, quaternary salts derived from 4-phenylpyridine were examined. 4-Phenyl-1-propylpyridinium salts show reversible behaviour for addition of the first electron at medium sweep rates in dimethyl formamide. The radical zwitterion has a shorter lifetime than that derived from *2a* and under our conditions $t_{1/2}$~4 s. At the same sweep rates 4-phenyl-1-propyl and 1-benzyl-4-phenylpyridinium salts show similar reversible behaviour for one electron addition on cyclic voltammetry (see Table 2). Thus carbon–nitrogen bond cleavage with loss of a benzyl radical is not observed for these compounds before the radical-zwitterion are destroyed by another process which is probably the dimerisation reaction.

The so far observed fast carbon–nitrogen bond cleavage reactions require a combination of assistance from 2,6-diphenyl substitution and a suitable leaving group such as benzyl or allyl which can stabilise the departing radical centre.

1,2-Dihalogenoalkanes are reduced in a two electron step to give an alkene and halide ions and similar reactions are known for other com-

Table 1. Cyclic voltammetry data for *N*-substituted 2,4,6-triphenylpyridinium salts (*2*) in *N,N*-dimethylformamide tetrapropylammonium fluoroborate (0.1 M), scan rate 0.125 V s^{-1}.

Compound *2* R	First electron wave, $-E_{pc}-E_{pa}-E^{\circ}$/ V *vs.* s.c.e.	Second electron wave $-E_{pc}$/V *vs.* s.c.e.
$CH_2CH_2CH_3$ [a]	0.96 0.90 0.93	1.52
Ph [b]	0.97 0.89 0.93	1.15
CH_2Ph [c]	0.92 – [e] –	1.25
$CH_2CH{=}CH_2$ [d]	0.90 – [e] –	–

[a] Ref. 15a. [b] Ref. 15b. [c] Ref. 15c. [d] Ref. 15d. [e] No anodic peak observable.

Table 2. Cyclic voltammetry data for *N*-substituted 4-phenylpyridinium salts (*3*) in *N,N*-dimethylformamide, tetrapropylammonium fluoroborate (0.1 M), scan rate 0.108 V s^{-1} .

Compound *3* R	First electron wave, V *vs.* s.c.e $-E_{pc}$	$-E_{pa}$	$-E^{\circ}$	i_{pa}/i_{pc}
$CH_2CH_2CH_3$	1.11	1.05	1.08	0.53
CH_2Ph	1.06	1.00	1.03	0.47

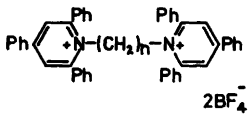
Table 3. Cyclic voltammetry data for the first two electron wave of *N,N'*-methylenebis (2,4,6-triphenylpyridinium) salts (*4*) in *N,N*-dimethylformamide, tetrapropylammonium fluoroborate (0.1 M), scan rate 25 mV s^{-1}.

Compound *4* *n*	Remarks	E_{pc}	E_{pa} V *vs.* s.c.e.	$E°$
2	Two overlapping waves	0.77, 0.88	0.72, 0.82	0.75, 0.85
3	Two overlapping waves	0.77, 0.95	0.72, 0.88	0.75, 0.92
4	One composite wave	0.94	0.86	0.90 [a]
5	One composite wave	0.96	0.90	0.93 [a]
6	One composite wave	0.96	0.90	0.93 [a]

[a] Observed $E°=(E_1°+E_2°)/2$, wave shape characteristic for $E_1°-E_2°=RT/F$ ln 4 (35.6 mV at 25 °C).

binations of leaving groups such as halogenohydrins and their esters,[8] halogenosulfones,[9] hydroxysulfones [10] and acetoxy thioethers.[11] We therefore examined the behaviour of the bispyridinium salt (*4*; $n=2$) in the hope of finding a related reaction. In fact this salt shows two partially overlapping one electron waves on cyclic voltammetry (see Table 3) in *N,N*-dimethylformamide and both waves show reversible behaviour. No cleavage of carbon–nitrogen bonds occurs on the time scale involved.

The cyclic voltammetry of the series of compounds (*4*) where $n=2$ to 6 was examined. All



4 n = 2 to 6

showed characteristics for the stepwise reversible addition of two electrons (see Table 3) in the region of potential where *2a* shows reversible formation of the radical-zwitterion. When $n=2$ or 3 the two one electron steps give rise to overlapping waves. As the alkyl chain becomes longer the two waves merge into one wave. If the current values at each point on this combined wave for the compounds (*4*; $n=5$ or 6) are divided by two then the resulting voltammogram, now at half sensitivity, is exactly superimposable on the voltammogram for *2a* at the same molar concentration and with the same electrode recorded at normal sensitivity.

When the alkyl chain is sufficiently long, each pyridinium ring behaves independently of the other and the standard enthalpy change for each redox process is the same. The entropy change is, however, not the same since there are two identical sites for addition of the first electron but only one site for its removal, also two identical sites for removal of the second electron but only one site for its addition. Such probability considerations require that $E_1°-E_2°=(RT/F)$ ln 4.[12] For this value of $E_1°-E_2°$, mathematical analysis indicates that the shape of the combined two electron voltammetric wave is that for a one electron process but with a current twice the value for a one electron process.[13] This theoretical result has received a previous practical demonstration for the reduction of α,ω-di(4-nitrophenyl)alkanes in aprotic solvents.[13] When $E_1°-E_2°=0$ the combined two electron voltammetric wave has a distinctly different shape.

When the alkyl chain is short as in *4* ($n=2$) the two positive centres interact so that $E°$ for the first electron addition is considerably less negative than for reduction of an isolated pyridinium ring in *2a*. The so formed radical-ion from *4* ($n=2$) has the positive charge located on nitrogen and the negative charge distributed over the π-system of one pyridine ring. This positive charge is still able to influence the value of $E°$ for addition of the second electron which is again less negative than $E°$ for the reduction of an isolated pyridine ring in *2a*. This electrostatic effect on the enthalpy of electron transfer disappears when $n=5$ or 6 and only the entropy, probability effect remains to distinguish $E_1°$ and $E_2°$.

EXPERIMENTAL

Cyclic voltammetry *N,N*-Dimethylformamide was kept over anhydrous calcium sulfate, then

anhydrous copper sulfate and distilled under nitrogen, b.p. 43 °C/1.6 kPa. Tetrapropylammonium fluoroborate (0.1 M) was used as supporting electrolyte and the substrate was 10^{-3} M. Cyclic voltammograms were recorded on a mercury coated platinum sphere electrode with a saturated calomel reference electrode isolated by a salt bridge containing the supporting electrolyte in dimethylformamide.

2,4,6-Triphenylpyryliumtetrafluoroborate.[14] A solution of chalcone (21 g, 0.2 mol) in acetophenone (12 g, 0.1 mol) was treated with sulfuric acid (17 g) and the mixture heated at 100 °C for 3 h, then cooled to 40 °C and diluted with ethanol (100 ml) and ether (300 ml). Fluoroboric acid (40 ml, 40 % solution) was added and the mixture let stand for 1 h. 2,4,6-Triphenylpyrylium tetrafluoroborate separated as yellow needles (15.5 g) which were collected and crystallised from ethanol, m.p. 223–225 °C (lit.[14a] m.p. 225–226 °C).

4-Phenylpyridinium salts. These were obtained by heating 4-phenylpyridine with excess of the alkyl bromide, evaporating the excess under reduced pressure and crystallising the residue from anhydrous ethanol and ether. The 1-propyl and 1-benzyl bromides formed hygroscopic solids.

2,4,6-Triphenylpyridinium salts. 2,4,6-Triphenylpyrylium tetrafluoroborate and a slight excess of the appropriate amine were reacted according to the usual conditions.[15] Not previously described is N,N'-*hexamethylenebis*(2,4,6-*triphenylpyridinium*) *tetrafluoroborate* which crystallised from aqueous pyridine as needles, m.p. 280–281 °C. (Found: C, 71.3; H, 5.3; N, 3.2. $C_{52}H_{46}B_2F_8N_2$ requires C, 71.6; H, 5.3; N, 3.2 %).

1-(2-Aminoethyl)-2,4,6-triphenylpyridinium tetrafluoroborate. 2,4,6-Triphenylpyrylium tetrafluoroborate (2 g) suspended in ethanol (20 ml) was treated with excess ethylene diamine (1 g) and the mixture refluxed for 2 h. Evaporation of the solvent under reduced pressure left a gum which crystallised from ethanol–ether as buff needles of the monopyridinium salt, m.p. 161–162 °C, (lit.,[16] m.p. 95–96 °C). (Found: C, 68.2; H, 5.4; N, 6.3. $C_{25}H_{23}BF_4N_2$ requires C, 68.5; H, 5.3; N, 6.4 %). ^{1}H NMR (250 MHz, $CDCl_3$): 2.62 (2H, t, CH_2NH_2), 4.56 (2H, t, $CH_2CH_2NH_2$), 7.5–7.8 (15H, m, aromatic), 7.83 (2H, s, pyridinium ring), NH_2 proton resonance not observed.

N,N'-*Ethylenebis(2,4,6-triphenylpyridinium)-tetrafluoroborate.* The above salt (300 mg) and 2,4,6-triphenylpyrylium tetrafluoroborate (300 mg) were refluxed in ethanol (10 ml) for 2 h. Addition of ether precipitated a lemon yellow solid, m.p. 196 °C. Crystallisation from chloroform–ether afforded almost colourless prisms of *N,N'*-ethylenebis(2,4,6-triphenylpyridinium)tetrafluoroborate, m.p. 195–196 °C. (Found: C, 70.5; H, 4.7; N, 3.3. $C_{48}H_{38}B_2F_8N_2$ requires C, 70.6; H, 4.7; N, 3.4 %). ^{1}H NMR (250 MHz, $CDCl_3$): 3.49 (4H, s, CH_2CH_2).

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Electroorganic Chemistry. 66. Electrochemical Oxidation of Aminals and Enamines Using a Mediatory System

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Electrochemical oxidation of aminals and enamines smoothly proceeded under the conditions using potassium iodide as a mediator to afford formamides and β-ketoamines, respectively. The reaction mechanisms of the formation of these products are discussed.

Electrochemical oxidation of organic compounds using mediators has been attracting much attention,[1] since (i) the oxidation of substrates with this system proceeds at lower potentials than those required for the direct anodic oxidation of the substrates, and (ii) the oxidation is efficiently achievable under mild conditions. Accordingly, it is important to exploit new mediatory systems being effective to organic synthesis.

We have already found some mediatory systems which are applicable to the Hofmann rearrangement [2] of amides *1* to carbamates *2*, and to the oxidation [3] of secondary alcohols *3* to ketones *4* and primary alcohols *5* to esters *6*.

$$\underset{1}{RCONH_2} \xrightarrow[KBr\text{–}CH_3OH]{-e} \underset{2}{RNHCO_2CH_3} \quad (1)$$

$$\underset{3}{\begin{matrix}R\\R'\end{matrix}\!\!>\!CHOH} \xrightarrow[KI]{-e} \underset{4}{\begin{matrix}R\\R'\end{matrix}\!\!>\!C{=}O} \quad (2)$$

$$\underset{5}{2\,RCH_2OH} \xrightarrow[KI]{-e} \underset{6}{RCO_2CH_2R} \quad (3)$$

In this report, we describe the electrochemical oxidation of aminals *7* and enamines *8* using potassium iodide (KI) as a mediator. The electrochemical oxidation of hexahydro-1,3,5-triazines to triazines has been reported.[4]

$$\underset{7}{CH_2(NR'_2)_2} \qquad \underset{8}{RCH{=}CHNR'_2}$$

7 R′: alkyl group *8* R,R′: alkyl groups

The oxidation of *7* leads to a convenient synthesis of *n,n*-dialkylformamides, while the latter compounds are oxidatively transformed to β-ketoamines.

RESULTS

Preparation of N,N-*dialkylformamides.* Constant current electrolysis of an aqueous solution of *N,N,N′,N′*-tetramethylmethanediamine (*9*) containing one-fifth equivalent of KI gave *N,N*-dimethylformamide (DMF) in 75 % yield at the time when 4 F/mol of electricity was passed (eqn. (4)).

$$\underset{9}{(CH_3)_2NCH_2N(CH_3)_2} \xrightarrow[KI]{-e,\ 4\ F/mol} \underset{75\ \%}{DMF} \quad (4)$$

Also, the electrolysis of an aqueous solution containing equimolar amounts of *9* and formaldehyde resulted in the formation of DMF in 176 % yield based on *9* (eqn. (5)).

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Table 1. Electrochemical synthesis of *N,N*-dimethylformamide.[a]

Run	Mediator (mmol)	[Aldehyde]/ [Mediator]	Yield (%)[b]
1	KI (1.7)	30	76
2	(5)	10	91
3	(10)	5	95>
4	(10)	5	95>[c]
5	KBr (10)	5	25
6	KCl (10)	5	15
7	NaI (10)	5	95>
8	NaBr (10)	5	30
9	Et_4NI (10)	5	59
10	KOH (10)	5	15

[a] HCHO: 50 mol, Me_2NH: 55 mol. Anode, Pt; cathode, carbon. [b] Yields based on aldehyde were obtained by GLC method. [c] Carbon rods were used as cathode and anode.

$$9 + CH_2O \xrightarrow[\text{KI},\ 4\ \text{F/mol}]{-e} \text{DMF}\quad 176\ \% \qquad (5)$$

The latter result implies that it is not necessary to isolate *9* as the starting material for the synthesis of DMF from dimethylamine and formaldehyde. In fact, the preparation of DMF was satisfactorily achieved by passing electricity through an aqueous solution* of dimethylamine (1 equiv.) and formaldehyde (1 equiv.) containing KI (0.2 equiv.) (run 3 in Table 1). The effects of the amount of KI (runs 1–3), the type of mediators (runs 5–10) and anode materials (run 4) on the yields of DMF were also examined. The results are summarized in Table 1 showing that less than one-tenth equivalent of KI is sufficient for almost perfect oxidation, and that the yields of DMF increased in the order of $I^- \gg Br^- > Cl^- \sim OH^-$. A carbon rod instead of platinum was usable as an anode.

Although DMF has been prepared by several methods using formic acid,[6] carbon monoxide,[7] methyl formate [8] or hydrogen cyanide [9] as the precursor of the formyl moiety, our mediatory method utilizing formaldehyde provides a new facile route for the production of DMF.

Formamides of primary and secondary amines other than dimethylamine were similarly synthesized using potassium iodide as a mediator (Table 2).

Preparation of β-ketoamines. In contrast to the reaction of formaldehyde, the electrolysis of a solution containing higher aldehydes *10* and amines *11* did not give the corresponding amides but yielded β-ketoamines *12* as main products together with some unidentified products. Results of the synthesis of β-ketoamines from heptaldehyde and citronellal are shown in Table 3.

$$\underset{10}{RCH_2CHO} + \underset{11}{R'_2NH} \xrightarrow[\text{KI, } H_2O\text{–}t\text{-BuOH}]{-e} \underset{12}{RC(=O)CH_2NR'_2} \qquad (6)$$

The structures of β-ketoamines were identified by IR, NMR and MS data. Some of the β-ketoamines were also identified by comparison with authentic samples independently prepared as follows.

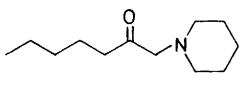
* The evolution of heat observed in the procedure indicates the formation of *9* in the solution. In fact, the ethereal extract of this solution gave *9* (Synthesis of *9*, see Ref. 5).

Table 2. Electrochemical synthesis of *N*-alkyl- and *N,N*-Dialkylformamides.[a]

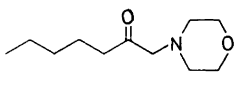
Run	Amine	Amide	F/mol	Yield (%)[b]
1	Et_2NH	Et_2NCHO	4.9	85
2	$(n\text{-}Bu)_2NH$	$(n\text{-}Bu)_2NCHO$	4.5	57
3	Morpholine	Morpholinecarboxaldehyde	4.2	83
4	Piperidine	1-Piperidinecarboxaldehyde	4.5	93
5	Pyrrolidine	1-Pyrrolidinecarboxaldehyde	4.8	75
6	$n\text{-}BuNH_2$	n-BuNHCHO	4.3	75

[a] HCHO: 20 mmol, amine: 20 mmol, KI: 10 mmol. [b] Isolated yields based on amines.

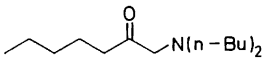
Table 3. Electrochemical synthesis of β-ketoamines.



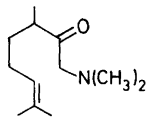
16c



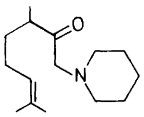
16d



16e



17a



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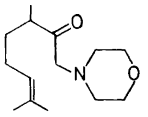
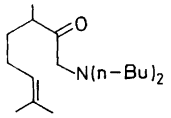


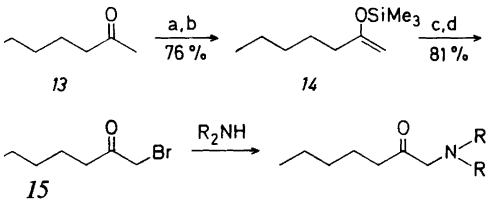
17d



17e

Amine	Product	Yield (%)[a,b,c]
Aldehyde: heptanal		
$(CH_3)_2NH$	*16a*	56
Pyrrolidine	*16b*	17
Piperidine	*16c*	41
Morpholine	*16d*	39
n-Bu_2NH	*16e*	48
Aldehyde: citronellal		
$(CH_3)_2NH$	*17a*	31
Pyrrolidine	*17b*	53 (39)[d]
Piperidine	*17c*	55 (33)[d]
Morpholine	*17d*	50 (35)[d]
n-Bu_2NH	*17e*	60 (35)[d]

[a] Determined by GLC method. [b] Electricity: 4 F/mol. [c] Based on aldehyde. [d] Isolated yield.

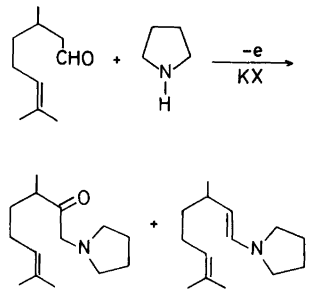


(7)

16a, R=CH_3, 60 %
16b, R=$-(CH_2)_4-$, 79 %

(a) LDA, THF; (b) TMSCl; (c) Br_2,CCl_4; (d) H_2O

The effect of mediators on the yields of β-ketoamines was examined in the reaction of citronellal with pyrrolidine (eqn. (8)).



(8)

17b *18*

KX		
KI	(53 %)	100: 0
KBr	(40 %)	79: 21
KCl	(61 %)	0:100

Although KI gave *17b* exclusively, the formation of *18* and a mixture of *17b* and *18* was observed in the reaction using KCl and KBr, respectively.

DISCUSSION

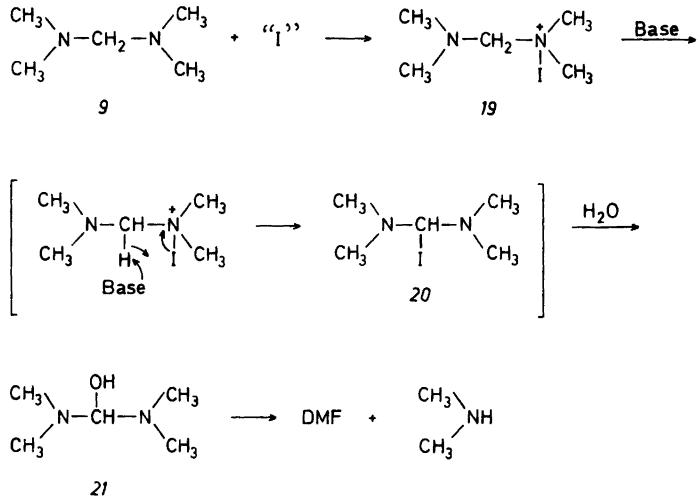
Reaction mechanism of formation of DMF. In the mediatory system using KI, the oxidation of I^- to active species tentatively denoted as "I" (eqn. (9))* is the initiation step. The fact that the yield of DMF obtained at a controlled potential (0.6 V *vs.* SCE) was reasonable (~60 %) also supports the oxidation of I^- in the initiation step, since the oxidation waves of *9*, dimethylamine and formaldehyde in aqueous solution containing Et_4NOTs were not observed at lower potential than 1.0 V *vs.* SCE.

$$I^- \xrightarrow{-2e} \text{"I"} \qquad (9)$$

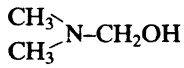
Thus, the attack of "I" on *9* followed by a base-catalyzed conversion of an intermediate *19* to *21* through *20* (Scheme 1) would reasonably explain the formation of DMF,** though the

* What "I" consists of is unknown.

** The mechanism shown in Scheme 1 is a working hypothesis which may be depicted in different patterns. The mechanism proposed in oxidative fragmentation of trimethylenediamine with chlorine dioxide [10] is, for instance, similar to Scheme 1.



Scheme 1.



22

mechanism involving the attack of "I" on an *N*-hemiacetal *22*[11,**] is not necessarily ruled out if *22* exists in the system in equilibrium with *9*. When the reaction is carried out in the presence of formaldehyde, dimethylamine formed in Scheme 1 gives *9* again, so that all the dimethylamine is transformed to DMF.

The base which catalyses the conversion of *19* to *20* can be generated by the reaction of water with potassium which is generated from potassium cation on the cathode.* Accordingly, the overall process of this mediatory system can be schematically represented as Fig. 1 in which the reaction system involves the reactions described in Scheme 1.

In connection with this mechanism, it is interesting to examine whether simple tertiary amines can be oxidized in this mediatory system according to the mechanism similar to that described in Scheme 1. Preliminary experiments carried out for *N,N*-dimethylbenzylamine (DMBA) using a two-phase system indicated the possibility that such mediatory oxidation of DMBA took place, since *N*-benzyl-*N*-methylformamide was obtained in 23 % yield.*

In the system using mediators other than KI, the yield of DMF was low probably due to the difference of reactivity of active halonium ion

* Although it has not been established that reduction of water in the presence of K^+ proceeds through the reductive formation of alkali metal, the result that using tetraethylammonium iodide instead of KI or NaI brought about a decrease in yield (59 %, run 9, Table 1) suggests that K^+ or Na^+ plays an important role in this oxidation.

** *N*-Hydroxymethylnortropane has been postulated as an intermediate in the electrochemical oxidation of tropane to *N*-formylnortropane.[12]

* Although the electrochemical oxidation of DMBA has been extensively studied,[13] indirect oxidation of DMBA using mediators is not known so far.

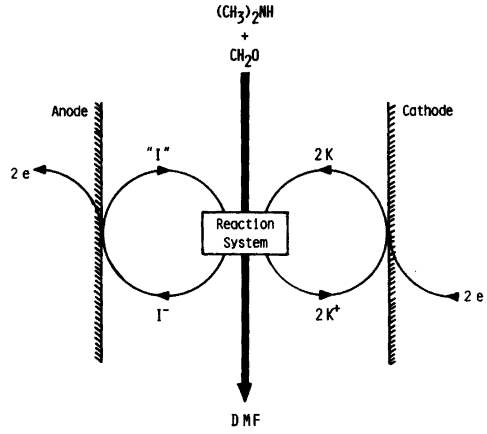
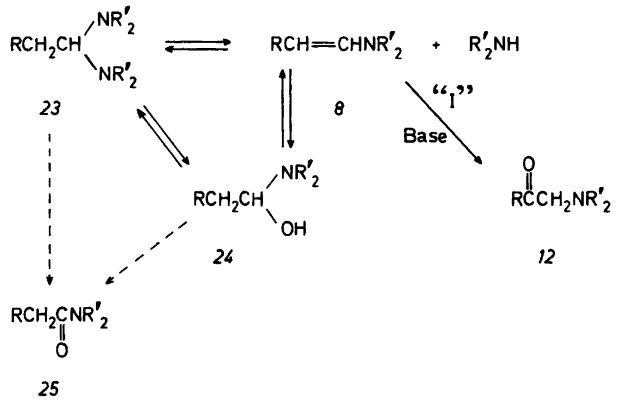


Fig. 1. Schematic representation of electrochemical preparation of DMF using KI as a mediator.

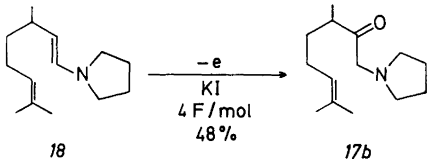


Scheme 2.

"X", and also the direct oxidation of *9* may contribute to a certain extent to the formation of DMF.

Reaction of mechanism of formation of β-ketoamines. It has been shown that equilibrated mixtures of enamines *8*, aminals *23*, and *N*-hemiacetals *24* form when aliphatic aldehydes and amines are allowed to react in solvents.[14] Although these three types of compounds have the possibility to be oxidized by "I" in our mediatory system, *8* is the most plausible precursor of *12*, because the oxidation of *23* or *24* must yield amides *25* through a similar mechanism to the formation of DMF from *9* (Scheme 2).

In fact, the electrochemical oxidation of an independently prepared enamine *18*, using KI as a mediator, gave *17b* (eqn. (10)), but *25* was not observed in the products.

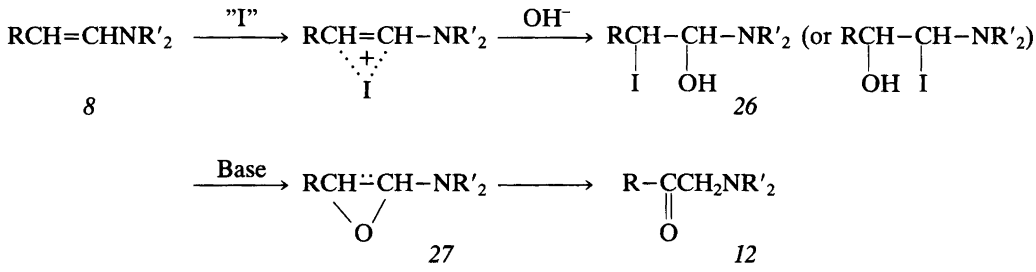


18 *17b* (10)

These results suggest that the addition of "I" to *8* is much faster than that to *23* or *24* (Scheme 2). The oxidation of *8* to *12* is reasonably explained as follows: Thus, "I" adds to the double bond of *8* to yield an iodohydrine *26* which is converted by base to α-aminoepoxide *27*. The conversion of α-aminoepoxides to β-ketoamines has been known [15] (Scheme 3).

The formation of β-ketoamines in the mediatory system using KBr is also explicable by a similar mechanism to that described in Scheme 3. Using KCl as a mediator, it is assumed that the anode potential [16] was not enough to oxidize Cl^-,* or that an active species generated by the oxidation of Cl^- did not work as an oxidant like "I".

* Although the direct oxidation of enamines in methanol has been known,[17] it would not take place in this mediatory system using two-phases since enamines with high molecular weights may be present in an organic phase.



Scheme 3.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian Associates EM-390 or EM-360 using tetramethylsilane as an internal standard. IR spectra were taken with a Hitachi 215 spectrometer. Mass spectra were recorded on a JEOL IMS-DX300 mass spectrometer. Elemental analysis was determined by the Center for Instrumental Analysis of Kyoto University. Constant current electrolyses were carried out using DC Power Supply (GPO 50–2) of Takasago Seisakusho, Ltd. A potentiostat HA-104 of Hokuto Denko, Ltd. was used for a controlled potential electrolysis. Gas chromatographic analyses were performed on a Yanaco GCG-550T gas chromatograph. Chemicals other than those described were commercially available and used without purification.

Preparation of N,N-*dimethylformamide. General.* Into a cell equipped with carbon rod cathode (8 mmϕ) and platinum anode (2 cm×2 cm) were added potassium iodide (1.66 g, 10 mmol), a 37 % aqueous solution of formaldehyde (4.05 g, 50 mmol) and a 50 % aqueous solution of dimethylamine (4.9 g, 55 mmol), succesively, without cooling. At that time, considerable exothermic reaction took place. After the solution was cooled to room temperature, water (2 ml) was added, and a constant current electrolysis (0.5 A, 0.24 A/cm^2) was carried out. After 2 F/mol of electricity was passed, DMF [18] was isolated by distillation *in vacuo* from the reaction mixture, and identified by spectroscopic method. Since DMF is missible with water, the isolated yield was low (~50 %). The yields shown in Table 1 were obtained by GLC method (Si DC550; 120 °C, internal standard; Et_2NCHO). The electrolysis of *9* [5] (1.02 g, 10 mmol) in water (5 ml) containing KI (0.332 g, 2 mmol) or a mixture of *9* (1.02 g, 10 mmol) and formaldehyde (10 mmol) in water (5 ml) containing KI (0.332 g, 2 mmol) was carried out in a similar way as above. Also, this method was similarly applied to mediators other than KI.

Preparation of N,N-*dialkylformamides. N,N*-Dialkylformamides shown in Table 2 were prepared by a similar method to the synthesis of DMF except for the following points. A 37 % aqueous solution of formaldehyde (1.62 g, 20 mmol), amines (20 mmol), KI (1.66 g, 10 mmol) and water (8 ml) were added into the cell. After the electricity shown in Table 2 was passed with a constant current (0.5 A), the reaction mixture was extracted with CH_2Cl_2, and the extract was washed with an aqueous solution of sodium thiosulfate and dried over $MgSO_4$. Products, *N,N*-diethylformamide,[18] *N,N*-dibutylformamide,[18] 4-morpholinecarboxaldehyde,[19,20] 1-piperidinecarboxaldehyde,[20] 1-pyrrolidinecarboxaldehyde,[20] and *N*-butylformamide [20] were isolated by distillation and identified by comparison of their spectroscopic data with those of authentic samples.

Electrochemical synthesis of β-ketoamines (16 and 17). Into a cell equipped with carbon rod cathode (8 mmϕ), platinum anode (2 cm×2 cm), and a dropping funnel was placed a solution of secondary amines (40 mmol) and KI (1.66 g, 10 mmol) in water (10 ml). A solution of aldehydes (20 mmol) in *tert*-butanol (5 ml) was added dropwise into the cell over a period when 2 F/mol of electricity was passed, the reaction mixture was poured into a solution of saturated sodium thiosulfate, extracted with CH_2Cl_2 and dried over $MgSO_4$. Products (*16* and *17*) were isolated by distillation or by GLC after crude distillation and identified by IR, NMR and MS data.

Products *16a* and *16b* were identified by comparison of their spectra with those of independently prepared samples.

16a. B.p. 100 °C (0.5 mmHg); IR (neat) 2920, 2855, 2820, 2770, 1705, 1640, 1450, 1400, 1350, 1265, 1150, 1130, 1040 cm^{-1}; NMR (CCl_4) δ 0.89 (3H, br t, 6 Hz), 1.00–1.90 (6H, m), 2.00–2.55 (2H, m), 2.23 (6H, s), 2.96 (2H, s); mass spectrum, *m/e* 157 (M^+).

16b. B.p. 50–55 °C (0.5 mmHg); IR (neat) 2920, 2860, 2780, 1705, 1460, 1410, 1378, 1350, 1313, 1293, 1262, 1205, 1150, 1128, 1092, 1053, 903, 880, 722 cm^{-1}; NMR (CCl_4) δ 0.91 (3H, t, 6 Hz), 1.10–1.60 (4H, m), 1.60–2.00 (4H, m), 2.20–2.74 (6H, m), 3.18 (2H, s); mass spectrum, *m/e* 183 (M^+), 84 ($^+CH_2N(CH_2)_4$).

16c. B.p. 70 °C (0.7 mmHg); IR (neat) 2920, 2845, 2780, 1700, 1440, 1298, 1275, 1252, 1205, 1155, 1120, 1038, 995, 860 cm^{-1}; NMR (CCl_4) δ 0.90 (3H, br t, 6 Hz), 1.05–1.90 (12H, m), 2.00–2.60 (6H, m), 2.92 (2H, s); mass spectrum, *m/e* 197 (M^+), 196 (M^+–1), 98 ($^+CH_2N(CH_2)_5$).

16d. B.p. 64–65 °C (0.5 mmHg); IR (neat) 2840, 2800, 1695, 1442, 1375, 1290, 1270, 1240, 1210, 1110, 1065, 1033, 1005, 897, 862, 795, 715 cm^{-1}; NMR (CCl_4) δ 0.90 (3H, br t, 6 Hz), 1.06–1.90 (6H, m), 2.25–2.60 (6H, m), 3.00 (2H, s), 3.40–3.77 (4H, m); mass spectrum, *m/e* 199 (M^+), 100 ($^+CH_2N(C_2H_4)_2O$).

16e. B.p. 120 °C (0.8 mmHg); IR (neat) 2950, 2920, 2855, 2800, 1705, 1455, 1350, 1300, 1260, 1150, 1121, 1081 cm^{-1}; NMR (CCl_4) δ 0.90 (9H, br t, 6 Hz), 1.05–1.90 (14H, m), 2.20–2.60 (6H, m), 3.02 (2H, s); mass spectrum, *m/e* 241 (M^+), 240 (M^+–1), 142 ($^+CH_2N(C_4H_9)_2$).

17a. B.p. 86–88 °C (0.4 mmHg); IR (neat) 2920, 2865, 2820, 2770, 1703, 1443, 1375, 1270, 1150, 1095, 1040, 1008, 855, 825 cm^{-1}; NMR (CCl_4) δ 1.04 (3H, d, 7.2 Hz), 1.20–2.20 (4H, m),

1.57 (3H, s), 1.62 (3H, s), 2.20 (6H, s), 2.50 (1H, m), 2.98 (2H, s), 5.00 (1H, m); mass spectrum, *m/e* 197 (M^+), 58 ($^+CH_2N(CH_3)_2$).

17b. B.p. 72–75 °C (0.9 mmHg); IR (neat) 2960, 2920, 2870, 2780, 1718, 1708, 1450, 1410, 1375, 1348, 1310, 1290, 1260, 1200, 1122, 1043, 1022, 980, 930, 880, 828, 800 cm^{-1}; NMR (CCl_4) δ 1.04 (3H, d, 6.6 Hz), 1.00–2.20 (8H, m), 1.57 (3H, s), 1.67 (3H, s), 2.20–3.90 (5H, m), 3.22 (2H, s), 5.03 (1H, m); mass spectrum, *m/e* 223 (M^+), 84 ($^+CH_2N(CH_2)_4$).

17c. B.p. 83–86 °C (2.5 mmHg); IR (neat) 2920, 2850, 1703, 1443, 1375, 1300, 1278, 1255, 1155, 1110, 1038, 985, 893, 860, 792 cm^{-1}; NMR (CCl_4) δ 1.02 (3H, d, 6.8 Hz), 1.59 (3H, s), 1.67 (3H, s), 1.00–1.20 (10H, m), 2.20–2.70 (4H, m), 2.40–3.10 (1H, m), 3.00 (2H, s), 5.03 (1H, m); mass spectrum, *m/e* 237 (M^+), 98 ($^+CH_2N(CH_2)_5$).

17d. B.p. 97–100 °C (0.9 mmHg); IR (neat) 2955, 2920, 2850, 2805, 1702, 1448, 1378, 1290, 1270, 1115, 1070, 1000, 865 cm^{-1}; NMR (CCl_4) δ 1.02 (3H, d, 6.8 Hz), 1.00–2.20 (4H, m), 1.60 (3H, s), 1.69 (3H, s), 2.44 (4H, m), 2.65 (1H, m), 3.06 (2H, s), 3.58 (4H, m), 5.02 (1H, m); mass spectrum, *m/e* 239 (M^+), 100 ($^+CH_2N(C_2H_4)_2O$).

17e. B.p. 96–103 °C (2 mmHg); IR (neat) 2960, 2930, 2880, 2815, 1708, 1455, 1380, 1308, 1250, 1160, 1090, 1030, 943, 825, 730 cm^{-1}; NMR (CCl_4) δ 0.98 (3H, d, 6.6 Hz), 0.65–2.20 (18H, m), 1.57 (3H, s), 1.65 (3H, s), 2.40 (4H, m), 2.50–3.00 (1H, m), 3.09 (2H, s), 4.99 (1H, m); mass spectrum, *m/e* 281 (M^+), 142 ($^+CH_2N(C_4H_9)_2$).

Using KBr as a mediator, the ratio of *17b* to enamine *18* was obtained by GLC method. When KCl was a mediator, enamine *18* was isolated by distillation from the ethereal extract of the reaction mixture. Enamine *18* was also prepared by the method of Mannich.[21]

18. 61 % yield; B.p. 96–98 °C (0.6 mmHg); IR (neat) 2960, 1640, 1440, 1362, 1298, 1125, 1030, 930, 880, 823, 778, 738 cm^{-1}; NMR (CCl_4) δ 0.95 (3H, d, 6.4 Hz), 1.00–2.35 (11H, m), 1.58 (3H, s), 1.66 (3 H, s), 2.93 (4H, m), 3.82 (1H, d d, 8.0 and 13.6 Hz), 5.04 (1H, m), 5.96 (1H, d, 13.6 Hz). Anal. calc. for $C_{14}H_{25}N$: C, 81.09; H, 12.15; N, 6.76. Found: C, 81.03; H, 12.23; N, 6.52.

*Synthesis of β-ketoamines (*16a *and* 16b*) from 1-bromo-2-heptenone.* Trimethyl[(1-pentylvinyl)oxy]silane (*14*) was prepared from 2-heptanone (*13*) by the method of H. O. House.[22] Into a solution of *14* (2.2 g, 11.8 mol) in CCl_4 (3 mol) was added slowly bromine (1.9 g, 11.8 mmol) at –10 °C. After the solution was stirred for 1 h, ice-water was added to the solution, and the organic portion was extracted with ether. The ethereal extract was dried over $MgSO_4$, and subsequent evaporation of the extract *in vacuo* followed by distillation gave 1-bromo-2-heptanone (*15*) in 81 % yield.

15. B.p. 98–100 °C (20 mmHg) (lit.[23] 96 °C (14 mmHg)); IR (neat) 2920, 2860, 1703, 1455, 1395, 1175, 1120, 1048 cm^{-1}; NMR (CCl_4) δ 0.92 (3H, br t, 5 Hz), 1.05–2.10 (6H, m), 2.63 (2H, t, 7.2 Hz), 3.74 (2H, s).

Into an aqueous solution of dimethylamine (40 %, 1.68 g, 9.6 mmol) was added 1-bromo-2-heptanone (*15*) (0.5 g, 2.59 mmol). After the solution was stirred at room temperature for 1.5 h, the organic portion was extracted with ether. Product *16a* was isolated by distillation from the ethereal extract: 36 % yield. The synthesis of *16b* from 1-bromo-2-heptanone and pyrrolidine was also achieved: 60 % yield, The NMR and IR spectra of these products were identical with those of electrochemically prepared *16a* and *16b*.

Electrolysis of enamine 18. A solution of KI (0.83 g, 0.5 mmol) in water (5 ml) was placed into a cell similar to that used in the preparation of β-ketoamines. Into this cell was added dropwise a solution of *18* (2.07 g, 10 mmol) in *t*-butanol (2 ml) over the period that 2 F/mol of electricity was passed. After 4 F/mol of electricity was passed, the working up similar to the electrochemical oxidation of a mixture of aldehydes and amines gave *17b* in 48 % yield.

Electrochemical oxidation of N,N-*dimethylbenzylamine (DMBA).* Into a cell equipped with a platinum anode (2 cm×2 cm) and a carbon rod cathode (8 mmϕ) were placed water (30 ml) containing KI (0.83 g, 5 mmol) and a solution of DMBA (1.35 g, 10 mmol) in CH_2Cl_2 (10 ml). The solution was stirred and cooled with ice-water. After 5 F/mol of electricity was passed (0.5 A), the organic portion was separated, and the aqueous solution was extracted with CH_2Cl_2. After the combined extract was dried, the evaporation of the solvent followed by distillation of the residue gave *N*-benzyl-*N*-methylformamide [24] (23 %) together with DMBA (29 %). Those yields were determined by GLC method.

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Indirect Electrochemical Processes. 14.* Indirect Electrochemical Oxidation of Benzylic Alcohols with Organic Mediators

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Benzylic alcohols can be oxidized with good selectivity, high material yield, and at low potentials to form benzaldehydes, if catalytic amounts of electrogenerated and regenerated triarylamine cation radicals are used as oxidizing agents. The reaction can either take place in acetonitrile or methanol in the presence of 2,6-lutidine or Na_2CO_3 as bases using a divided cell or in an undivided cell, if methanol is used as a solvent. In the latter case the aldehyde is formed as the major product, if small amounts of Na_2CO_3 are present. In the absence of an added base, however, the dimethyl acetal predominates. The cathodic formation of dimeric products from the benzaldehyde can be suppressed by the use of a platinum cathode and by working under neutral conditions. The formation of methyl benzoate as a side product is dependent on the conversion of the substrate.

Anodic oxidation of benzylic alcohols takes place at rather positive potentials [1] and in most cases is accompanied by electrode passivation. In the case of 4-methoxybenzyl alcohol this passivation can be prevented and 4-methoxybenzaldehyde is formed in 72 % yield, if pyridine is present as a base.[1] In other cases Na_2CO_3 in wet acetonitrile was used as base and potential pulsing prevented fouling of the electrode surface.[2,3] In the case of benzylic alcohol the aldehyde (30 %) was accompanied by benzoic acid (40 %). By application of a nickel gauze anode in aqueous alkali 1,4-bis(hydroxymethyl)benzene can be transformed to terephtalic acid in high yield.[4] In a similar way benzylic alcohol forms benzoic acid,[5] while using a two phase system of water and benzene (1:1) yields benzaldehyde (57 %) and benzoic acid (14 %).[6] An effective alternative is the use of electrochemically generated hypobromide in a two phase system under phase transfer catalysis conditions leading to benzaldehydes in 70–100 % material yield and 30–80 % current yield.[7,8]

In previous studies we have shown that benzylic ethers [9–11] and esters [12] can be oxidized to form benzaldehydes and aliphatic alcohols, respectively acids, with high selectivity under mild conditions, low potentials and without electrode fouling, if electrochemically, respectively chemically generated, triarylammoniumyls are used as oxidizing agents and organic or inorganic bases are present. The electrochemical procedure allows the use of catalytic amounts of the mediator by its continuous regeneration. The purpose of this investigation was the application of this procedure to the oxidation of benzylic alcohols to form benzaldehydes selectively in acetonitrile or methanol as solvents according to eqn. (1).

RESULTS AND DISCUSSION

Electroanalytical studies. The chance of success for the indirect electrochemical reaction can be evaluated by the cyclic voltammetric method (CV) by which not only values of the redox potentials are measured (Table 1) but also the presence of an electrocatalytic mechanism can be

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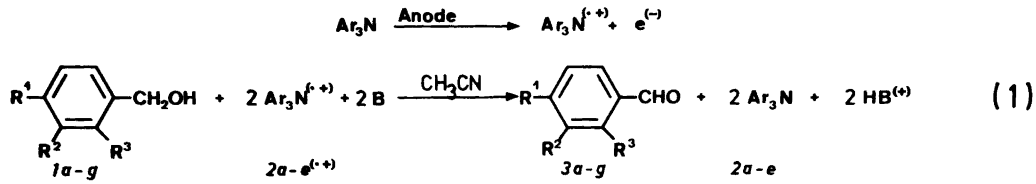
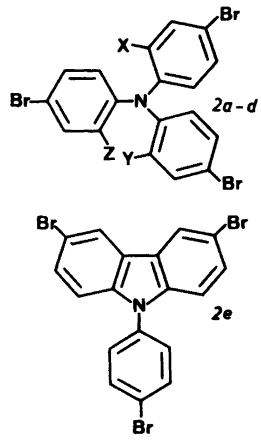


(1)

Compound	R^1	R^2	R^3
1a, 3a	OCH_3	H	H
1b, 3b	H	H	H
1c, 3c	Br	H	H
1d, 3d	Cl	H	H
1e, 3e	Cl	Cl	H
1f, 3f	Cl	H	Cl
1g, 3g	CH_2OH	H	H



Compound	X	Y	Z
2a	H	H	H
2b	Br	H	H
2c	Br	Br	H
2d	Br	Br	Br

indicated by the so-called catalytic effect. In the case of an indirect electrochemical oxidation the anodic peak current for the electron transfer agent (mediator) is increased and the cathodic peak current lowered by the amount of a catalytic current.[13] The size of the catalytic current is dependent on the potential scan rate, the benzylic alcohol concentration and the overall reaction rate for the regeneration of the mediator in its reduced form. The reaction rate is not only dependent on the difference of the standard redox potentials of the mediator and the benzylic alcohol which determines the equilibrium constant for the homogeneous electron transfer step but also by the rate of the follow-up chemical reaction, the deprotonation of the benzylic alcohol cation radical. Therefore the catalytic effect is expected to show a large influence of the kind and amount of the added base. To examine the size of the catalytic effect cyclic voltammograms have been recorded for different potential scan rates, concentrations of the alcohols and amounts of added bases.

Table 1. Redoxpotentials of compounds *1a–g* and *2a–c* in acetonitrile/0.2 M $LiClO_4$ against the normal hydrogen electrode.

Compound	E_{pa}/V [a]	$E°$/V [b]
1a	1.72	
1b	2.49	
1c	2.35	
1d	2.44	
1e	2.50	
1f	2.52	
1g	2.23	
2a		1.30
2b		1.42
2c		1.56
2d		1.74
2e		1.72

[a] Anodic peak potential, extrapolated for scan rate 0 V/s; first peak. [b] Standard redox potentials; first wave only.

Fig. 1 shows cyclic voltammograms of the triarylamine *2a* in acetonitrile in the presence of 4-methoxybenzyl alcohol *1a* in the absence and presence of 2,6-lutidine. While in the absence of the base no catalytic effect is observed (Fig. 1B), the electrocatalytic process is initiated by the addition of 2,6-lutidine which is indicated by the strong catalytic current (Fig. 1C, D).

In acetonitrile 4-chlorobenzyl alcohol *1d* only shows a very small catalytic effect in the presence of *2d* and Na_2CO_3 as a base. The main reason is the low solubility of Na_2CO_3 in the solvent. In

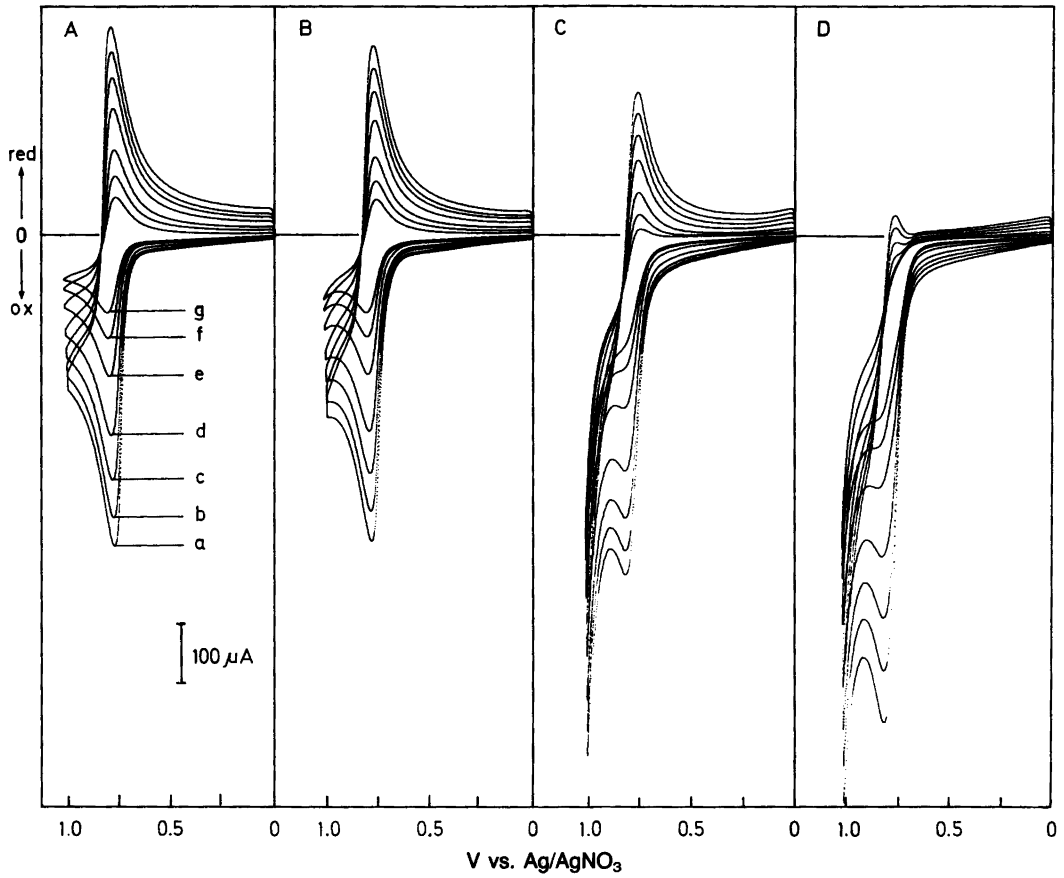


Fig. 1. Cyclic voltammograms of *2a* in the absence and presence of 4-methoxybenzyl alcohol (*1a*) and 2,6-lutidine in acetonitrile. a: 400 mV/s; b: 320 mV/s; c: 240 mV/s; d: 160 mV/s; e: 80 mV/s; f: 40 mV/s; g: 20 mV/s. Platinum anode (0.5 cm^2). A: 0.8 mmol/l *2a*; B: 0.8 mmol/l *2a*, 80 mmol/l *1a*; C: 0.8 mmol/l *2a*, 80 mmol/l *1a*, 80 mmol/l 2,6-lutidine; D: 0.8 mmol/l *2a*, 80 mmol/l *1a*, 800 mmol/l 2,6-lutidine.

methanol, however, even in the absence of a base such a strong catalytic current is observed (Fig. 2B) that addition of Na_2CO_3 does not have further influence.

It therefore can be concluded that preparative scale indirect electrochemical oxidations of benzylic alcohols *1b–1g* by triarylamine mediators *2d* and *2e* will be possible in acetonitrile/Na_2CO_3 at a rather slow to moderate rate while *1a* should react much faster with *2a* in acetonitrile/2,6-lutidine.* In methanol/Na_2CO_3, however, also reactions of *1b–1g* with *2d* or *2e* should take place at a fast rate.

* 2,6-Lutidine cannot be used together with $2d^{\cdot+}$ because it is oxidized.

Preparative oxidation in acetonitrile in a divided cell. Benzylic alcohols *1a–1f* can be oxidized by electrochemically generated and regenerated triarylamine cation radicals $2a^{\cdot+}$ (*1a*), respectively $2d^{\cdot+}$ and $2e^{\cdot+}$ (*1a–1f*), (molar ratio of *1* and *2* =5:1) in base containing acetonitrile to form benzaldehydes *3a–3f* in high yield (Table 2).

The applied potential for the generation of the oxidizing agent is in all cases considerably lower than the half-wave potentials of the substrates. In this way the oxidation can take place under very mild conditions and high selectivity. The selectivity is demonstrated by the absence of side products, for example acids or esters. The oxidizing power of $2a^{\cdot+}$ is too low to attack *1b–1f* to any extent while the better oxidizing agents $2d^{\cdot+}$

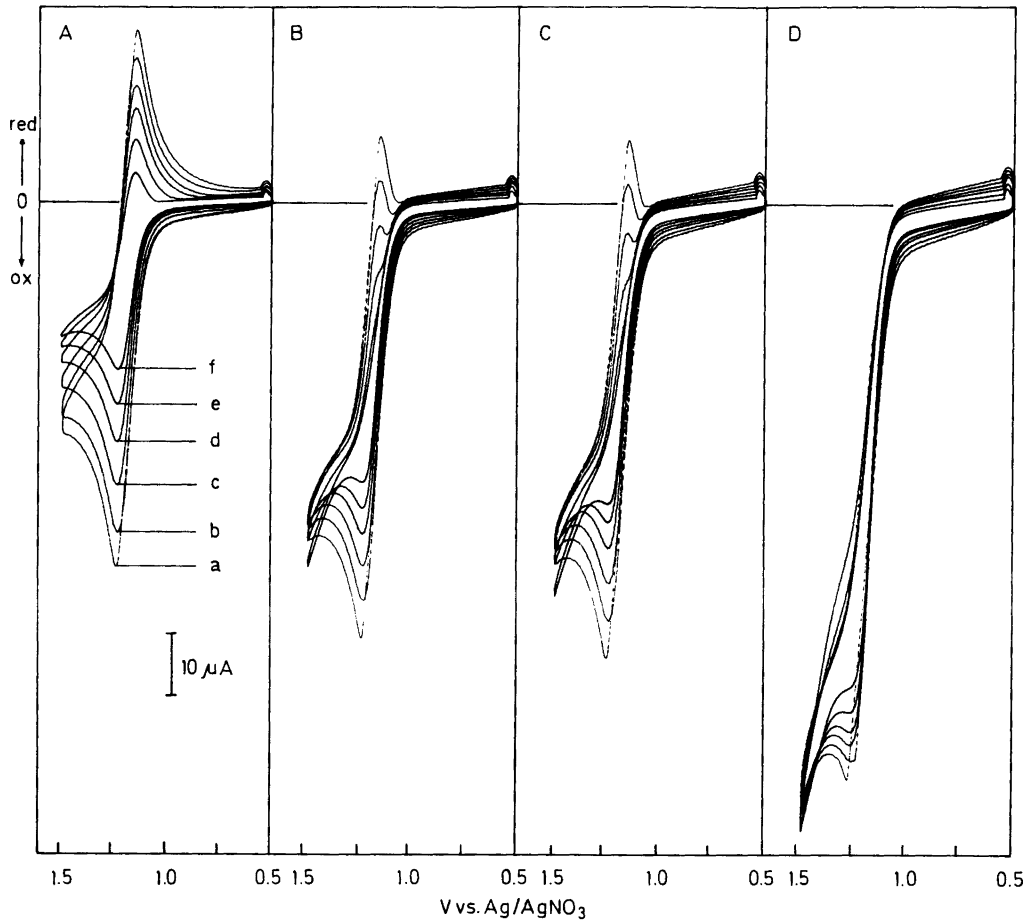
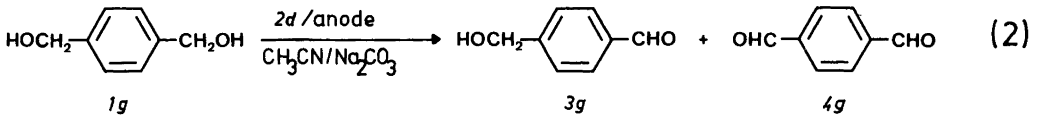


Fig. 2. Cyclic voltammograms of *2d* in the absence and presence of 4-chlorobenzyl alcohol *1d* and Na_2CO_3 in acetonitrile/methanol (2:1). a: 1280 mV/s; b: 980 mV/s; c: 720 mV/s; d: 500 mV/s; e: 320 mV/s; f: 180 mV/s. Glassy carbon anode (0.05 cm^2). A: 1 mmol/l *2d*; B: 1 mmol/l *2d*, 10 mmol/l *1d*; C: 1 mmol/l *2d*, 10 mmol/l *1d*, 100 mmol/l Na_2CO_3; D: 1 mmol/l *2d*, 20 mmol/l *1d*, 100 mmol/l Na_2CO_3.

Table 2. Results of the indirect electrochemical oxidation of benzylic alcohols *1a–1f* by electrogenerated triarylamine cation radicals in acetonitrile using a divided cell.

Educt *1*	Mediator *2*	Base	Conversion of *1*	m.y.[a] *3* (%)	c.y.[b] *3* (%)
1a	*2a*	lutidine	100	96	80
1b	*2a*	lutidine	5	5	<5
1b	*2d*	Na_2CO_3	77	95	65
1c	*2d*	Na_2CO_3	92	90	50
1d	*2d*	Na_2CO_3	85	98	61
1d	*2e*	Na_2CO_3	85	72	45
1e	*2d*	Na_2CO_3	78	100	63
1e	*2e*	Na_2CO_3	86	82	50
1f	*2d*	Na_2CO_3	90	84	50

[a] Material yield of *3* with respect to converted *1*. [b] Current yield of *3*.



and $2e^{\cdot+}$ lead to high material yields. Current densities in these cases are in the order of 3-4 mA/cm^2 while in the case of *1a*/*2a* 15 mA/cm^2 can be reached. The current yields with $2d^{\cdot+}$ and $2e^{\cdot+}$ are lower as compared with $2a^{\cdot+}$ because in a side reaction residual water is oxidized by those mediators. Most electrolyses are stopped after about 85 % conversion. Otherwise the current yield will further decrease.

In the case of 1,4-bis(hydroxymethyl)benzene *1g*, total conversion to form terephthalaldehyde *4g* in 75 % material yield and 46 % current yield can be performed by $2d^{\cdot+}$ according to eqn. (2). By continuous monitoring of the progress of the reaction (see Fig. 3) it was shown that at 50 % conversion the ratio of the monoaldehyde *3g* to dialdehyde *4g* is about 10:1 (44 %:4 %). From 50–70 % conversion the yield of the monoaldehyde still increases faster than for the dialdehyde indicating a faster oxidation rate for the diol. This is further supported by the indirect electrochemical oxidation of *1g* by *2b* as mediator in acetonitrile/2,6-lutidine leading to a considerably higher selectivity in *3g*.

In correspondence with previously detected similar reactions [9–11] one could postulate the following mechanism:

$$Ar_3N \overset{\text{anode}}{\rightleftharpoons} Ar_3N^{\cdot+} + e^-$$

$$Ar_3N^{\cdot+} + PhCH_2OH \rightleftharpoons PhCH_2OH^{\cdot+} + Ar_3N$$

$$PhCH_2OH^{\cdot+} + B \rightarrow Ph-\dot{C}H-OH + HB^+$$

$$Ph-\dot{C}H-OH + Ar_3N^{\cdot+} \rightleftharpoons Ar_3N + Ph-\overset{+}{C}H-OH$$

$$Ph-\overset{+}{C}H-OH + B \rightarrow Ph-CHO + HB^+$$

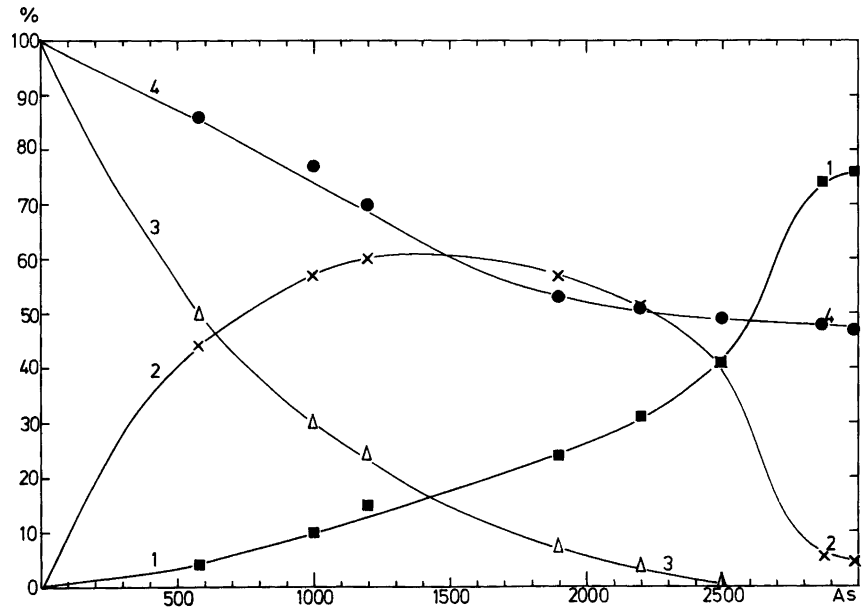
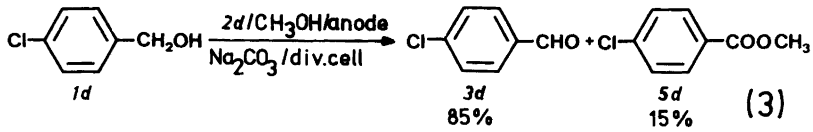


Fig. 3. Product distribution during the course of the indirect electrochemical oxidation of 1,4-bis(hydroxymethyl)benzene *1g* by *2d* as mediator in acetonitrile/Na_2CO_3 using a divided cell. Curve 1 (■): Material yield of dialdehyde *4g*; Curve 2 (×): Material yield of monoaldehyde *3g*; Curve 3 (△): Unconverted educt *1g*; Curve 4 (●): Current yield of *3g* and *4g*.



(3)

An alternative reaction path which possibly could take place by H-atom abstraction from benzylic alcohol by $Ar_3N^{\cdot+}$ could be excluded in the case of the cleavage of benzylic esters because the reaction rate of benzhydryl esters towards $2d^{\cdot+}$ is lower as compared with 4-methoxybenzyl esters.[12] This is in contradiction to a radical abstraction mechanism but in agreement with the oxidation potentials of the esters (4-(MeO)Ph–CH_2OCOR: $E_{1/2}$=+1.89 *vs.* NHE; $(Ph)_2$CHOCOR: $E_{1/2}$=2.70 V *vs.* NHE).

A mechanism based on the oxidation of the alcoholate anion by triarylammoniumyls could perhaps better accomodate a "non-bonded" electron transfer.[14] However, in this case an oxy-radical would be formed which either could react under hydrogen atom abstraction leading to the same problems as pointed out above, or it must further be oxidized. In both cases the observed potential dependence of the reaction rate indicating that the first electron comes from the benzylic part of the molecule is difficult to explain. Besides that, as the reaction also takes place under neutral conditions only small amounts of $PhCH_2O^-$ would be present.

To accommodate the large potential differences between mediator and substrate also an inner-sphere or "bonded" electron transfer mechanism [14] has to be taken into consideration.

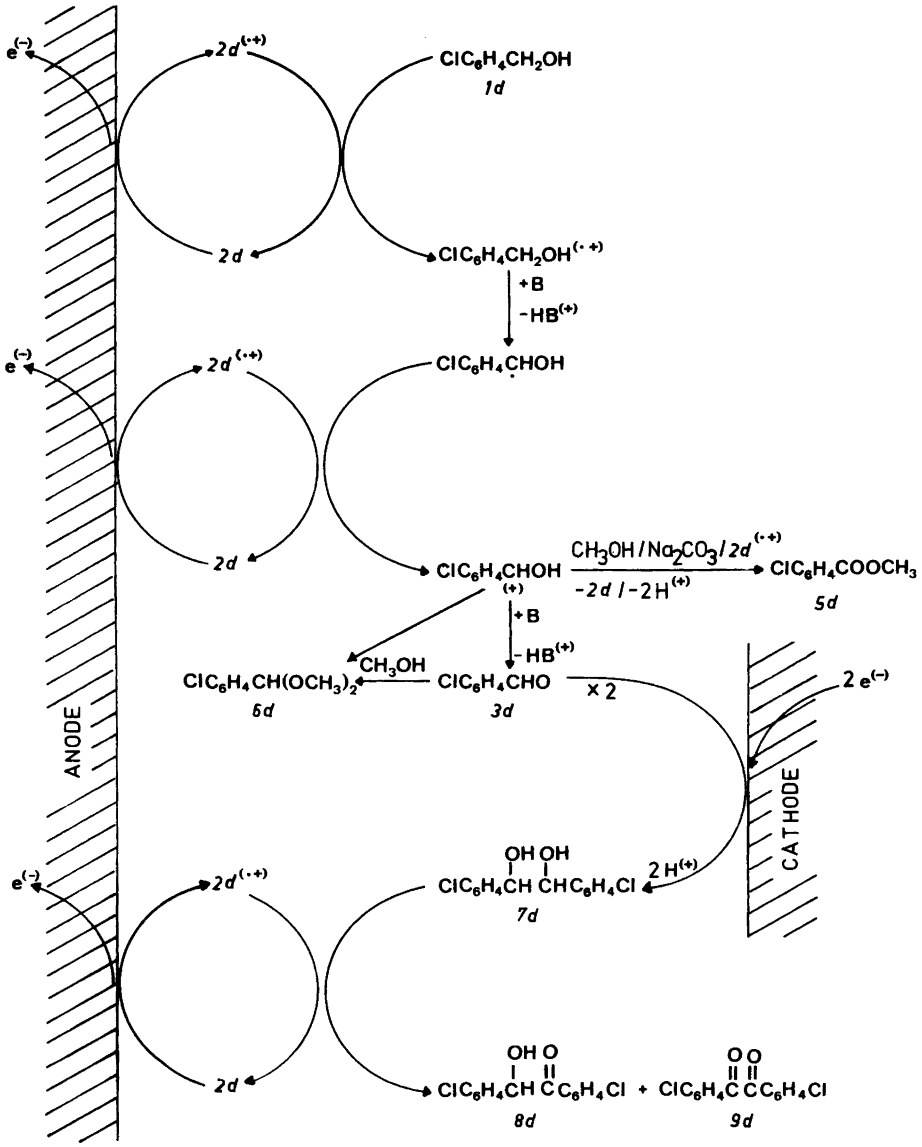
Preparative oxidation in methanol in a divided cell. Preparative oxidation of benzylic alcohols is also possible in methanol/Na_2CO_3 using a divided cell. In this case, as indicated by the electroanalytical experiments, a higher current density can be obtained as compared with acetonitrile. For example, 4-chlorobenzyl alcohol *1d* is anodically oxidized in the presence of *2d* (*1d*:*2d*=10:1) as a mediator using a CH_3OH/CH_2Cl_2 (3:1) solvent system to form 4-chlorobenzaldehyde *3d* and the corresponding methyl 4-chlorobenzoate *5d* in 85 %, respectively 15 %, material yield and 37 %, respectively 14 %, current yield after a conversion of 80 % (eqn. (3)). The current density reaches 5 to 10 mA/cm^2. Under identical conditions benzyl alcohol *1b* is transformed to yield benzaldehyde *3b* and methyl benzoate *5b* in 63 %, respectively 4 %, material yield and 30 %, respectively 4 %, current yield.

Preparative oxidation in methanol using an undivided cell. The indirect electrochemical oxidation of benzylic alcohols in an undivided cell using methanol as a solvent is complicated by the possibility of the cathodic reduction of the generated benzaldehyde to form the hydrodimer. The hydrodimer is oxidation labile forming benzil and benzoin. The direct cathodic pinacolization of benzaldehydes is favoured in alcoholic KOH at a mercury cathode.[15] The reaction pathways for

Table 3. Results of the indirect electrochemical oxidation of 4-chlorobenzyl alcohol *1d* by electrogenerated $2d^{\cdot+}$(*1d*:*2d*=10:1) in methanol/methylene chloride (3:1) in an undivided cell.

Exp. No.	Cathode material	Na_2CO_3 (g)	Conversion (%)	Aldehyde *3d* (%) [a]	Acetal *6d* (%) [a]	Ester *5d* (%) [a]	Dimers *7d*+*8d*+*9d* [b]
1	graphite	4	78	47 (18)	–	4.6 (3.5)	30
2	V4A-steel	4	88	39 (13)	–	23 (15)	20
3	platinum	4	59	55 (11)	–	40 (15)	<2
4	platinum	0	73	7 (3)	75 (29)	18 (17)	<0.5
5	graphite	0	82	10 (4)	62 (25)	21 (25)	3
6 [c]	graphite	0.5	79	69	–	10	20
	graphite	0.5	85	67 (28)	–	14 (12)	

[a] Material yield with respect to converted educt; current yields in parentheses. [b] Because of uncertainties in the GC-calibration of the dimers only *circa* values are given; the ratio of *7d*:*8d*:*9d* is about 2:1:1; *7d* is produced as *meso*- and *dl*-form in almost equal amounts. [c] Ratio *1d*:*2d*=20:1.



Scheme 1. Reaction scheme for the indirect electrochemical oxidation of 4-chlorobenzyl alcohol *1d* by *2d* as mediator in methanolic solution using an undivided cell.

the indirect electrochemical oxidation in the case of the system *1d*/*2d* are shown in Scheme 1.

In Table 3 the influence of the reaction parameters on the product distribution is summarized. It could be shown that the dimer formation is influenced by the cathode material and the pH of the solution. Changing the cathode material from graphite to platinum with a lower hydrogen overvoltage results in drastic reduction of the dimerization (Exp. Nos. 1 and 3). The same is true if one goes from basic to neutral conditions (Exp. Nos. 1 and 5). A different approach is the use of cathodes with a small active area as, for example, a steel rod in connection with a large anode area. Using a carbon felt anode together with a steel rod cathode benzyl alcohol *1b* under

the conditions of experiment No. 5 leads to the formation of only 10 % dimers together with 12 % benzaldehyde, 42 % benzaldehyde dimethyl-acetal and 23 % of methyl benzoate. Under neutral conditions the dimethyl-acetal is the major product (Exp. Nos. 4 and 5) while under basic conditions no acetal formation is observed and the aldehyde predominates. The formation of the methyl benzoates is favoured with increasing conversion (Exp. No. 6). There is also a complex influence of the cathode material and the pH on the ester production which, however, could not be resolved to date.

EXPERIMENTAL

The benzylic alcohols *1a–1g* were commercially available and purified by distillation, respectively recrystallization. The triarylamines *2a–2d* and the 9-phenylcarbazole derivative *2e* were synthesized as previously described.[16] Acetonitrile (Merck, for synthesis) was dried over P_2O_5 and K_2CO_3 and further purified by distillation. Methylene chloride was purified by distillation while methanol (Merck, p.a.) was used without further purification.

$LiClO_4$ (Fluka, Buchs) was applied as obtained. Products were identified by mass spectra and NMR spectra which were identical with those reported in the literature.[17,18] Yields were either determined by isolation or by GLC on a 4 % FFAP/Chromosorb W AWDCMS 100/120 mesh column (2 m×2 mm) using methyl *n*-alkanoates as internal standards.

Cyclic voltammetry. As current source a Wenking potentiostat, model PCA 72 L (Bank Elektronik, Göttingen), coupled to a function generator, model 133 LF (Wavetek) was applied. Current/voltage curves were recorded on Hewlett-Packard, model 7045, XY-recorder. A model EA 876-5 (Methrohm, Herisau) analytical cell was used together with a platinum (0.5 cm^2) or glassy carbon (0.05 cm^2) working electrode, a platinum counter electrode and an $Ag/AgNO_3$ (0.1 M in CH_3CN) reference electrode. Solvent supporting electrolyte was either CH_3CN (0.1 M $LiClO_4$) or CH_3CN/CH_3OH (2:1) (0.1 M $LiClO_4$).

Equipment for preparative electrolyses. Preparative electrolyses were performed either using a Wenking potentiostat, model ST 72 (Bank Elektronik, Göttingen), or a stabilized current source, model NTN 700M-200 (FUG, Rosenheim), modified as potentiostat together with a digital coulometer based on voltage-to-frequency conversion.*

* Design by H. Luftmann, Münster.

Preparative electrolyses in acetonitrile using a divided cell. General procedure. The mediators *2a–2e* (1 mmol) dissolved in 80 ml CH_3CN/CH_2Cl_2 (4:1)/(0.2 M $LiClO_4$) together with the base 2,6-lutidine (20 mmol), respectively Na_2CO_3 (3 g), were transferred to the anode chamber of a two-compartment beaker-type glass cell with cooling mantle. The cathode compartment was formed by a glass cylinder closed by a G-4 glass frit and filled with 20 ml CH_3CN (1 M $LiClO_4$) as catholyte. As electrodes were used a platinum sheet anode (12 cm^2), a carbon-felt cathode and an $Ag/AgNO_3$ (0.1 M) reference electrode together with a salt bridge. The cell was kept at 15 °C by water cooling. After application of the working potential for the oxidation of the mediator a pre-electrolysis was performed until 50 As had been passed and the colour of the triarylamine cation radical had appeared. Subsequent addition of the benzylic alcohols *1a–1g* (5 mmol) resulted in the disappearance of the colour and the increase of the current to 35–50 mA, respectively 180 mA in the case of *1a*. Electrolysis was continued until the current dropped to about 15 mA (*ca.* 1200–1500 As). For separation of the benzaldehydes from unreacted benzylic alcohols after evaporation of most of the solvent, the benzaldehydes were converted into the Schiff base salts of sodium ε-aminocaproate according to the literature [19] and extracted into the aqueous phase. After acidification (pH 5–6) the aldehydes could be extracted by ether and further purified by bulb-to-bulb distillation. Yields determined by GLC were nearly identical to the isolated ones. The products were identified by melting points and NMR spectra which in all cases were identical with literature values. The formation of side products could be excluded by GLC analysis of the crude solution. In the case of *1g* the progress of the electrolysis was also followed by GLC.

Preparative electrolysis in methanol in a divided cell. The mediator *2d* (0.5 mmol) dissolved in 80 ml CH_3OH/CH_2Cl_2 (3:1)/0.2 M $LiClO_4$ together with 2 g Na_2CO_3 was transferred to the anode chamber of a divided beaker-type glass cell (see above). The cathode compartment was filled with 20 ml CH_3OH/0.3 M $LiClO_4$. As electrodes were used a glassy carbon anode (12 cm^2), platinum cathode (3 cm^2) and Ag/AgCl-reference electrode together with a salt bridge. The cell was kept at 15 °C by water cooling. After application of the working potential of +1.56 V a pre-electrolysis was performed until 50 Coulombs had been passed. By addition of 5 mmol *1d* the current increased to about 80–90 mA. After consumption of 2000 Coulombs the electrolysis was worked up as described above.

Preparative electrolysis in methanol in an undivided cell. General procedure. The mediator *2d* (1 mmol) (Exp. No. 6: 0.5 mmol) dissolved in 100 ml CH_3OH/CH_2Cl_2 (3:1)/0.2 M $LiClO_4$ together with the amount of base shown in Table 3 was transferred to an undivided beaker-type glass cell which was equipped with a glassy carbon anode (18 cm^2) and Ag/AgCl reference electrode. The material for the cathodes (18 cm^2) was varied according to Table 3. The cell was kept at 15 °C by water cooling. After application of the working potential of +1.56 V a pre-electrolysis was performed until 50 Coulombs had been passed. By addition of 10 mmol *1d* the current increased to 100 mA. After consumption of about 4000 Coulombs the electrolyses were worked up. The yields were determined by GLC and the products isolated by HPLC on a 50 cm×1.6 cm Si-60 (7 μm, Merck) column using CH_2Cl_2/ethyl acetate (15:1) as eluent. The products were identified by comparison of their MS spectra, respectively NMR spectra, with literature data.

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Electrogenerated Bases. VI. Reaction of Electrogenerated Superoxide with Some Carbon Acids. II.*

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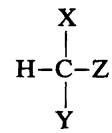
Electrogenerated superoxide and molecular oxygen were allowed to react sequentially with a number of esters, nitriles, *N,N*-dialkylamides, sulfones, and aliphatic nitro compounds. The α-methyl groups in these compounds bore aliphatic and/or aromatic substituents. When the electron-withdrawing group (EWG) of these carbon acids could be displaced intact (nitrile *2*, $ArSO_2$ *4*, nitro) good to excellent yields of the corresponding carbonyl compounds could be obtained. The efficiency of the transformation depended upon the nature of the substitutents: α,α-diphenyl (*e.g.*, *2a*) >α-methyl-α-phenyl (*2b*)>α,α-dimethyl. By conducting the electrolysis in the presence of acetic anhydride it was shown that the known conversion of phenylacetonitrile (*2d*) to benzoic acid did indeed proceed *via* benzaldehyde. When the EWG itself could be cleaved (esters *1*, *N,N*-dialkyl-amides *3*), this methodology produced α-hydroxylated compounds and the products resulting from fragmentation of the EWG and also from its complete displacement. The effects of the α-substituents were similar to those above.

Superoxide may function as a radical, an electron-transfer agent, a nucleophile, or a base. Examples of these behaviors are cited in Ref. 2. As part of a broader program in synthetic utilization of electrogenerated bases (EGB), we have here focused upon the behavior of carbon acids RH (electroinactive in these systems) of adequately low pK_a toward a stream of O_2 which is partially reduced to form the EGB $O_2^{\cdot-}$, *i.e.*, upon the sequential reactions $RH+O_2^{\cdot-}\rightarrow R^-$, $R^-+O_2\rightarrow$products. (The deprotonation of the *O*-acid n-BuOH (pK_a 33.3) is reported in Ref. 3.) For RH to be amenable to deprotonation by $O_2^{\cdot-}$ it must have within R one or more electron-withdrawing groups, so that a more appropriate general structural formula encompassing the types of compounds whose behavior is reported here is given by *1*:



X=H, alkyl, aryl, RO ArO, *etc.*
Y=Z or H, alkyl, aryl
Z=electron-withdrawing group

1a, X=H, Y=CH_3, Z=COOEt
1b, X=H, Y=C_6H_5, Z=COOEt
1c, X=Y=CH_3, Z=$COOCH_3$
1d, X=Y=C_6H_5, Z=$COOCH_3$
1e, X=H, Y=Me_2CH, Z=COOEt
1f, X=H, Y=$EtOCOCH_2$, Z=COOEt
1g, X=H, Y=$EtOCH_2CH_2CH_2$, Z=COOEt

It was anticipated [2] that the nature of the reaction sequence would depend upon the identity of Y, particularly whether it could be displaced intact (*e.g.*, $-NO_2$) or in part (*e.g.*, COOR) or not at all, upon the pK_a of *1*, and upon steric and electronic effects inherent in X and Y. For a series of malonate esters (1, Y=Z=COOR)

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reactions depended upon structure; products representing either oxygenation of the intact molecule or oxygenation and cleavage were obtained. A monoalkyl acetate was usually only saponified in a well precedented reaction [4] while an alkyl monophenyl acetate was oxygenated and cleaved.[2] In all cases when X or Y in *1* was H difficulties arose.

We report here an extension of this methodology to selected additional esters, nitriles, *N,N*-dialkylamides, sulfones, and nitro compounds. New, convenient and useful syntheses of oxygenated species have emerged from this work; elucidation of mechanisms remains to be accomplished.

Esters. The behavior of alkyl *mono*-substituted acetates (*1a*, X=H, Y=CH_3, Z=COOEt; *1b*, X=H, Y=C_6H_5, Z=COOEt) has been reported.[2] It must be added that *1a* gave erratic results with $O_2^{\cdot-}+O_2$, sometimes showing no reaction [2] and sometimes yielding a mixture of ethyl lactate (15 %) and lactic acid (35 %); the latter obviously is a product of α-hydroxylation and saponification. We have not yet resolved these discrepancies.

Alkyl *di*-substituted acetate (*1c*, X=Y=CH_3, Z=$COOCH_3$; *1d*, X=Y=C_6H_5, Z=$COOCH_3$) gave more promising yields of α-hydroxylated compounds: *1c* was hydroxylated and saponified to 40 % of 2-hydroxy-2-methylpropionic acid, *1d* was hydroxylated and cleaved to methyl 2,2-diphenyl-2-hydroxyacetate (45 %) and benzophenone (10 %), respectively. Cleavage when α-phenyl-groups are present seems to be usual.[2]

Mono-esters which are dialkylated at positions remote from the α-position (*e.g.*, *1e*, X=H, Y=Me_2CH, Z=COOEt) gave only, inseparable mixtures of compounds.

Unlike *gem*-diesters (malonates) but like acetates, α,ω-diesters in which the COOR groups were separated by n(CH_2) reacted poorly: diethyl succinate (*1f*, X=H, Y=$EtOCOCH_2$, Z=COOEt) yielded only 4 % of *dl*-tartaric acid while diethyl adipate (*1g*, X=H, Y=$EtOCH_2CH_2CH_2$, Z=COOEt) did not react at all.

The new data for esters are summarized in Table 1. The limitations of $O_2^{\cdot-}$ as an EGB are pointed out by contrasting our results with those obtained when methyl esters were converted at −75 °C to their enolate anions by the very strong base lithium *N*-cyclohexyl−*N*-isopropylamide and then oxygenated: good yields (43−69 %) of α-hydroperoxy esters resulted. Depending upon structure, the latter decomposed thermally to mixtures of products including the α-hydroxy esters.[5]

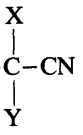
Nitriles. To the best of our knowledge there is only one report in which $O_2^{\cdot-}+O_2$ have been allowed to react with nitriles:[6] phenylacetonitrile

Table 1. Behavior [a] of selected esters toward $O_2^{\cdot-}+O_2$.

Ester (No.)	Products	Isolated yield, %
$MeCH_2COOEt$ (*1a*)	$MeCH(OH)COOEt$	15
	$MeCH(OH)COOH$	35
$Me_2CHCOOMe$ (*1c*)	$Me_2C(OH)COOH$ (MW 104)	40
$Ph_2CHCOOMe$ (*1d*)	$Ph_2C(OH)COOMe$ (MW 242)	45
	Ph_2CO (MW 182)	10
Me_2CH COOMe (*1e*)	complex mixture	–
$(CH_2COOEt)_2$ (*1f*)	CH_2COOEt *dl-*	
	$HOCHCOOEt$ *meso-*	4
$(CH_2CH_2COOEt)_2$ (*1g*)	no reaction	

[a] Divided cell, Hg cathode, Pt anode. Dry DMF+0.1 M Et_4NBr. Ester and stream of O_2 in catholyte, cyclohexene in anolyte. T=20 °C. Cathode voltage −0.9 to −1.0 *vs.* SCE. 1 F/mol was passed.

was converted to benzoic acid, presumably *via* PhCHO. Superoxide is capable of deprotonating CH_3CN but in the work done the $(CH_2CN)^-$ was not exposed to oxygen but rather trapped by PhCHO.[7] Anions of *sec*-nitriles were (a) prepared at −78 °C *via* lithium diisopropylamide (LDA); (b) the anions reacted with O_2 to yield (c) after quenching with aqueous acid or acetyl chloride, hydroperoxide derivatives which were (d) reduced by Sn(II) to cyanohydrins; the latter on treatment (e) with alkali provided the final product ketones in excellent yields.[8] Aromatic nitriles [4] are inert toward $O_2^{\cdot-}$.



2a, X=Y=Ph
2b, X=Ph, Y=Me
2c, X=Y=Me
2d, X=H, Y=Ph
2e, X=H, Y=Me
2f, X=Y=H

Electroreduction of O_2 in the presence of selected nitriles *2* under our "standard" conditions (see Experimental) provides a convenient "one-pot" method for oxidative decyanation. The data are gathered in Table 2.

Good yields of ketones were obtained from *sec*-nitriles containing at least one aromatic substituent (*2a, 2b*). Isobutyronitrile (*2c*) gave only 20 % of acetone. Due to the anticipated instability in this system of aldehyde intermediates produced from primary nitriles,[6] electrolyses in these cases were carried out both in the absence and in the presence of acetic anhydride. Primary nitriles (*2d, 2e*) were converted to carboxylic acid in the absence of Ac_2O and to the aldehyde diacetates in its presence. Acetonitrile (*2f*) behaved similarly. In all cases conversions were poor when only aliphatic substituents or H were at the α-position; the inadequately low pK_a of these derivatives toward reaction with $O_2^{\cdot-}$ is of greater importance than the small steric effects of the Ph groups in *2a–2b*, and *2d*.

In the case of the nitriles *2* and of the classes of compounds discussed below it was of interest to attempt to determine the fate of the leaving group (Z in *1*). For this purpose the products of

Table 2. Reaction of $O_2^{\cdot-}+O_2$ with selected nitriles.[a]

Nitrile (No.)	*F* mol	Products	Yields (%)
Ph_2CHCN (*2a*)	0.8	Ph_2CO	95
		MeNCO and trimer [b]	75
$PhCH(CH_3)CN$ (*2b*)	1.0	$PhCOCH_3$	72
		2b	20
Me_2CHCN (*2c*)	1.0	$(CH_3)_2CO$	20
		2c	74
$PhCH_2CN$ (*2d*)	1.8	PhCOOH	89
	1.8 [c]	PhCOOH	27
		$PhCH(OAc)_2$	54
CH_3CH_2CN (*2e*)	1.0	CH_3COOH	15 [d]
	1.0	CH_3COOH	5 [d]
		$CH_3CH(OAc)_2$	18 [d]
CH_3CN (*2f*)	0.22	HCOOH	0.18 [e,f]
	0.22 [c]	HCOOH	0.04 [e,f]
		$CH_2(OAc)_2$	0.14 [e,f]

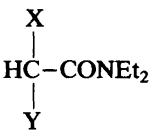
[a] H-cell, Hg cathode, Pt anode. DMF/Bu_4NBr. O_2 stream, −1.0 V *vs.* SCE. 4–5 mmol of substrate. [b] After addition of MeI to the catholyte. See text. [c] Ac_2O present. [d] 75–80 % of *2e* recovered. [e] mol/F. [f] 70–73 % of *2f* was recovered.

the reaction of *2a* which had given the cleanest conversion to ketone were carefully examined. Addition of methyl iodide at the end of the electrolysis to react with *in situ* $R_4N^+CN^-$ or $R_4N^+CNO^-$ yielded only MeNCO and its trimer, trimethyl isocyanurate. No conclusions, however, can be drawn about the initial expelled fragment since control experiments showed that in the absence of *2*, the CN^- of Bu_4NCN is inert to O_2 but is oxidized to the same end products by $O_2^{\cdot-}$.

N,N-*Dialkylamides*. Both benzamide and *N*-(*α*-phenylethyl)acetamide have been reported [4] to be inert toward $O_2^{\cdot-}$. However, when *N*-substituted amides were first converted to their anions by LDA at 0 or −78 °C and then oxygenated, they yielded *α*-hydroperoxides which were reduced by aqueous sodium sulfite to give excellent overall yields of *α*-hydroxy-*N*-substituted amides. No C–C cleavage products were found nor presumably were C–N cleavage products.[9] The sequence succeeded even with RCH_2CONMe_2.

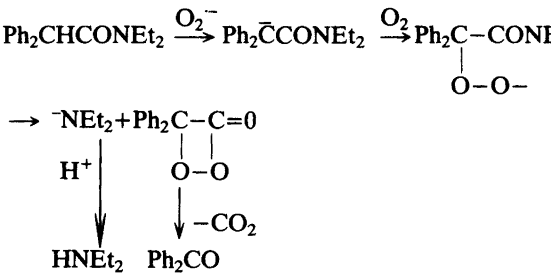
One of the mechanisms proposed for the hydrolysis of esters by superoxide involved initial nucleophilic attack upon the carbonyl of the ester group with expulsion of ^-OR. It was of interest to us, *inter alia*, to determine whether a similar cleavage (with the less likely expulsion of $^-NR_2$) would occur with *N,N*-disubstituted amides.

As was the case with esters, the amides *3*, depending upon structure,



3a, X=Y=Ph
3b, X=Ph, Y=Me
3c, X=Y=Me
3d, X=Ph, Y=H

yielded *α*-hydroxylated and cleavage products or did not react at all (*3c* in Table 3). The similarity in behavior of *3d* and *1d* (Table 1) is striking; the fragmentation products (Et_2NH and CO_2) were collected and identified. A possible sequence of reactions, similar to those proposed previously for the cleavage of ethyl phenylacetate,[2] is



Of potentially great synthetic importance is the report that *α*-hydroperoxides of esters, amides, ketones and nitriles epoxidize a number of

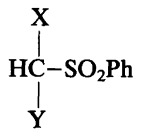
Table 3. Reaction of $O_2^{\cdot-}+O_2$ with selected *N,N*-diethylamides.[a]

Amide (No.)	*F* mol	Products	Yields (%)
$Ph_2CHCONEt_2$ (*3a*)	1.5	Ph_2CO	54
		$Ph_2C(OH)CONEt_2$	40
		CO_2	30
		Et_2NH	21
$Ph(CH_3)CHCONEt_2$ (*3b*)	1.2	$PhCOCH_3$	trace
		$Ph(CH_3)$ C(OH) $CONEt_2$	53 [b]
Me_2 $CHCONEt_2$ (*3c*)	1.5	no reaction	–
		3c	80
$PhCH_2CONEt_2$ (*3d*)	1.2	PhCOOH	5
		$PhCH(OH)CONEt_2$	30
		unknown	–

[a] H-cell, Hg cathode, Pt anode. DMF/Bu_4NBr. 4.0 mmol substrate. O_2 stream; −1.0 V *vs.* SCE. [b] 30 % of starting material was recovered.

olefins, albeit sometimes very slowly.[10] The presumed hydroperoxide anion intermediates formed by the action of $O_2^{\cdot-}+O_2$ on the compounds discussed in our paper participate in several competitive followup reactions: transfer of oxygen to the parent carbanion,[2] nucleophilic displacement of a leaving group, reduction to alcohol; their effectiveness as epoxidizing agents will require that means be found for suppressing all other available routes. Up to the present, very small yields (<5 %) of epoxides have been obtained from styrene and stilbene, respectively, by including these olefins in electrolytic experiments with *3a*. Styrene alone with $O_2^{\cdot-}$ is reported [11] to yield no oxygenated products.

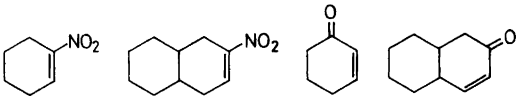
Sulfones. Only two representatives of *4* were studied in order to augment the generality of the behaviour of *1* when Z is an intact leaving group.



4a, X=Y=Ph
4b, X=Me, Y=Ph

4a, after passage of 2 F/mol, yielded 90 % of benzophenone; *4b* (1.5 F/mol) gave 25 % of acetophenone. Addition of MeI to the catholyte after electrolysis in the presence of *4a* produced 65 % of methyl benzenesulfonate; the latter is also formed from sulfinate (as $Bu_4N^+SO_2Ph^-$) with O_2 alone or $O_2+O_2^{\cdot-}$. These results contrast with those reported above for *2a*.

Nitro compounds. The very facile conversion by this methodology of a variety of *sec*-aliphatic nitro compounds, including those containing other functionalities such as keto-, ester, aryl to their corresponding ketones has been reported separately.[1] Additional examples, incorporating new features, have now become available. *Primary nitro compounds* (I, X or Y=H, Z=NO_2), as do other active methylene compounds with α-methylene rather than α-methinyl, reacted poorly. 1-Nitrononane after short electrolysis (0.62 F/mol) yielded 36 % nonanal. Starting material (16 %) was recovered but no carboxylic acid was found. Longer electrolysis (1 F/mol) yielded an unresolved mixture of products not containing acid. An attempt to intercept the intermediate aldehyde by reducing O_2 in the presence of 1-nitropentane and Ac_2O was unsuccessful: 16 % of pentanoic acid and 33 % of starting material was obtained but no aldehyde diacetate. Nitrocycloolefins (*e.g.*, *5*, *6*) were converted to the



5 *6* *7* *8*

respective α,β-unsaturated ketones in good yield. Nitro compounds under basic conditions are known [24] to form hydroxamic acids which decompose on hydrolysis to carboxylic acids.

EXPERIMENTAL

Study of esters.[12] A conventional H-cell with a G5 sintered glass diaphragm was used. The cathode was a 20 cm^2 Hg pool, the anode a Pt foil. The potentiostat was manufactured by Ultraschalltechnik Co. of Halle (DDR) The ^1H NMR spectra (in $CDCl_3$ with hexamethyldislane or tetramethylsilane as internal standards) were obtained using an 80 MHz instrument BS 847C of Tesla; the mass spectra employed a Varian Match 6. For thin layer chromatography (0.2 mm) DC Alufolien Kieselgel 60 (Merck) was used; for preparative chromatography (PC) 2 mm. Developing agents were benzene (B), ethyl acetate (E) and methanol (M).

The *general electrolysis procedure* was as follows. About 1–3 g of starting ester, 100 ml of dry DMF together with 10 g of activated neutral Al_2O_3 dried at 400 °C at 5 Torr for at least 5 h [13] and 0.01 mol of Et_4NBr were charged into the catholyte. The anolyte contained solvent/supporting electrolyte and 5 ml of cyclohexene. A stream of dry O_2 was bubbled through the catholyte. Electrolysis at −0.9 to −1.0 V *vs.* SCE was stopped after passage of 1 F/mol. Workup followed one or more of these procedures: (A) The DMF was removed from the catholyte *in vacuo;* the residue was extracted repeatedly with ether; the ether extract was washed with water, dried and evaporated to remove solvent. (B) like (A) except that the water washes were re-extracted with ether+$CHCl_3$, the extracts washed with a minimum of water, dried and combined with the above extracts. (C) The DMF was removed under N_2. The residue at 0 to −5 °C was brought to pH 10 and taken to dryness. The new residue was dissolved in the minimum amount of H_2O, adjusted to pH 1 with dilute HCl, and extracted several times with $CHCl_3$.

The extracts were washed minimally with water, dried and the solvent evaporated. (D) The catholyte at 0 to −5 °C was added to 200 ml of 3.5 % HCl. Distillation under N_2 to 155 °C yielded a distillate containing DMF, HCl/H_2O and products. The distillate was brought to pH 10 with 1N NaOH and evaporated to dryness under N_2. The residue was then treated as in (C).

Methyl isobutyrate (1c). 2.05 g of ester, after electrolysis, yielded 0.97 of crude product which after purification by PC using B:E:M in the ratio 1:2:1 yielded purified α-hydroxyisobutyric acid (R_F 0.35−0.45). After sublimation at 49 °C the product had R_F 0.39. The IR spectrum showed OH at 3450 cm^{-1} and >C=O at 1695 cm^{-1}. The MS (70 eV) showed *m/e* (%): 104(3), 73(100), 59(82). ^1H NMR ($CDCl_3$): δ 2.72 (S, 3H, CH_3), 2.82 (S, 3H, CH_3) and 7.94 (S, 1H, OH). The product was identical with an authentic sample.

Methyl diphenylacetate (1d). Electrolysis of 2.26 g at −0.95 V was followed by workup (A) to yield 1.25 g of crude. Purification by PC using B:E of 10:1 yielded *1d* (R_F 0.23), methyl α-hydroxydiphenylacetate (R_F 0.5) and benzophenone (R_F 0.7) The purified hydroxy-exter (R_F 0.52), a viscous yellow oil, was 45 % of the crude mixture. In the IR it showed absorption at 1725 cm^{-1} (>C=O) and at 3450 cm^{-1} (OH). The ^1H NMR (δ) showed a singlet for 3H of OCH_3, a singlet at 4.11 (OH, exchangeable with D_2O) and a multiplet for 10 aromatic H at 7.15−7.50. The MS (70 eV) gave *m/e* (%): 242(12), 184(23), 183(100), 165(7), 152(8), 106(20), 105(95), 78(12) and 77(16). The benzophenone, 10 % of the crude after further purification, had R_F 0.68 and IR spectrum identical to that in the literature.[14] The MS (12 eV) showed *m/e* (%): 182(100), 105(81), 77(66).

Diethyl succinate (1f). Electrolysis at −0.9 V and workup (B) gave 0.8 g crude product purified by PC using B:E:M at 1:2:1. About 4 % of *dl*- and *meso*-diethyl tartrate was obtained with IR spectrum identical to that reported.[15]

Study of the other carbon acids. Equipment. An H-cell with medium porosity sintered glass separator was used. The cathode chamber was provided wth ports for gas inlet (glass frit), luggin capillary leading to an SCE, and removal of samples. The cathode was Hg (*ca.* 7.1 cm^2) and the anode a Pt foil (6.25 cm^2). Each compartment held *ca.* 60 ml. The potentiostat was a Wenking 70 HV1/90 and the digital coulometer model 640 supplied by the Electrosynthesis Co. Analytical gas chromatography employed the Hewlett Packard model 5830A equipped with the HP 18850 data system; the column used was 1/8 in. ×0.5 m. 6 % SE-30. ^1H NMR spectra were taken on a Varian T-60 spectrometer (Me_4Si as internal standard); IR spectra on a Perkin-Elmer 283, and mass spectra on a VG ZAB-2F. For preparative column chromatography there were used (a) a gravity glow silica gel column (Merck, silica 60, 70−230 or 230−280 mesh), (b) a Chromatotron (Harrison Research, Model 7924) with a 2 mm thick silica gel rotor (Merck, silica 60, PF-254) and (c) an Aerograph A-90P preparative GLC unit equipped with a 6 mm×1.5 m 5 % SE-30 column whose temperature was 150 °C. For thin layer chromatographic analysis Aldrich phosphomolybdic acid and an UV handlamp were used.

Materials. Acetonitrile (Mallinckrodt, spectral grade) was purified only by passage through a column of Sigma neutral alumina before use; the same solvent from other sources was first distilled under N_2 from P_2O_5 before the alumina treatment. Dimethylformamide was vacuum distilled first from calcium hydride then from anhydrous $CuSO_4$. It was stirred over freshly activated 3A molecular sieves and passed through a column of alumina directly before use. Tetrabutylammonium bromide (Southwestern Analytical Chemicals, electrometric grade) was used as received. Molecular oxygen (Linde, 99.3 %) was passed through a 2×30 cm tube containing Drierite and NaOH pellets and then through 10A Molecular Sieves (Sigma) before it was admitted into the cell. The nitriles *2* were purchased from Aldrich and purified by distillation or recrystallization as appropriate. Bu_4NCN in DMF was made by double decomposition of Bu_4NCl (Aldrich) and NaCN (Mallinckrodt) in this solvent and used directly after removing the NaCl by filtration. The *N,N*-dialkylamides *3* were prepared from the acid chlorides and diethylamine in ether [16] and purified by distillation under reduced pressure. Each showed only one component by GLC. The sulfones *4a* [17] and *4b* [18] were prepared according to the literature. IR, ^1H NMR and MS data as well as melting points were in agreement with the structures indicated. Tetrabutylammonium benzenesulfonate was prepared by acidifying the sodium salt (Aldrich), extracting into ether, evaporating to dryness, neutralizing with 40 % Bu_4NOH (Aldrich) and again concentrating and drying *in vacuo*. 1-Nitropentane [19] and 1-nitrononane [20] were prepared according to the literature as were 1-nitro-1-cyclocyclohexane (*5*) and bicyclo[4.4.0]nitro-$\Delta^{2,3}$-decene (*6*).[21]

General procedure. The oven-dried H-cell was charged under Ar with 60 ml of solvent and 0.2 M n-Bu_4NBr in each compartment. The solutions were pre-electrolyzed at −1.2 or −1.9 V *vs.* SCE until the background current decayed to 10 and 1 mA, respectively. Cyclohexene (*ca.* 1 ml) was added to the anolyte to trap liberated bromine and *ca.* 4 mmol of substrate was added to the

catholyte. Oxygen was bubbled through the catholyte; the cathode potential was controlled at −1.0 V. The consumption of the starting material was monitored by periodic TLC or GLC analysis. The workup procedure depended on the solvent that had been used: (a) when it was DMF the catholyte was poured into 60 ml of ice-water, the solution extracted with 5×100 ml of ether, the ether extracts washed with water, dried over $MgSO_4$, filtered and taken to dryness; when it was CH_3CN, about 80 % of the solvent from the catholyte was removed using a rot-o-vap with water aspirator, ether was added, the combined solutions washed with water and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Deviations from this general procedure, necessitated by the physical properties of the starting material or products, are noted below.

Diphenylacetonitrile (2a). The general procedure (DMF) was followed. The ether residue after workup was benzophenone identical with an authentic sample. In other experiments designed to identify the composition of the leaving fragment, the electrolyzed catholyte (pH 10) was brought to pH 8–9 by addition of HOAc. Argon was bubbled through the solution to displace excess O_2 and MeI was added. After one hour's stirring GLC analysis (SE-30 column) showed the presence of a small quantity of methyl isocyanate. After addition of water to the catholyte, a white precipitate was formed which, after recovery by filtration and recrystallization from ether–methanol, was shown to be trimethyl isocyanurate, m.p. 176 °C, unchanged by admixture with an authentic specimen.[22]

2-Phenylpropionitrile (2b). Electrolysis under the general conditions (DMF) yielded 72 % of acetophenone and 20 % of unchanged starting material which were separated by column chromatography (ethyl ether–pentane). Each component was identical to a respective authentic sample.

Isobutyrnitrile (2c). Since it was anticipated that the expected product, acetone, would be swept out of the cell by the O_2 stream, a trap containing aqueous 2,4-dinitrophenylhydrazine sulfate was attached to one of the ports of the cathodic chamber. After the electrolysis, the derivative was found in the trap and no acetone, as judged by the same test, remained in the catholyte. Aqueous workup of the latter yielded the amount of starting material indicated (Table 2).

Phenylacetonitrile (2d). The starting material (3 mmol) was entirely consumed after 1.8 F/mol had been passed according to the general procedure (DMF). The workup procedure depended upon whether or not Ac_2O (3 mmol) had also been added to the catholyte. In the absence of the anhydride the electrolyzed catholyte was brought to pH 3 by dilute HCl and extracted with several portions of ether. The ether extracts were washed, dried over $MgSO_4$, filtered, and evaporated to dryness yielding authenticated PhCOOH. When Ac_2O had been included in the catholyte, the latter was diluted with cold water and directly extracted with ether. From the extract authenticated benzaldehyde diacetate was isolated.[23] The catholyte was then acidified to pH 3, extracted with ether, *etc.* to recover PhCOOH.

Propionitrile (2e) and acetonitrile (2f). In each case, a Dry Ice–acetone cold finger was attached to the cathode chamber. The results given in Table 2 are typical of many experiments in which the system DMF/n-Bu_4NBr was used. The ratio of Ac_2O to substrate was varied from 0 to 10. Generally a current of 85–110 mA was passed until 2000–2500 coulombs had been used. Products and unchanged starting materials were determined by GLC.

Tetrabutylammonium cyanide. The DMF solution of this cyanide as prepared above was charged to the cathode compartment of the cell. Bu_4NBr was added and O_2 was bubbled through for 2 h. A sample was removed, treated with MeI and then examined by GLC. MeCN but no MeNCO or isocyanurate was detected. The remainder of the catholyte was electrolyzed similarly to *2a*; after displacement of O_2 the catholyte was treated with MeI, *etc.*, yielding MeNCO and its cyclic trimer.

N,N-*Diethyl diphenylacetamide* (3a). Two traps, one with saturated $Ba(OH)_2$ the other with 1 M HCl were attached to the cathode chamber. Standard electrolysis (DMF) with 4 mmol of *3a* was carried out until the starting material had been consumed (1.5 F/mol). $BaCO_3$ and $Et_2NH \cdot HCl$ were recovered from the respective traps. Usual workup of the catholyte followed by column chromatography of the ether extracts on silica gel allowed separation of 2.2 mmol (54 %) of benzophenone and 1.6 mmol (40 %) of the α-hydroxylated *N,N*-diethylamide, m.p. 90 °C, identical with a sample prepared according to the literature.[9]

N,N-*Diethyl 2-phenylacetamide* (3b). The procedure was similar to that used with *3a* except that only 1.2 F/mol was arbitrarily used for the solution containing 4 mmol of *3b*. The α-hydroxyamide, m.p. 84 °C, was identical to the sample prepared according to Ref. 9.

N,N-*Diethyl phenylacetamide* (3d). Electrolysis was as in *3a*, using 4 mmol of substrate and 1.8 F/mol. Workup of the acidified catholyte yielded the PhCOOH and the α-hydroxyamide,[9] m.p. 43 °C.

Diphenylmethyl phenylsulfone (4a). Standard electrolysis of a DMF solution containing 4 mmol of *4a* was run until all the starting material had been consumed. The O_2 was displaced by Ar, MeI was added and the solution stirred for 1 h. Volatile materials were removed under reduced pressure. Addition of ether to the residue caused precipitation of n-Bu_4NBr which was removed by filtration. Column chromatography of the ether solution followed by elution with ether–pentane yielded 3.7 mmol (91 %) of benzophenone and 2.4 mmol (60 %) of methyl benzenesulfonate.

1-Phenylethyl phenyl sulfone (4b). 1 F/mol was used for the electrolysis of 1.6 mmol of *4b*. The usual workup afforded 0.4 mmol (25 %) of acetophenone and unchanged starting material (0.91 mmol, 64 %).

Tetrabutylammonium benzenesulfinate dissolved in DMF+Bu_4NBr and treated with O_2 alone or O_2+$O_2^{\cdot-}$ yielded after methylation methyl benzenesulfonate.

Nitro compound 5. The SSE was CH_3CN/n-Bu_4NBr. Electrolysis in the presence of 2.66 mmol of *5* at −1.1 V *vs.* SCE for 43 mins (1F/mol) yielded 73 % of cyclohexenone (*7*) as determined by GLC analysis on a 1.0 m 15 % SE-30 column using biphenyl as an internal standard and 46 % isolated yield (Kugelrohr distillation at 170 °C). ^1H NMR and IR spectra agreed with the expected. More by-products were obtained at a less negative cathode potential.

Nitro compound 6. The same SSE system as above was used. Electrolysis using 1.2 mmol of *6* at −1.0 V *vs.* SCE for 25 min (0.86 F/mol) yielded 59 % of the enone *8* and 20 % recovered *6*. The compounds were separated by column chromatography using 15 % ether–Skelly Solve. The ^1H NMR and the IR spectra for *8* were satisfactory. Further, *8* was reduced completely with $NaBH_4$ in 1:1 ether–ethanol to the saturated alcohol identical by TLC, IR and GLC criteria with an authentic sample; the saturated alcohol on oxidation by the Jones Reagent yielded 2-decalone identical with a commerically available sample.

1-Nitrononane and 1-nitropentane. The same procedures as above were followed. The results have been presented in the Discussion.

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Electroreduction of Organic Compounds. 4.* Electrochemical Reduction of Mono- and Bis-dithiobenzoate Esters in the Presence of Bi- or Monofunctional Electrophiles

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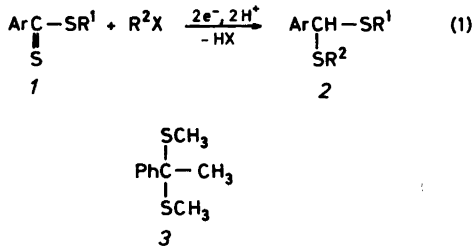
Co-electroreduction of methyl dithiobenzoate (*1a*) and 1,3-dimesyloxypropane (*5*) in acetonitrile yields the tetrahydrodithiepine *6*, whereas the thiirane *9* is formed from trimethylene-1,3-bis-dithiobenzoate (*7a*) and methyl iodide. The bis-thioacetals *8* are obtained, if *7a* and its homologue *7b* are electrolized together with methyl iodide; the heterocyclic bis-thioacetal *10* is formed from *7c* as a mixture of the *meso*- and *d,l*-diastereomers in acetonitrile.

Alkyl dithiobenzoates (*1*) are easily electroreduced in the presence of alkylating agents according to eqn. (1). Under various conditions thioacetals (*2*) are formed as main products in reasonable yields,[1,2] whereas C-alkylation occurs only in dry DMF at a Pt-cathode yielding acetophenone thioacetal (*3*).[3]

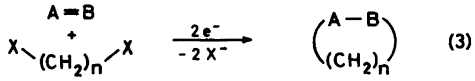
On the other hand, the stilbene derivatives *4* which are formed as minor by-products in the presence of alkyl iodides [1,2] are obtained in good yields, if the latter are absent [4] (eqn. (2)).

Stimulated by the work of C. Degrand and coworkers,[5] who established the cyclization shown in eqn. (3) as an interesting type of electroorganic reaction, we became interested in the products – possibly heterocycles – that could be formed, if bifunctional electrophiles and bis-dithioesters were used in the electrolyses.



(1)

(2)



(3)

ELECTROLYSES

Methyl dithiobenzoate and bis-electrophiles. Electroreduction of methyl dithiobenzoate (*1a*) in dry acetonitrile in the presence of 1,3-dimesyloxypropane (*5*) yielded 30 % of a pale yellow crystalline compound, which could easily be isolated from the catholyte by extraction with light petroleum. Elemental analyses and spectroscopic data (*cf.* Experimental) were consistent with the structure of dithiepine *6*. This could be further confirmed by independent synthesis from benzoin and propane-1,3-dithiol (eqn. (4)).

* Part 3. See Ref. 4.

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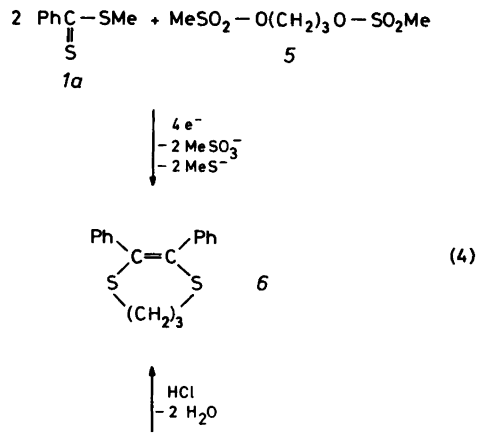
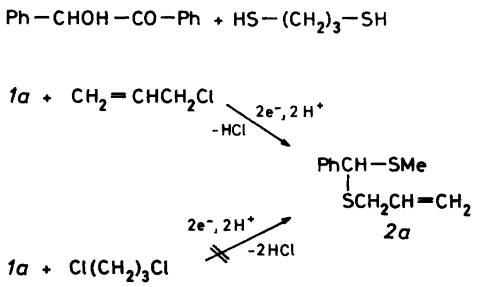
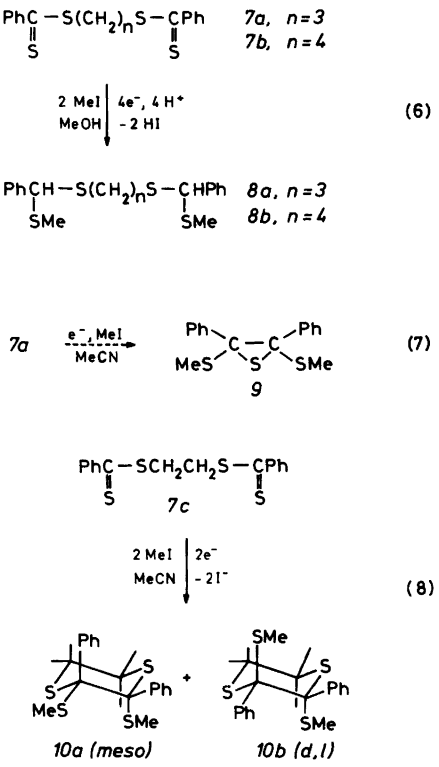






No electrolysis products could be identified when 1,2-dichloroethane, 1,3-dichloropropane or 1,4-dichlorobutane were used as bifunctional electrophiles. Especially the formation of the allyl thioacetal *2a* which could have been formed during co-electroreduction of *1a* with 1,3-dichloropropane by elimination of hydrogen chloride, was excluded. [Co-electroreduction of thiobenzomorpholide with 1,3-dichloropropane yields the *S*-allyl-*S,N*-acetal PhCH(NR_2)–$SCH_2CH{=}CH_2$].[6] *2a* was easily obtained if *1a* and allyl chloride were electroreduced (eqn. (5)).

Bis-dithiobenzoate esters. The bis-dithiobenzoates *7* were electrolysed in the presence of methyl iodide using the protic solvent methanol.

As one would expect, the bis-thioacetals *8* were formed (eqn. (6)). These compounds were accompanied by a variety of less interesting by-products (*cf.* Experimental). The isolation and purification of the oily and non-distillable liquids were difficult and the yields were low. The structures of *8a* and *8b* were established by NMR spectroscopy.

When, on the other hand, *7a* and methyl iodide were electroreduced in dry acetonitrile, no thioacetal could be identified. Preparative layer chromatography of the product mixture on silica gel yielded the thiirane *9* (28 %) as a colourless crystalline compound (eqn. (7)).

Quite a different result was obtained, when ethylene bis-dithiobenzoate (*7c*) was electroreduced in dry acetonitrile in the presence of methyl iodide. The six-membered cyclic bis-thioacetal *10* was formed (74 %) as a 1:1-mixture of the two possible diastereomers *10a* and *10b* (eqn. (8)). Separation of *10a* and *10b* was easily and completely accomplished by preparative layer chromatography. We were able to assign the two configurations unequivocally by high-resolution ^{1}H NMR spectroscopy. The four methylene protons in *10a* are non-equivalent. Therefore a complex multiplet results even at 400 MHz. On the other hand, *10b* exhibits a twofold axis of symmetry, and the methylene signal appears as an AA'XX'-system with a diaxial coupling $J_{AA'}$=12.2 Hz, a diequatorial coupling $J_{XX'}$=4.2 Hz, a geminal coupling J_{AX}=−15.5 Hz and an axial-equatorial coupling $J_{AX'}$=1.3 Hz.

DISCUSSION

The seven-membered heterocycle *6*, obtained from *1a* is not the expected product. According to eqn. (3) the thiolane *11* should have been formed but C-alkylation failed to take place. Obviously cyclization, which should be favoured by entropy effects, does not even overcome competing reactions that lead to products without attack of the electrophile on the central carbon atom.

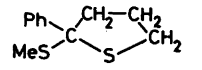
On the other hand, C–C-coupling reactions between two thiocarbonyl carbon atoms do occur during electroreduction of the dithiobenzoate esters. Even though the electrochemical mechanism has not really been studied, some considerations about the course of the reactions will be given.

Primarily the radical anions of the dithioesters which are moderately persistent [7] are formed at the applied protentials by a one-electron step. The data of Table 1 show that the electron transfer is not reversible in the case of the bis-dithiobenzoates *7a* and *7c*. Apparently fast irreversible steps are involved. Ethylene dithiobenzoate (*7c*) is easier reduced than *1a* which probably results from the electron-withdrawing effect of the second thiobenzoylthio group in the β-position. A second wave due to formation of a dianon or bis-radical anion *12* is well separated in the polarogram of *7a* and *7c*. Nevertheless, the two-electron reduction product can be present in a small equilibrium concentration and may play a significant role in the overall reaction. The reduction potentials are shifted in the positive direction in the protic solvent methanol. Trimethylene dimesylate (*5*) as a model

Table 1. Electroanalytical data on the dithiobenzoate esters.

Comp.	$E^{(1)}_{1/2}$ [a]	$E^{(1)}_{1/2}$ [b]	$E^{(1)}_{1/4} - E^{(1)}_{3/4}$ [c]	$E^{(2)}_{1/2}$ [b]	i_{pa}/i_{pc} [d]
1a	−1.26	−1.32	52	−1.91	0.86
7a	−1.09	−1.31	127	−2.02	0.55
7b	−1.13				
7c		−1.15	58	−1.52	<0.4
5		−1.96	150	−	

[a] In methanol, V *vs.* s.c.e. [b] In acetonitrile, V *vs.* s.c.e. [c] In acetonitrile, mV. [d] In acetonitrile at a sweep rate of 200 mVs^{-1}.



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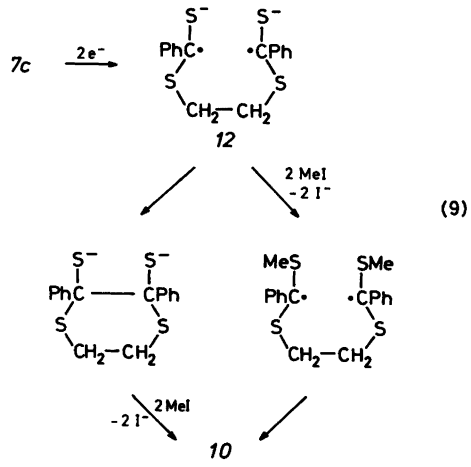
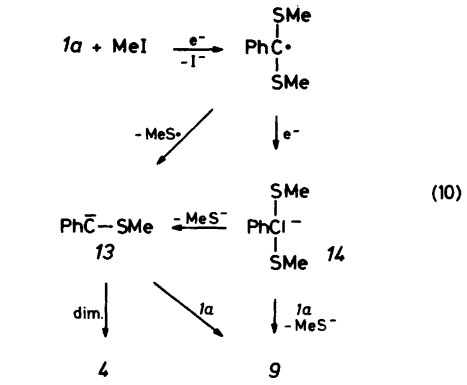


electrophile is reduced at a far more negative potential, so that its reduction can be excluded as the primary step and even its electrocatalytic reduction is unlikely.

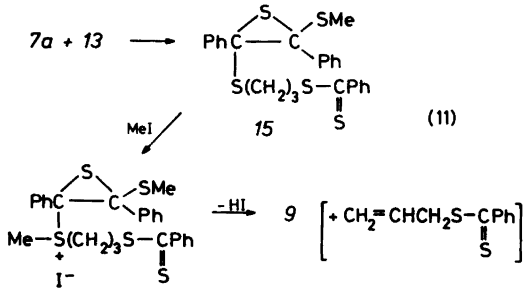
Formation of the open-chain thioacetals *8* from the radical anions by protonation, further reduction and alkylation is rather straightforward.[1] However, our data do not allow one to decide which step of this sequence is the first one or whether steps occur more or less simultaneously. The formation of the heterocyclic thioacetal *10* is explained quite similarly. Initially *7c* is reduced to the bis-radical anion *12* which can first undergo radical dimerization and then alkylation (eqn. (9)).

This assumption is supported by the fact that the first cyclovoltammetric peak of *7c* is not even reversible in the absence of methyl iodide and is not significantly shifted on addition of a large excess of methyl iodide (ΔE=+30 mV at molar ratios up to 200:1). However, the second possibility, first methylation and then dimerization of the neutral biradical (eqn. (9)), cannot be completely excluded.

The occurrence of the thiirane *9* as a precursor of *4* during electroreduction of *1a* has been presumed.[4] Its formation from a carbene *13* or the corresponding carbenoid *14* and *1a* (eqn. (10)) seems reasonable and is in accordance with

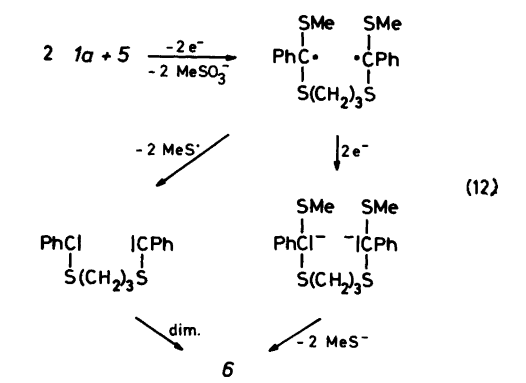


(10)



(11)

Seebach's results [8] on the behaviour of $(RS)_3C$-radicals, -anions (carbenoids), and carbenes of the type $(RS)_2C$. The formation of *9* and *7a* which again shows no significant dependence of the cyclovoltammetric peak potential on the methyl iodide concentration needs, however, a special explanation. Loss of the trimethylene chain can take place by a Hofmann-type elimination from an intermediate *15* which is formed similarly to *9* from *7a* and *13* (eqn. (11)) or after initial dimerization and thiirane formation.



(12)

Finally, the formation of *6* which is a cyclic analogue of *4* should take place by dimerization of a carbene or reaction of the corresponding carbenoid according to eqn (12).

EXPERIMENTAL

Apparatus. Melting points (uncorrected): Leitz-Heiztischmikroskop. Molecular distillation: Büchi GKR 50. IR: Perkin-Elmer 297 and 399. ^{1}H NMR: Varian T 60, Bruker WH 270 and WM 400. ^{13}C NMR: Bruker WP 80. MS: Varian MAT CH 7.

The electrochemical equipment has been described elsewhere.[9,10] All potentials are referred to the aqueous SCE. *Electrolyses in methanol* were performed in a simple beaker-type cell, shown in Fig. 1. *Preparation of the cellulose diaphragm:* 50 g fine copper wire were put into 1 l conc. aqueous ammonia, and air was passed through for 20 h. 30 g of cotton wool were dissolved with shaking in the deep blue tetrammine-copper-hydroxide solution. Soxhlet thimbles were impregnated with the obtained cellulose solution, dried first at room temperature (8 h), then at 120 °C (3 h). The thimbles were treated with dilute hydrochloric acid (20 ml conc. HCl/5 l water) for 3 h at 40–60 °C, then twice rinsed with warm water in the same way and again dried at 120 °C (3 h). This impregnation was repeated a few times. Finally the colourless diaphragms had a permeability of 2–3 ml/min, when they were half-filled with methanol.

Materials. Methanol, tetraethylammonium bromide, methyl iodide, allyl chloride, 1,2-dichloroethane, 1,3-dichloropropane, 1,4-dichlorobutane, ethane-1,2-dithiole, propane-1,3-dithiole, butane-1,4-dithiole and benzoin are commercially available and were used as received.

1,3-Dimesyloxypropane (*5*) [11] and tetra-n-propylammonium perchlorate [12] were prepared according to literature procedures. Acetonitrile was purified as described earlier.[9]

Methyl dithiobenzoate (1a). 4.6 g (0.2 mol) of sodium were dissolved in 100 ml dry methanol. 6.4 g (0.2 mol) of sulfur and 12.6 g (0.1 mol) of benzyl chloride were added. The mixture was stirred at 70 °C for 4 h and then evaporated *in vacuo*. The residue was dissolved in 100 ml water and extracted with chloroform to remove unpolar material. 12.6 g (0.1 mol) of dimethyl sulfate were added dropwise to the aqueous solution of the sodium dithiobenzoate at room temperature. After 1 h of stirring the product was extracted with light petroleum, washed with water, dried

over sodium sulfate and after removal of the solvent distilled.

Yield 8.4 g (50 %), red oil. B.p. 125–127.5 °C/ 0.53 kPa; lit.[13] b.p. 118 °C/0.4 kPa.

Ethylene-1,2-bis(dithiobenzoate) (7c). 5.3 g (25 mmol) of carboxymethyl dithiobenzoate [14] and 2 g sodium hydroxide were dissolved in 50 ml water. After addition of 1.2 g (12.5 mmol) of ethane-1,2-dithiole the mixture was stirred under N_2 for 1 h at room temperature. The red crystals of *7c*, which separated from the solution, were filtered off and recrystallized from ether.

Yield 2.3 g (55 %), m.p. 103–105 °C. Anal. $C_{16}H_{14}S_4$: C, H, S. ^{1}H NMR (60 MHz, $CDCl_3$): δ 3.75 (4H, s), 7.43 (6H, m), 7.93 (4H, m). IR (KBr): 1265 (ss) cm^{-1} (C=S).

Trimethylene-1,3-bis(dithiobenzoate). (*7a*) was obtained as a red oil analogously to *7c* from 15.3 g (75 mmol) of carboxymethyl dithiobenzoate and 4.1 g (37.5 mmol) of propane-1,3-dithiole. *7c* was purified by column chromatography on silica gel with light petroleum (60–70 °C)/ethyl acetate 9:1 as eluant.

Yield 9.0 g (79 %). Anal. $C_{17}H_{16}S_4$: C, H, S. ^{1}H NMR (60 MHz, CCl_4): δ 2.12 (2H, qn), 3.45 (4H, t), 7.35 (6H, m), 8.00 (4H, m).IR (film): 1230 (ss) cm^{-1} (C=S).

Tetramethylene-1,4-bis(dithiobenzoate). (*7b*) was obtained analogously to *7c* from 15.3 g (75 mmol) of carboxymethyl dithiobenzoate and 4.6 g (37.5 mmol) of butane-1,4-dithiole. *7b* was purified by recrystallization from methanol/chloroform.

Yield 7.4 g (62 %), red crystals, m.p. 64–65 °C. Anal. $C_{18}H_{18}S_4$: C, H, S. ^{1}H NMR (60 MHz, CCl_4): δ 1.98 (4H, m), 3.50 (4H, m), 7.49 (6H, m), 8.09 (4H, m).IR (KBr): 1215 (ss) cm^{-1} (C=S).

2,3-Diphenyl-4,5,6,7-tetrahydro-1,4-dithiepin (6). 3.6 g (17 mmol) benzoin are dissolved in 100 ml acetic acid. 4 ml of water and 4.7 g (43 mmol) of propane-1,3-dithiole are added. The solution is saturated with gaseous hydrogen chloride at 0 °C (1 h). The product precipitates from the mixture. It is filtered with suction, washed with water, dried over potassium hydroxide in a desiccator and recrystallized from light petroleum (60–70 °C).

Yield 2.7 g (54 %), yellowish leaflets, m.p. 142 °C. Anal. $C_{17}H_{16}S_2$: C, H, S. ^{1}H NMR (60 MHz, $CDCl_3$): δ 2.15 (2H, qn, J=6 Hz), 3.72 (4H, t, J=6 Hz), 7.1 (10H, m). IR (KBr): 1520 (m) cm^{-1} (C=C).

Electroreductions in acetonitrile.[9] 1.2 g (7 mmol) *1a* and 1.6 g (21 mmol) allyl chloride were reduced at −1.4 V, n=1.75 F mol^{-1}. *Allylthiomethylthio-phenylmethane* (*2a*) was isolated.

Yield 0.6 g (40 %, NMR-spectroscopically: 63 %), red oil, b.p. 110–120 °C/2.5 kPa. Anal. $C_{11}H_{14}S_2$: C, H, S. ^{1}H NMR (60 MHz, CCl_4): δ 1.93 (3H, s), 3.03 (2H, m), 4.53 (1H, s), 4.6–5.9 (3H, m), 6.8–7.2 (5H, m).IR (film): 1615 (s) cm^{-1} (C=C).

1.2 (7 mmol) *1a* and 4.1 g (18 mmol) *5* were reduced at −1.4 V, n=1.7 F mol^{-1}. *6* was isolated by rapid column chromatography on silica gel with CCl_4.

Yield 0.3 g (15 %) yellowish leaflets, m.p. 141–143 °C (ether). Anal. $C_{17}H_{16}S_2$: C, H, S. MS [IP 70 eV; *m/e* (% rel int.)]: 284 (100, M), 210 (60, *9*), 178 (70, PhC≡CPh), 121 (59, Ph–CS), 106 (57, $C_3H_6S_2$). ^{1}H NMR and IR: identical with *6* from benzoin. ^{13}C NMR (20.15 MHz, $CDCl_3$): 29.0 (CH_2), 32.4 (CH_2S), 127.0 (C=C), 127.6, 131.0, 131.6, 142.2 (Ar).

1.0 g (3 mmol) *7a* and 1.27 g (9 mmol) methyl iodide were reduced at −1.55 V, n=3.1 F mol^{-1}. *2,3-Bis(methylthio)-2,3-diphenylthriirane* (*9*) was isolated by preparative layer chromatography on silica gel with CCl_4 (3 elutions).

Yield 0.25 g (28 %), colourless crystals, m.p. 150–152 °C. (light petroleum). Anal. Found: C 61.79; H 6.33; S 30.40. Calc. for $C_{16}H_{16}S_3$: C 63.11; H 5.30; S 31.59; decomposition occurs during recrystallization. MS [IP 70 eV, *m/e* (% rel int.)]: 272 (2, M–S), 195 (96, M–C_6H_5S), 121 (100, Ph–CS), 77 (26, C_6H_5). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.69 (6H, s), 1.77 (6H, s), 7.1–7.4 (2OH, m); *E*- and *Z*–*9* are obtained as a mixture. IR: No C=C-band occurring.

1.0 g (3 mmol) *7c* and 1.27 g (9 mmol) methyl iodide were reduced at −1.55 V, n=3.3 F mmol^{-1}. 0.75 g of *10* crystallizes from the light petroleum extract on standing at 4 °C for several days. NMR-yield: 74 %. Chromatography on silica gel plates (Merck) with CCl_4 (3 elutions) and subsequent recrystallization gave equal amounts of *meso*-(R,S)-*2,3-bis(methylthio)-2,3-diphenyl-1,4-dithiane* (*10a*): Colourless crystals, m.p. 164–166 °C (light petroleum). Anal. $C_{18}H_{20}S_4$: C, H, S. ^{1}H NMR (400 MHz, $CDCl_3$): 1.74 (6H, s), 3.28 (2H, m), 3.56 (2H, m), 7.2 (10H, m), and d,l-(R,R+S,S)-*2,3-bis(methylthio)2,3-diphenyl-1,4-dithiane* (*10b*): Colourless crystals m.p. 214–216 °C (light petroleum). Anal. $C_{18}H_{20}S_4$: C, H, S. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71 (6H, s), 2.84 (2H, ν_A), 3.92 (2H, ν_X) [$J_{AA'}$=12,2 Hz, J_{AX}=$J_{A'X'}$=−15.5 Hz, $J_{AX'}$=$J_{A'X}$=1.3 Hz, $J_{XX'}$=4.2 Hz], 7.15 (10H, m).

Electroreductions in methanol. The Pb-cathode was polished and rinsed with methanol before use. 650 ml of a 0.2 molar solution of tetraethylammonium bromide in methanol were filled into the cathodic compartment of the cell (Fig. 1). After 30 min waiting for compensation of the

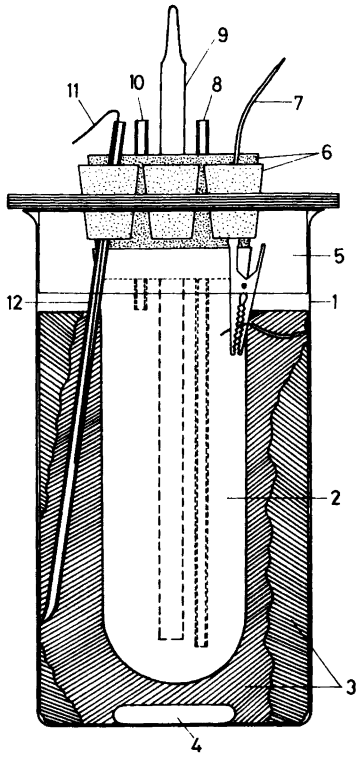


Fig. 1. Electrolysis cell for the reduction of dithio esters in methanol. 1. 800 ml-beaker, 2. Soxhlet-thimble, 3. lead sheet (cathode), 4. stirring rod, 5. polyethylene ring, 6. rubber stoppers, 7. lead for the cathode, 8. suction tube, 9. graphite rod (anode), 10 anode compartment opening, 11. silver wire, 12. Luggin-capillary.

levels the dithioester and alkylating agent were introduced with stirring. The electroreduction was performed without cooling at a current of 1A (current density 5 mA cm^{-1}). After completion of the electrolysis the anolyte was quickly removed by suction. An aliquot (20 ml) of the catholyte was mixed with 50 mg of 1,2-diphenylethane and used for the quantitative determination of yields by NMR spectroscopy.[15] The remainder of the catholyte was diluted with 250 ml of water and extracted with five 100 ml-portions of light petroleum (60–70 °C). The extract was dried with magnesium sulfate and evaporated.

8.0 g (23 mmol) *7a* and 7.8 g (55 mmol) methyl iodide were reduced at −1.25 V, n=5 F mol^{-1}. 7.3 g yellow oil were isolated, which exhibited at least six spots on a thin layer chromatogram. Subsequent column and layer chromatography on silica gel with CCl_4/CH_2Cl_2 (1:1) gave a small amount of the main product, which was not analytically pure, exhibited the expected NMR spectrum of *2,4,8,10-tetrathia-3,9-diphenylundecane* (*8a*) (yellow liquid).

^{1}H NMR (60 MHz, CCl_4): δ 1.89 (6H, s), 1.6–3.8 (6H, m), 4.70 (2H, s), 7.3 (6H, m), 7.95 (4H, m).

Methyl benzoate, *O*-methyl thiobenzoate and benzaldehyde were detected as by-products by their characteristic NMR signals.

6 g (16.6 mmol) *7b* and 5.6 g (39.7 mmol) methyl iodide were reduced at −1.2 V, n=7.5 F mol^{-1}. 4.76 g yellow oil were obtained. Column chromatography on silica gel with light petroleum/CH_2Cl_2 (4:1) gave *2,4,9,11-tetrathia-3,10-diphenyldodecane* (*8b*).

Yield 26 % (by NMR), yellow liquid.Anal. Found: C 61.20; H 6.71; S 31.37. Calc. for $C_{20}H_{26}S_4$: C 60.86; H 6.64; S 32.50. MS [IP 70 eV; *m/e* (% rel. int.)]: 137 (25, $PhCH(SCH_3)$), 136 (34), 135 (12), 122 (5, PhCHS), 121 (31, PhCS). ^{1}H NMR (60 MHz, CCl_4): δ 1.63 (4H, m), 2.00 (6H, s), 2.43 (4H, m), 4.82 (2H, s), 7.3 (10H, m).

Methyl benzoate, *O*-methyl thiobenzoate, benzaldehyde and dibenzyldisulfide were detected as by-products by their characteristic NMR signals.

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Electrochemical Synthesis of Heterocyclic Compounds. XV.* Anodic Synthesis of *s*-Triazolo[4,3-*a*]pyridine Derivatives

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Anodic oxidation of several heterocyclic hydrazones (*1a–9a*) prepared from 2-hydrazinopyridine of 2-hydrazino-4-nitropyridine and the corresponding aromatic aldehyde was performed in a $CH_3CH-Et_4NClO_4$ solution with the addition of 60 % $HClO_4$ on a platinum electrode, using controlled potentials. As a result of two-electron oxidative cyclizations, several *s*-triazolo[4,3-*a*]pyridine derivatives (*1b–9b*) were prepared in yields ranging from 55 to 92 %. A mechanism rationalizing the formation of the 3-phenyl-*s*-triazolo[4,3-*a*]pyridine, *1b*, has been studied by LSV, CPSV, RDE, coulometry and preparative scale electrolysis.

Oxidative cyclization of heterocyclic hydrazones is a widely used route for the preparation of *s*-triazoloazines or *s*-triazoloazoles and may be carried out with a variety of oxidizing agents,[2–6] among the most widely used of which are bromine and metallic compounds.

We have previously reported [7] a new route to 3-phenyl-3-triazolo[4,3-*a*]pyridine by electrochemical oxidation of benzaldehyde 2-pyridylhydrazone. However, we have found that the yield of products is dependent upon the solvent used. In order to gain a better understanding of the factors which influence the distribution of the products and to find the electrolysis conditions which give the highest yield of *s*-triazolo[4,3-*a*]pyridines we have now studied the anodic oxidation of a series of structurally different heterocyclic hydrazones. As a result, we can report a convenient new synthesis of several *s*-triazolo[4,3-*a*]pyridine derivatives.

RESULTS AND DISCUSSION

Synthetic aspects. In order to optimize the yield of cyclic products (*1b*) anodic oxidations of benzaldehyde 2-pyridylhydrazone (*1a*) were performed in "neutral", basic and acidic media. Electrochemical oxidations were carried out on a platinum anode by controlled potential electrolysis in acetonitrile with tetraethylammonium perchlorate as supporting electrolyte in a divided cell. The electrolysis was complete after about 1–2 h, when the current dropped to 1–5 % of its starting value. The products *1b*, *1c* and *1d* were isolated, and hydrazide *1e* was detected by TLC. Two other products, pyridine (*1f*) and benzaldehyde (*1g*), were determined by means of GLC. The structures of all products obtained are shown in Scheme 1.

The results presented in Table 1 are mean values obtained from three electrolyses. The best yield of *1b* was obtained in acidic media in which electrolyses proceeded smoothly without inhibition of the electrode. The electrolysis, with the addition of pyridine as a base or in "neutral" media, resulted in a lower yield of *1b*, and the filming of the electrode occurred during the reaction.

Anodic oxidation of heterocyclic hydrazones prepared from 2-hydrazinopiridine (*1a–6a*) or 2-hydrazino-4-nitropyridine (*7a–9a*) and the corresponding aldehyde was typically performed in

* Part XIV. See Ref. 1.

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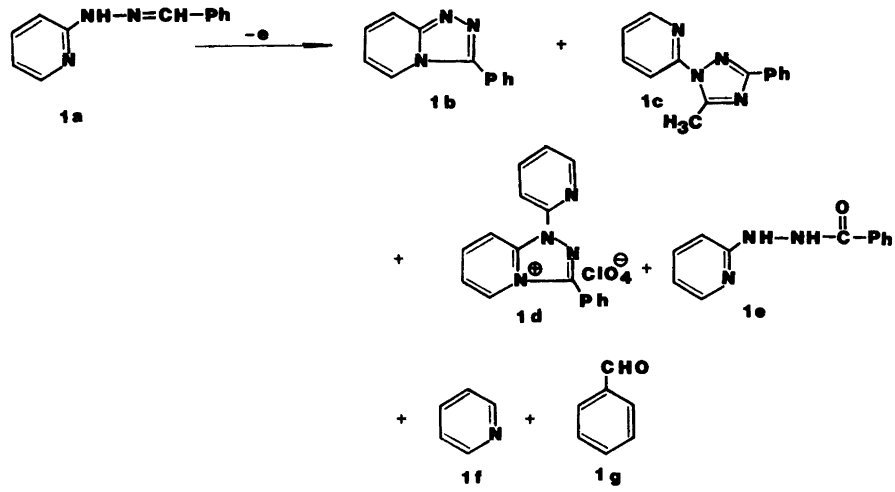


Scheme 1.

an acetonitrile solution (100 ml) containing tetraethylammonium perchlorate (0.1 M) with the addition of 60 % perchloric acid (2 ml), using controlled potential electrolysis. All electrochemical syntheses were carried out in a divided cell on a Pt-gauze anode (3×5 cm) giving rise to *s*-triazolo[4,3-*a*]pyridine derivatives as shown in Scheme 2.

Voltammetric and preparative data for the anodic oxidation of heterocyclic hydrazones (*1a–9a*) are given in Table 2. RDE voltammograms, run in CH_3CN–0.1M Et_4NClO with the addition of 60 % $HClO_4$, showed one wave. Coulommetry at the applied potential showed that the overall electrode reaction was a two-electron oxidation in all cases examined.

The oxidation products (*1b–9b*) were isolated in good yields ranging from 55 to 92 % and, after purification, their elemental analyses, IR, NMR and mass spectra were in agreement with the assigned structures.

The intramolecular cyclodehydrogenation of heterocyclic hydrazones leading to the *s*-triazolo[4,3-*a*]pyridines is usually performed using various oxidants. Our present anodic synthesis provides a useful, equally convenient alternative.

Mechanistic rationalization. The oxidations of benzylidene 2-pyridylhydrazone, *1a*, were studied in some detail. All of the electrochemical studies were conducted using CH_3CN–0.1M Et_4NClO_4. A single compartment cell containing a saturated calomel electrode was employed, the counter electrode was a platinum sheet and the working electrode was a polished disc of platinum (diameter=1.0 or 3 mm) or a platinum bead electrode.

Current-potential curve of *1a*, obtained with a

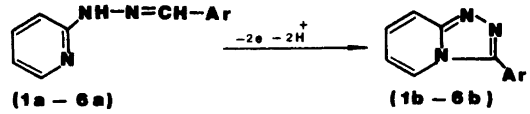
Table 1. Composition of the reaction mixture after oxidation of benzaldehyde-2-pyridylhydrazone, *1a*.

Solution conditions	$E_{1/2}$ (V *vs.* SCE)	Applied potential (V *vs.* SCE)	*n*-Value	*1b* (%)	*1c* (%)	*1d* (%)	*1e* (%)	*1f* (%)	*1g* (%)
CH_3 CN	0.82	1.4	2.2	62	10	–	traces	7	3
CH_3CN+ 60 % $HClO_4$ (2 ml)	1.28	1.4	2.1	72	25	–	traces	traces	traces
CH_3CN+ pyridine (2 ml)	0.92	1.0	3.6	26	–	45	–	–	traces

Table 2. Conditions and results of electrochemical cyclodehydrogenation.

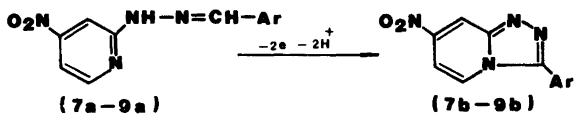
Conversion	RDE [a] voltammetry results, $E_{1/2}$ (V *vs.* SCE)	Applied potential (V *vs.* SCE)	*n*-Value [b]	Yield of product (%)	M.p.[c] (solvent)	Molecular formula or lit. m.p. (°C)	Elemental analysis Calc./found		
							% C	% H	% N
1a→1b	1.28	1.4	2.1	72	172–173° (A)	172–173 Lit. 8			
2a→2b	0.93	1.0	2.3	65	162–164° (A)	$C_{13}H_{11}N_3$ [d] (209.13)	74.65 74.27	5.25 5.19	20.08 20.31
3a→3b	0.95	1.0	2.6	55	143–144° (A)	$C_{13}H_{11}N_3$ [e] (209.13)	74.65 74.69	5.25 5.20	20.08 20.02
4a→4b	0.70	0.8	2.4	63	124–125° (A)	$C_{13}H_{11}N_3O$ [f] (225.13)	68.01 68.35	4.88 5.01	18.65 18.79
5a→5b	0.85	1.2	2.4	60	166–168° (A)	$C_{15}H_{15}N_3O_3$ [g] (285.15)	63.17 63.20	5.26 5.25	14.72 15.01
6a→6b	1.40	1.5	2.3	75	296–298° (B)	$C_{12}H_{18}N_4O_2$ [h] (240.12)	60.01 59.98	3.33 3.30	23.32 23.81
7a→7b	1.10	1.2	2.2	90	162–163° (C)	$C_{13}H_{10}N_4O_3$ [i] (270.13)	57.79 58.04	3.70 3.58	20.73 21.05
8a→8b	0.72	0.8	2.1	92	231–233° (D)	$C_{14}H_{13}N_5O_2$ [j] (283.14)	59.38 59.03	4.59 4.37	24.79 24.45
9a→9b	0.91	1.0	2.3	83	181–182° (C)	$C_{15}H_{14}N_4O_5$ [k] (330.15)	54.56 54.79	4.24 4.19	16.96 17.22

[a] Pt-electrode; 600 r.p.m.; substrate concentration 1 mM; compounds *1a–9a* were run in CH_3CN–0.1 M Et_4NClO_4 in the presence of 60 % $HClO_4$ (C=0.1 M). [b] Determined by coulometry at controlled potential. [c] A=benzene–cyclohexane (1:1); B=CH_3CN; C=EtOH; D=EtOH-acetone (1:1). [d] IR (KBr): 3080, 2970, 1630, 1500, 1470, 1410, 1370, 1320, 1075, 1010, 830, 750, 740, 720, 700 cm^{-1}. ^1H NMR (DMSO-d_6): δ=2.4 (s,3H,CH_3), 6.8–8.5 (m, 8H, arom.) p.p.m. MS [IP 70 eV; *m/e* (% rel. int)]: 209(100), 208(63), 194(6), 181(6), 143(6), 116(9), 92(23), 91(6), 90(6), 89(5), 66(6), 75(26). [e] IR (KBr): 3180, 2980, 1600, 1570, 1435, 1315, 1140, 990, 765, 755 cm^{-1}. ^1H NMR (DMSO-d_6): δ=2.2 (s,3H,CH_3); 6.8–8.05 (m, 8H, arom.) p.p.m. MS [IP 70 eV; *m/e* (% rel. int)]: 209(100), 208(29), 207(5), 180(4), 117(6), 116(10), 106(4), 105(6), 92(5), 91(4), 90(8), 89(15), 78(8), 76(4), 66(5), 65(23). [f] IR (KBr): 3080, 3000, 2850, 1635, 1610, 1540, 1500, 1470, 1380, 1310, 1255, 1015, 835, 750, 730, 700 cm^{-1}. ^1H NMR (DMSO-d_6): δ=3.9 (s,3H,CH_3O); 6.9–8.6 (m, 8H, arom.) p.p.m. MS [IP 70 eV; *m/e* (% rel. int)]: 225(100), 224(29), 211(15), 210(29), 182(10), 107(15), 84(41), 76(20). [g] IR (KBr): 3080, 3000, 1630, 1590, 1500, 1470, 1430, 1370, 1320, 1300, 1240, 1190, 1085, 1010, 880, 850, 750, 710 cm^{-1}. MS [IP 70 eV; *m/e* (% rel. int)]: 285(45), 270(27), 242(5), 85(18), 84(56), 83(15), 69(65), 56(72), 55(76), 43(34), 42(56), 41(100), 40(13). [h] IR (KBr): 3100, 1640, 1600, 1570, 1540, 1490, 1440, 1420, 1350, 1270, 1150, 850, 770, 760 cm^{-1}. MS [IP 70 eV; *m/e* (% rel. int)]: 240(100), 210(8), 194(4), 194(23), 193(10), 140(10), 92(15), 76(10), 66(10), 65(28). [i] IR (KBr): 3000, 1645, 1610, 1500, 1475, 1340, 1100, 1030, 820 cm^{-1}. MS [IP 70 eV; *m/e* (% rel. int)]: 270(100), 255(6), 222(7), 221(16), 209(3), 179(13), 181(3), 119(5), 103(7), 76(5), 64(6), 63(34), 44(23), 43(7). [j] IR (KBr): 2910, 1640, 1605, 1500, 1345, 1300, 1190, 830 cm^{-1}. MS [IP 70 eV; *m/e* (% rel. int)]: 283(59), 237(11), 210(9), 209(50), 193(10), 146(55), 145(100), 133(10), 132(84.5), 131(9), 180(16), 129(18), 115(11), 105(16), 103(19.5), 89(13), 78(23), 77(13), 65(8). [k] IR (KBr): 2940, 2840, 1640, 1590, 1350, 1305, 1230, 1130, 1005, 880, 855, 830 cm^{-1}. ^1H NMR (DMSO-d_6) at 150°: δ=3.82 (s, 3H, CH_3O); 3.9 (s, 6H, CH_3O); 7.46 (s, 2H, H_2H_6); 7.85 (d, 1H, H_6. J=9.3 Hz); 8.25 (d,d, 1H, H_5); 9.78 (d, 1H, H_8, J=1.9 Hz). MS [IP 70 eV; *m/e* (% rel. int.)]: 330(100), 315(45), 287(7), 272(7), 257(12), 241(12), 165(7), 114(18), 72(6), 63(10).



(1a – 6a) (1b – 6b)

1a–b, Ar=C_6H_5–; 2a–b, Ar=p–H_3C–C_6H_4–;
3a–b, Ar=o–CH_3–C_6H_4–; 4a–b, Ar=p–CH_3O–C_6H_4–;
5a–b, Ar=3,4,5–$(CH_3O)_3$–C_6H_2–;
6a–b, Ar=p–NO_2–C_6H_4–;



(7a–9a) (7b–9b)

7a–b, Ar=p–CH_3O–C_6H_4–; 8a–b, Ar=p–$(CH_3)_2N$–C_6H_4–;
9a–b, Ar=3,4,5–$(CH_3O)_3$–C_6H_2–;

Scheme 2.

rotating disc platinum electrode, shows the presence of two waves at +0.82 and 1.62 V, respectively, for oxidation in acetonitrile (Fig. 1a). The first wave corresponds to the oxidation of the parent molecule and the second to the *1a* protonated by protons liberated along the first wave. After the addition of pyridine as a base, the first wave doubled in height (Fig. 1b). The addition of perchloric acid (60 % in water) reduces the initial oxidation wave at 0.82 V and increases the more anodic wave (Fig. 1c). Thus, the experiments described confirm that the first wave represents oxidation of the parent hydrazone, *1a*, and the second represents oxidation of the protonated hydrazone. The current function, $i_L/w^{1/2}C$, obtained in the presence of perchloric acid was constant with a rotation rate in the range from 1.6 to 50 rev. s^{-1}, and showed two-electron behaviour by comparison of the values of the current function with that obtained for ferrocene, a compound known to undergo reversible one-electron oxidation. The reaction order (the log current-log concentration ratio) in the diffusion plateau region was about one.

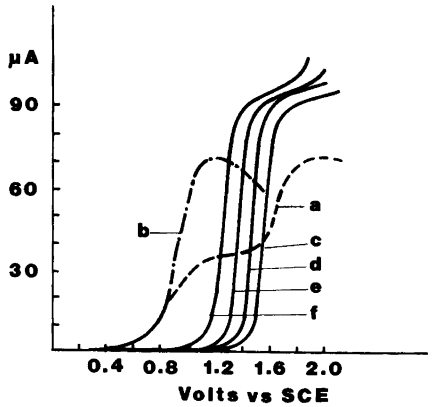


Fig. 1. RDE voltammograms of 1 mM solution of *1a* 0,1 M Et_4NClO_4–CH_3CN at rotation rate of 900 rev min^{-1}; Pt-electrode ($2r$=1 mm); (a) benzylidene-2-pyridylhydrazone, *1a*; (b) *1a* plus pyridine (C=0.001 M); (c) *1a* plus 60 % $HClO_4$ (C=0.001 M); (d) *1a* plus 60 % $HClO_4$ (C=0.01 M); (e) *1a* plus 60 % $HClO_4$ (C=0.1 M); (f) *1a* plus 60 % $HClO_4$ (C=0.1 M) plus H_2O (C=0.1 M).

Coulometry performed by exhaustive preparative electrolysis at a controlled potential for compound *1a* confirmed (n=2.1) that the wave shown in Fig. 1c corresponded to two electrons.

The effect of increasing acid concentration is shown in Figs. 1c, 1d and 1e; the major effect observed is the shift of the oxidation wave of the protonated hydrazone *1a* cathodically. The addition of water affects further cathodic movement of the wave (Fig. 1f). A similar effect was observed in the oxidation of 1-phenylpyrazolidin-

Table 3. Results of CPSV and LSV of *1a*.[a]

CPSV [b] Logarithmic analysis Number [c]	Slope (mV)	LSV [d] $Ep-Ep/2$ (mV)	$\partial Ep/\partial \log v$ [e] (mV)	$\partial Ep/\log C$ [f] (mV)
1	60 (2)			
2	58 (2)	58	*ca.* 20	*ca.* −20
3	83 (2)			

[a] Concentration of *1a*, 1 mM; CH_3CN–0.1 M Et_4NClO_4 plus 60 % $HClO_4$ (C=0.1 M). [b] v=0.15 V s^{-1}; Pt-electrode ($2r$=3 mm). [c] 1=log [($|_1-|$) i^{-1}]; 2=log [($|_1-|$) $i^{-2/3}$]; 3=log [($|_1-|$) $i^{-2/3}|^{-1/3}$]. [d] Platinum bead electrode. [e] Correlation coefficient: 0.994. [f] Correlation coefficient: 0.929; v=0.1 V s^{-1}.

3-one in acidic media.[9]

Using the same arguments the cathodic shift of the wave can be explained in terms of the Nernst equation and the equilibrium process according to Scheme 3.

$$\begin{array}{ccc} RH+H_3O^+ & \rightleftharpoons & RH_2^+ + H_2O \\ & & \uparrow\downarrow -e \\ RH^{+\cdot}+H_3O^+ & \rightleftharpoons & RH_2^{+\cdot +} + H_2O \end{array}$$

Scheme 3.

The water present in acetonitrile acts as a base because of the fact that acetonitrile is a very weak base compared with water.[10]

In order to learn more about the mechanism for conversion *1a*→*1b*, we have used the diagnostic criteria given by Andrieux and Savéant for cyclization reaction [11,12] using linear sweep voltammetry (LSV). Peak potentials on a small platinum bead electrode were measured for concentrations, from 0,2 to 2 mM at v=0.15 V s^{-1}, and sweep rate variations in the range of sweep rate from 0.05 to 1 V s^{-1}. The results are presented in Table 3.

The data given are the result of linear least squares analysis of over 15 voltammograms. It is worth noting that cyclic voltammetry of *1a* does not show reversibility for the sweep range from 0.02 to 80 V s^{-1}, indicating a fast chemical reaction which follows electron transfer.[13]

It is seen that the rate of variation of E_p with log v is 20 mV dec^{-1} and the variation of the peak potential with the concentration is also 20 mV dec^{-1}. According to the diagnostic criteria previously established, three mechanistic schemes are possible: e-c-D-p-p, e-c-p-D-p and e-D-c-p-p (the capital letter designates the rate determining step, e=electron transfer on the electrode, d=a solution electron transfer, c=cyclization step and p=deprotonation). The formation of products *1c*, *1d* and *1e*, in preparative electrolysis of *1a* under various solvent conditions (see Scheme 1), is a good indication that the cyclization reaction in conversion *1a*→*1b* occurs after the second electron has been transferred. This observation ruled out the first two mechanistic possibilities. The remaining possibility was e-D-c-p-p, involving a solution electron transfer as a rate-deter-

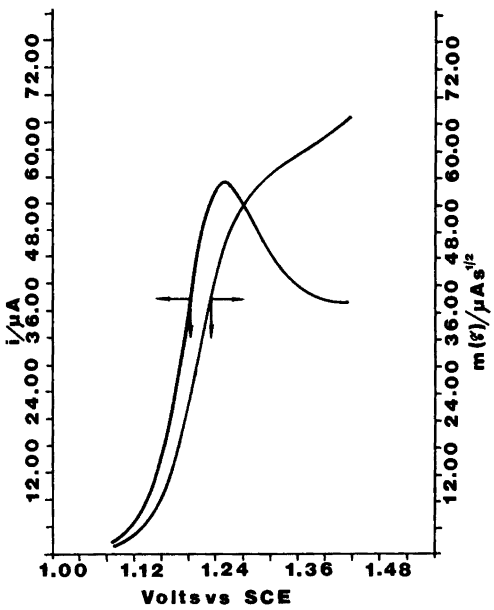


Fig. 2. LS and CPS voltammograms of *1a* (C=1 mM) with 60 % $HClO_4$ added (C=0.1 M); Pt-electrode ($2r$=3 mm); v=0.15 V s^{-1}.

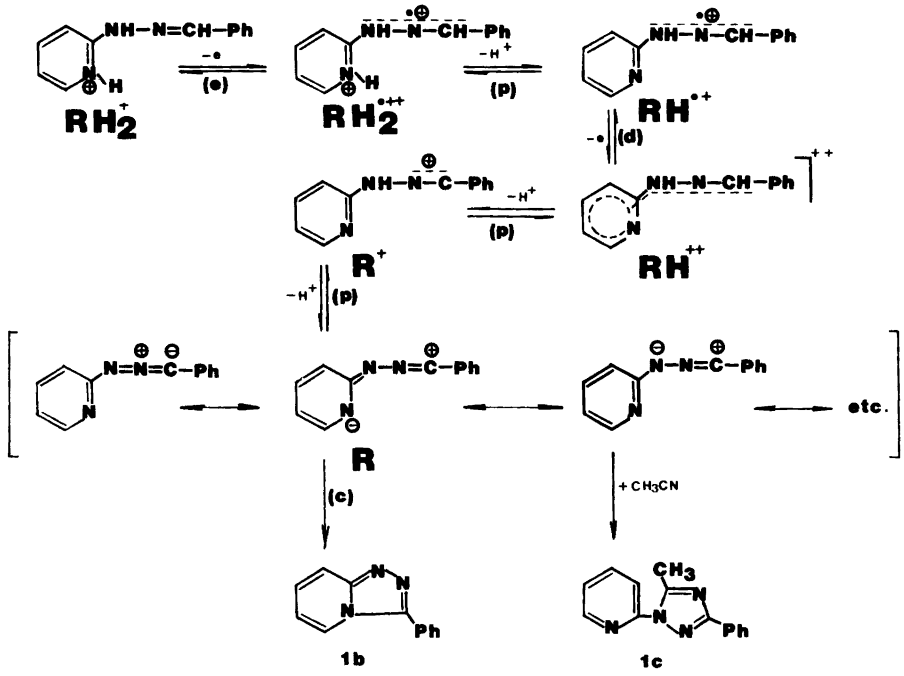
mining step.

Further mechanistic study of the reaction *1a*→*1b* was done by application of convolution potential sweep voltammetry (CPSV).[14] The technique of semi-integral analysis using the equation given by Vanden Born and Evans [15] applied to convolute i-E curves was described recently.[16] The linear sweep and the convoluted voltammogram are shown in Fig. 2.

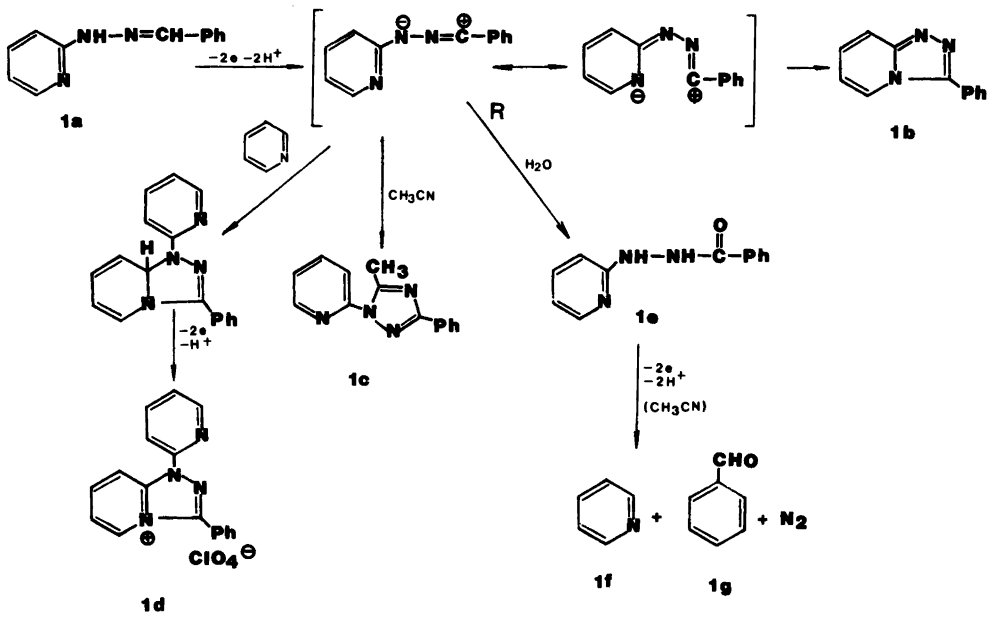
The logarithmic analysis decribed by Savéant *et al.*[17] were applied over the wave of the *I*–*E* curve, and the resulting slopes are given in Table 3. Comparison of the CPSV slope values in Table 3 shows that analysis number 2, with an experimental value of 58 mV, is closest to the theoretical values (58.6 mV).

The conversion of protonated *1a*→*1b* involves a loss of two electrons and three protons and a carbon–nitrogen bond formation. The values of $\partial e_p/\partial \log \nu$=20 mV and $\partial E_p/\partial \log C$=20 mV point to the occurrence of a second order rate-determining step (D-step) by comparison with diagnostic criteria for LSV [11] and CPSV.[17] Scheme 4 presents an overall picture of many of the mechanistic possibilities.

Oxidation of protonated benzaldehyde 2-pyridylhydrazone (RH_2^+) is initiated by the rapid, reversible formation of radical-dication ($RH_2^{\cdot 2+}$) which deprotonates as an acidic species leading to the formation of radical-cation ($RH^{\cdot +}$). A pair of $RH^{\cdot +}$ disproportionates in solution giving the parent compound *1a* (RH), which is protonated in acidic media, and dication (RH^{2+}). Our kinetic data obtained from LSV and CPSV approximately fit a second order rate low. According to the diagnostic criteria,[11,12] three reaction steps, after the rate-determining step, are included: (i) cyclization of dication (c-step), (ii) deprotonation (p-step) and (iii) deprotonation (p-step). Hence, since diagnostic criteria presented by Andrieux and Savéant [11] do not cover all possible mechanisms, it seems reasonable to propose that the relatively high acidity of RH^{2+} favours deprotonation leading to the cation (R^+). Also it is possible that the coulombic repulsion in the stage of dication is unfavourable to the cyclization. The cation (R^+) deprotonates, leading to the nitrilimine (R). One can anticipate that the intermediate nitrilimine (R) may either cyclize as a 1,5-dipolar species into 3-phenyl-*s*-triazolo[4,3-*a*]pyridine, *1b*, or may be trapped by acetonitrile in a concurrent



Scheme 4.



Scheme 5.

cyclization of nitrilimine as a 1,3-dipolar species giving 1-(2-pyridyl)-3-phenyl-5-methyl-1,2,4-triazol, *1c*. The final step in the formation of the fused triazolo-compounds is generally considered to involve nitrilimine as intermediates based on kinetic measurement data [18,19] or on trapping with 1,3-dipolarophiles.[6,20,21] Our results suggest also that the nitrilamine is the final intermediate in the electrochemical reaction *1a*→*1b*.

The possible formation of all products during anodic oxidation of *1a* under various solution conditions can be explained in terms of nitrilimine as an intermediate according to Scheme 5.

The intermediate nitrilimine (R) formed in "neutral" media as well as in the presence of perchloric acid preferentially undergoes the cyclization leading to *1b* (62 and 72 % yield, respectively). The slower 1,3-dipolar cycloaddition process leading to *1c*, and the nucleophilic attack of water leading to hydrazide *1e* are overrun by a faster nucleophilic attack of ring nitrogen to give a cyclic product *1b*. The hydrazide *1e* is further oxidized at the applied potential ($E_{1/2}$ of *1e* is 0.75 V *vs.* SCE) giving rise to pyridine, *1f*, and benzaldehyde, *1g*, as side products. The same type of cleavage of hydrazones performed by anodic oxidation has already been observed.[22] However, it seems that in basic media, with pyridine present, intermolecular 1,3-cycloaddition is faster than the intramolecular 1,5-cycloadition reaction leading to the higher yield of *1d* (45 % more than of *1b* (26 %)). One can envisage the formation of 1-(2-pyridyl)-3-phenyl-*s*-triazolo[4,3-*a*]pyridinium perchlorate, *1d*, through the formation of an intermediate bicyclic compound which is further oxidized at the applied potential by the loss of two electrons and one proton, giving rise to *1d*. This type of reaction has been used for annelation of pyridine rings *via* the anodic oxidation of some aldehyde hydrazones in the presence of pyridine.[23]

EXPERIMENTAL

Materials. Acetonitrile (Merck) was purified by refluxing over potassium permanganate for 1 h, followed by distillation over P_2O_5.[24] Tetraethylammonium perchlorate (Eastman) was recrystallized twice from water, then dried in an oven at 110 °C and kept in a desiccator over P_2O_5. Heterocyclic hydrazones (*1a*–*9a*) were prepared by refluxing 2-hydrazinopyridine or 2-hydrazino-4-nitropyridine and corresponding aldehyde in ethanol. All heterocyclic hydrazones

gave, after purification, a correct elemental analysis.

Apparatus and procedures. All melting points are uncorrected. The IR spectra (KBr pellets) were recorded on a Perkin-Elmer M-377 spectrophotometer, the NMR spectra were recorded on a Perkin-Elmer R 12A spectrometer using tetramethylsilane as an internal standard, and mass spectra were recorded on a Hitachi Perkin-Elmer RMV-6L mass spectrometer. GLC analysis was performed with a Perkin-Elmer F-11 equipped with a flame ionization detector at a constant temperature (100 °C) using 10 % Carbowax 20 M on Chromosorb W column.

The apparatus and cells for voltammetry, coulometry and preparative electrolysis have been described earlier.[25] The procedure used for CPSV was described recently.[16] The platinum electrode used in the voltammetric experiments was cleaned by keeping it for *ca.* 60 s in DMF, then rinsing it with acetone and drying it with paper tissue. This procedure was repeated prior to each measurement.

General procedure for preparation of s-*triazolo-[4,3-a]pyridine derivatives (*2b–9b*).* Into the anodic compartment of the divided cell with Pt-gauze anode (3×5 cm) and an Ni-cathode, filled with a 0.1 M solution of Et_4NClO_4 and 2 ml $HClO_4$ (60 %) in acetonitrile (200 ml) the heterocyclic hydrazone (*2a–9a*) was added (0.3–0.5 g). The potential was maintained at a fixed value (see Table 2) with initial currents generally 200–900 mA. Electrolysis was usually discontinued when the current dropped to 10–20 mA, which generally took 1–3 h. The solution was evaporated to *ca.* 10 ml and saturated aqueous sodium hydrogen carbonate (150 ml) was added. The pH of the solution was adjusted at about 10 by the addition of a 3N NaOH solution. After standing about 48 h at room temperature, the precipitated cyclization product (*2b–9b*) was isolated by filtration and recrystallized from the appropriate solvent (see Table 2).

Oxidation of benzaldehyde-2-pyridylhydrazone, 1a, *in "neutral" and acidic media.* The electrolysis of *1a* (0.3 g) was carried out in the same way as described in the general procedure, using controlled potential with or without the addition of perchloric acid (see Table 1). Electrolysis without the addition of perchloric acid, *e.g.* in "neutral" media, was accompanied by the passivization of the platinum electrode. The process was interrupted several times during the electrolysis and the electrode was washed in HNO_3 (1:1), water and acetone in order to "activate" it. After electrolysis at controlled potential, acetonitrile was evaporated to a volume of *ca.* 10 ml and saturated aqueous sodium hydrogen carbonate (150 ml) was added. The products *1b* and *1c* were isolated by extraction with chloroform (4×30 ml). After evaporation of the solvent the residue was subjected to column chromatography on a silica gel column using chloroform as an eluant. The compound *1c*, 1-(2-pyridyl)-3-phenyl-5-methyl-1,2,4-triazol, appeared as a first fraction. After evaporation of the solvent the compound *1c* and recrystallization from benzene–cycloxehane (1:2) gave m.p. 54–55 °C. Anal. Found: C. 71.64; H. 5.21; N. 24.01. Calc. for $C_{14}H_{12}N_4$; C. 71.20; H. 5.08; N. 23.71. IR (KBr): 2920, 1590, 1570, 1520, 1460, 1430, 1380, 1350, 1310, 1270, 1175, 1140, 1120, 1090, 1040, 995, 960, 925, 780, 730, 700, 665 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 2.9 (3H, s, CH_3); 7.1–8.5 (9H, m. arom.). MS IP 70 eV, *m/e* (% rel. int): 236(100), 235(24), 169(17), 195(63), 194(89), 149(33), 147(70), 133(91), 117(28), 105(43), 104(57), 103(24), 93(33), 92(35), 91(22), 76(54), 65(37).

The second fraction after evaporation of the solvent and recrystallization from benzene–cycloxehane (1:1) gave *1b* with m.p. 172–173 °C (Lit.[8] m.p.=172–173 °C).

Oxidation of benzaldehyde-2-pyridylhydrazone, 1a, *in basic media.* The electrolysis of *1a* (0.3 g) was carried out in the same way as described in the general procedure, using controlled potential with the addition of 2 ml of pyridine (see Table 1). During the electrolysis, passivization of the electrode occurred and the process was interrupted several times during the electrolysis and the electrode was washed in HNO_3 (1:1), water and acetone. After electrolysis, acetonitrile was evaporated to a volume of *ca.* 5 ml and water (100 ml), containing a few drops of perchloric acid, was added. After standing 5 days at room temperature the precipitated product *1d* was isolated by filtration and after recrystallization from $CH_3CN–H_2O$ (2:1), acidified with a few drops of perchloric acid giving m.p. 245–247 °C. Anal. Found: C. 55.01 H. 3.51; N. 15.07. Calc. for $C_{17}H_{13}N_4ClO_4$; C. 54.85; H. 3.48; N. 15.05. IR (KBr): 3100, 1640, 1590, 1570, 1500, 1470, 1440, 1340, 1320, 1290, 1100, 780, 700, 630 cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 7.3–8.8 (13 H, m, arom.).

The mother liquor was extracted with chloroform (3×40 m) and after evaporation of the solvent and recrystallization from benzene–cycloxehane, (1:1), it gave *1b* with m.p. 172–173 °C (Lit.[8] m.p.=172–173 °C).

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Z/E-Isomerization of Unsaturated Carboxylic Acids during the Kolbe Electrolysis*

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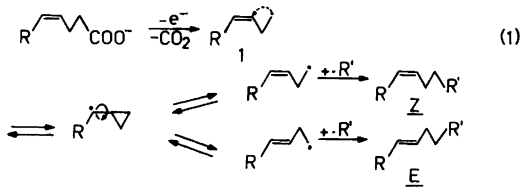
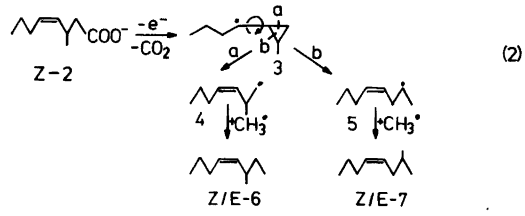
Z-4-Enoic acids partially isomerize to *E*-configurated products in the Kolbe electrolysis. The results from methyl and deuterium labelled carboxylic acids *2* and *16* support an isomerization *via* a reversible ring closure to cyclopropylcarbinyl radicals. The double bonds of *Z*-N-enoic acids with N≥5 fully retain their configuration in the Kolbe electrolysis; for N=6, 7 cyclic products are formed to some extent, which is in accord with the reactivity of 5- and 6-alkenyl radicals.

The Kolbe electrolysis has been successfully applied to the synthesis of pheromones.[2a–c] (for other pheromone syntheses, see Ref. 2d.). As the efficiency of synthetic pheromones to lure insects is very much influenced by the presence of the other geometric isomer,[3] it is the aim of every pheromone synthesis to obtain the olefins configurationally pure. Surprisingly, in the Kolbe syntheses of the *Cabbage looper* and the *Douglas fir tussock moths* pheromones: *Z*-7-dodecenyl acetate and *Z*-6-heneicosen-11-one, a double bond isomerization of the γ,δ-unsaturated carboxylic acid occurred.[4] Starting from pure monomethyl *Z*-4-octene dioate 6–15 % of *E*-configurated coupling products were found. As the recovered half ester was not isomerized, it was assumed that the *Z/E*-conversion occurred after the anodic decarboxylation at the alkenyl radical stage. The isomerization can only take place, when free rotation is possible by decoupling of the π-bond. The most plausible way for that is the reversible, intramolecular cyclization of the intermediate γ-alkenyl radical *1* (eqn. (1)). Such cyclizations have been found in Kolbe electrolyses [5a] (the coelectrolysis of *E*-4-nonen-6-ynoic acid with methyl glutarate afforded 33 % methyl 5-cyclopropyl-6-nonynoate [5b]) and are well known for alkenyl radicals generated from other sources.[6]



(1)



(2)

If a cyclopropylcarbinyl radical is an intermediate, it should be detectable by an appropriate labelling of the unsaturated carboxylic acid, *e.g.* by a β-methyl group in *2* (eqn, (2)). The ring opening of the cyclopropylcarbinyl radical *3*, which is now unsymmetrical, leads to a primary *4* and secondary radical *5*, depending on the position of the ring opening at *a* or *b*. *4* and *5* could be coupled with methyl radicals generated by coelectrolysis with acetic acid to afford *6* and *7*.

* Partly presented at the 10th Scandinavian Meeting on Electrochemistry at Sandbjerg, Denmark, 10–13th June, 1982. Anodic oxidation of *29*; for *28* see Ref. 1.

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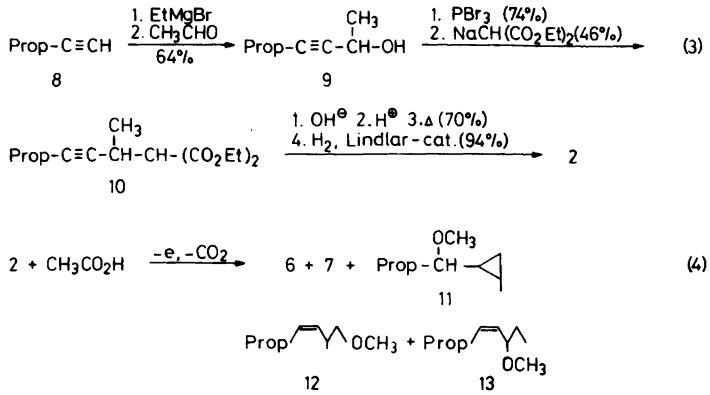


RESULTS

2 was prepared according to eqn. (3) from 1-pentyne (*8*). *8* was added to acetaldehyde to yield 64 % alcohol *9*, which was converted to the bromide and this substituted by sodio diethyl malonate to *10*. *10* was decarboxylated after hydrolysis and hydrogenated with a Lindlar catalyst to *2*. The overall yield of *2* was 14 %, the admixture of *E*-isomers was, as determined by capillary GLC, between 0–0.4 %. To make the product analysis simple, *2* was coelectrolyzed with an excess of acetic acid. This way only the unsymmetrical coupling products *6* and *7* should be yielded, besides ethane, whilst no dimers of *4* or *5* are expected because of the high methyl radical concentration. The electrolysis products (eqn. (4)) and their yields under different reaction conditions are summarized in Table 1.

The products were identified by capillary GLC-MS and ^{1}H NMR spectra or comparison with authentic reference compounds. After hydrogenation the four GLC peaks of *Z/E-6* and *7* merged to two isomeric methyloctanes. A reference sample for *7* was prepared by alkylation of lithio 1-pentyne with i-butylbromide to *14*, which was hydrogenated on a Lindlar catalyst to *Z-7*. *11* was obtained according to eqn. (5) by cyclopropanation of crotonaldehyde with the Simmons-

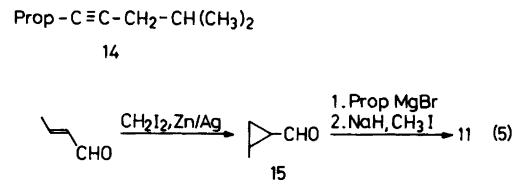
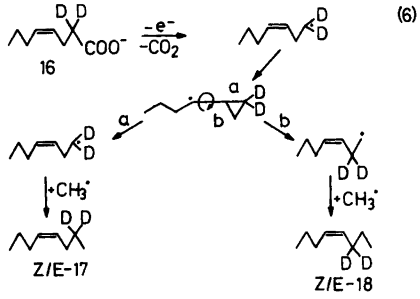


Table 1. Products of the mixed Kolbe electrolysis between *2* and acetic acid (molar ratio 1:10) at different conditions.

Current density (mA/cm^2) [a]	Temp. (°C)	Yield *6* (%)	*Z/E*-Ratio of *6*	Yield *7* (%)	Yield [b] *11* (%)
200	34	34	71/29	7	20
100	−16	35	91/9	2	18
120	0	19	88/12	2	31
120	0–5	21	90/10	2	30
250	25–27	46	85/15	8	7
200	25–30	50	85/15	8	5
200	40	38	79/21	8	16
250	40–43	42	80/20	12	10
200	44	37	75/25	12	14
110	46	40	70/30	21	7
250	60	43	78/22	16	7

[a] Degree of neutralization 5 %. [b] The yield of *12* and *13* was between 2 and 4 %.



(6)

Smith reagent. Subsequently the impure cyclopropanecarboxaldehyde *15*, which was too labile to purify by distillation, was added to propyl magnesiumbromide and the alcohol obtained was methylated with a 20-fold excess of sodium hydride and methyliodide to *11*.

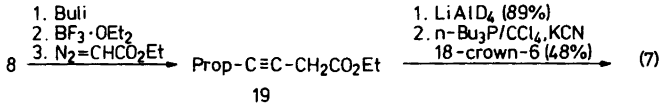
In order to exclude a possible influence of the methyl group in the ring opening of the cyclopropylcarbinyl radical,[7] the dideuterated carboxylic acid *16* (eqn. (6)) was coelectrolyzed with a 10-fold excess of acetic acid. Electrolysis products were to more than 85 % the alkenes *17* and *18* and contained, contrary to the electrolysis with *2*, only small amounts of methyl ethers. The ratio *17:18* was about 95:5; the portion of isomerized *E-17,18* is shown in Table 2. The amount of *18* was determined in two ways by MS and ^{1}H NMR spectroscopy. For the MS analysis *17, 18* were converted by ozonization, oxidative work-up and methylation to methyl butyrate; in the mass spectrometer this forms by a McLafferty rearrangement the fragment: $X_2C{=}C(OH){=}OCH_3^+$ as base peak: [*m/e*=74 (X=H), 76 (X=D)]. From the intensities of *m/e*=76, 74 the portion of *18* was calculated to be 5 %. From the integration of the ^{1}H NMR signals of a carefully purified sample of *17, 18* the ratio of *17:18* was determined to be 94:6. A control experiment confirmed that *16* did not exchange deuterium under the electrolysis conditions. *16* was prepared from 1-pentyne (*8*) according to eqn, (7). *8* was converted to tris(1-pentynyl)bor-

Table 2. Portion of *E-17/18* in the coelectrolysis of *16* with acetic acid (1:10) at different reaction conditions.

Temp. (°C)	Current density (mA/cm^2)	Portion of *E-17/18* (%)
37	200	5.0
39	120	5.8
45	160	8.7
48	170	9.5
52	180	11.5

ane which afforded with diazo ethyl acetate [8] 30 % ethyl 3-heptynoate (*19*). Subsequent reduction with $LiAlD_4$ and reaction with tributylphosphine/KCN under phase transfer conditions [9] yielded after column separation 48 % of the nitrile *20*, which was hydrolyzed and hydrogenated with a Lindlar catalyst to *16*, this *Z*-enoic acid had less than 1 % of the *E*-isomer admixed.

We were next interested in whether similar isomerizations, as found with *2* and *16*, occurred with *Z*-enoic acids *21*, where the distance between the double bond and the carboxyl group was increased. The electrolysis of *21* (*n*=3 to 7) with a 10-fold excess of acetic acid afforded the products *22–25* (eqn. (8), Table 3); the effect of the temperature is shown in Table 4. In all cases, no *Z/E*-isomerization was observed but cyclic products *23* were found for *21* (*n*=4, 5). The portion of Non-Kolbe products: *24, 25* increased with decreasing acidity of the electrolyte. For comparison with the *Z*-enoic acid *2 E-21* (*n*=2) was coelectrolyzed with acetic acid. It yielded 70 % 4-decene, which contained only 2 % of the *Z*-isomer. The products were identified by ^{1}H NMR and mass spectroscopy, *23* and *25* (*n*=4) by authentic reference compounds. Authentic *23* (*n*=4) was prepared by addition of cyclopentyl magnesiumbromide to 2-heptanone, dehydration of the alcohol and subsequent hydrogenation. *25* (*n*=4) could be obtained by reaction of cyclo-



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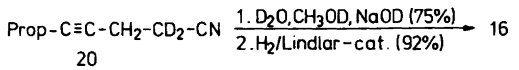
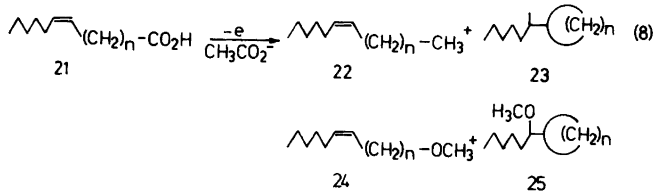




pentyl magnesiumbromide with hexanal and methylation of the alcohol. The carboxylic acids *21* were yielded by alkylation of 1-heptyne with 1,n-chlorobromoalkanes, subsequent substitution to the nitrile and its hydrolysis to the alkynoic acids *26* [*26* (*n*=3) 56 %; (*n*=4) 58 %]; furthermore by alkylation of 1-heptyne with 6-bromohexanoic acid [*26* (*n*=5) 70 %] or with 7-bromo-1-heptanol followed by CrO_3-oxidation [*26* (*n*=6) 34 %]. The alkynoic acids were then hydrogenated with a Lindlar catalyst to *21*.

DISCUSSION

The methyloctene *7* and the cyclopropylcarbinyl methyl ether *11* support the assumption that the double bond isomerization is caused by reversible cyclization of the intermediate alkenyl radical. A mechanism is proposed in eqn, (9).

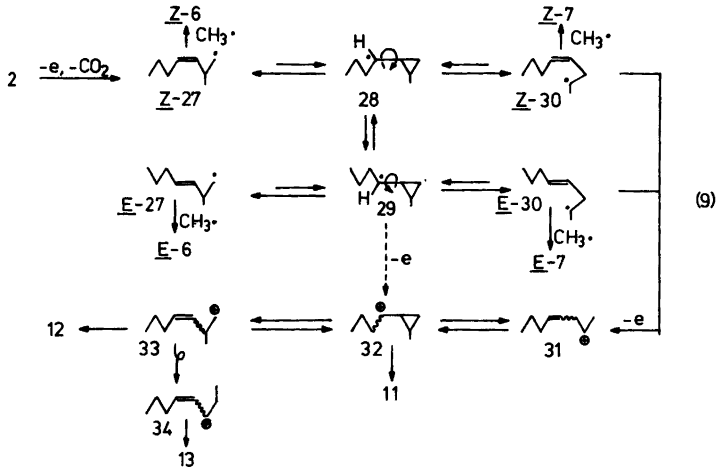
2 is decarboxylated to the alkenyl radical *Z-27*, which can couple with CH_3 to *Z-6* or cyclize to the cyclopropylcarbinyl radical *28*,[15a,b] free rotation around the single bond interconverts *28* and *29*, subsequent fast ring opening of the cyclopropylcarbinyl radicals [10b,11] yields *Z-27*, *30* and *E-27*, *30*, that react with the CH_3 radicals from acetic acid to *Z-6*, *7* and *E-6*, *7*. The *Z*:*E*-ratio of *7* (25:75) possibly reflects the thermodynamic equilibrium between *E*- and *Z-30*. The secondary alkenyl radicals *30* are to some extent oxidized to the alkenyl cation *31*, that can rearrange to *32* and *33*,[12] a subsequent methyl shift converts the primary cation *33* partly to its isomer *34*. *32*, *33*, *34* solvolyze to the methyl ethers *11*–*13*. The oxidation of *28*, *29* to *32* seems to be a less important reaction path to the Non-Kolbe products. With *16*, where only a cyclopropylcarbinyl- and a primary alkenyl radical are intermediates, the alkenes *17*, *18* are the

Table 3. Coelectrolysis of *Z-21* with acetic acid (1:10); reaction conditions and products.

Z-21 *n*	Current density [mA/cm^2]	*T* [°C]	Yield (%) *22*	*23*	*24*	*25*	Rates for ring closure [6b] (s^{-1})
3	200	53	68	–	9	–	7×10^{-1}
4	160	52	46	21	–	5	3.6×10^{5}
5	175	52	65	5	6	–	1.1×10^{4}
6	160	50	72	–	7	–	3.0×10^{2}
7	160	48	73	–	4	–	–

Table 4. Effect of temperature on the product distribution in the Kolbe electrolysis of *Z-21* (*n*=4) with acetic acid.

Temp. (°C)	Current density [mA/cm^2]	Rel. yield (%) *22*	*23*	*24*	*25*
18	100	75	18	2	5
49	170	62	29	1	8
52	160	64	29	–	7



predominant products, whilst the Non-Kolbe products are considerably decreased. Primary radicals, as *E,Z-27,* are normally not oxidized in a Kolbe electrolysis. From the mechanism, it is to be expected that there is a relation between the degree of *Z/E*-isomerization and the amount of *7* and, as Fig. 1 shows, this is indeed the case. Compared to the degree of isomerization the yield of *7* appears to be too low. This could be due to the oxidative drain: *30*→*31* and/or the sterically favoured, predominant cyclization to *trans-28,* that then mainly opens to the primary radical *27.*[7] The latter is suggested by the temperature dependence of the isomerization and of the formation of *7* (see Table 1). Lowering the temperature reduces the portion of *7* much more than the isomerization, which is predicted for *trans-28,* whose selectivity of ring opening to the primary alkenyl radical increases with decreasing temperature.[7] Coupling products of *28* with CH_3. radicals are not to be expected as the equilibrium [*k*(ring-opening) 1.3×10^8 s^{-1}, *k*(cyclization) 1.8×10^4 s^{-1} [6b, 10, 11]] strongly favours the acyclic radicals.

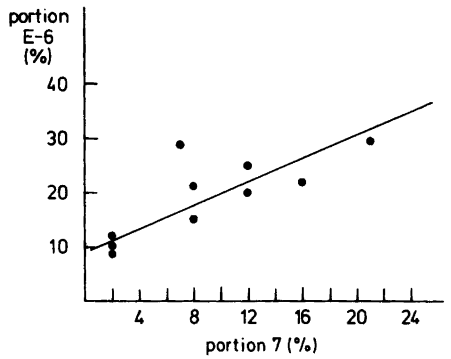
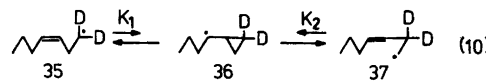
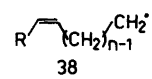


Fig. 1. Relation between double bond isomerization (*E-6*) and rearrangement (*7*).

The results with *2* are independently confirmed by those obtained with *16.* Here 5–11 % of double bond isomerization are accompanied by 4–8 % of rearranged products. As with *2* double bond isomerization increases with temperature. The effect of the methyl substituent in *2*, favouring the unrearranged product, is here excluded. The considerable decrease in Non-Kolbe products indicates that with *2* the secondary alkenyl radicals *30* are partly further oxidized, thus favouring the formation of *6* over this of *7.* With *2* the ratio *7*:*E-6* is 0.17 to 0.72 whilst with *16* the corresponding ratio *18*:*E-17, 18* is 0.72 to 0.8. If one assumes the same equilibrium constant for *35*⇄*36* and *37*⇄*36* (eqn. (10)) one can calculate from the ratio *18*:*E-17, 18* that about 40–30 % *Z-35, 37* and about 60–70 % *E-35, 37* alkenyl radicals are formed in the reversible cyclization. That the *E*-configurated radicals preponderate is also demonstrated by the results with *E-21* (*n*=2), where only 2 % isomerization to the





Z-alkene is found.

Extension of the Kolbe electrolyses to enoic acids *21*, with varying distances between the radical site and the double bond, shows that the *Z*/*E*-isomerization is limited to γ,δ-unsaturated carboxylic acids. For *21* ($n \geq 3$) no isomerization is found, except for a small to moderate portion of cyclic products with $n=5$, 4. These results are readily explained by the proposed mechanism [eqn. (9)]. The cyclization of the alkenyl radicals *38* ($n=3,6$) is 10^5 to 10^2 times slower than this of *38* ($n=2$),[6b] so it is sensible that the cyclization and thus the isomerization cannot compete with the Kolbe coupling. The radicals *38* ($n=4,5$) cyclize as fast as *38* ($n=2$), however, the ring opening of the cyclohexyl- and cyclopentylcarbinyl radicals is much slower, due to the lack of steric strain, than this of the cyclopropylcarbinyl radical,[13] so that the cyclic products are trapped by Kolbe coupling before ring opening.

The results demonstrate that the Kolbe electrolysis is a suitable method for the stereospecific introduction of olefinic units *via* enoic acids, except for γ,δ-unsaturated carboxylic acids, whose double bond can isomerize at the alkenyl radical stage by a reversible cyclization *via* cyclopropylcarbinyl radicals.

EXPERIMENTAL

IR spectra were recorded with the Perkin-Elmer spectrometers 177 and 441, the ^{1}H NMR spectra with the Jeol PMX 60, Varian HA 100 and Bruker WM 300 instruments, TMS was used as internal standard. Mass spectra were obtained with the Varian CH 7 model, the Varian GLC-MS System MAT 111 and for capillary GLC-mass spectroscopy the Varian MAT CH 7 A.

Gas chromatography. GLC analyses were done with the Varian Gaschromatograph 2740, combined with the Perkin-Elmer recorder 56, the Spectra Physics Integrator Vidar 6300 and the following columns; column 1: 1.7 m glass, Ø 2 mm, 4 % FFAP on Chromosorb W-DMCS; column 2: as 1 but with 4 % OV 225; column 3: 3 m glass, Ø 2 mm, 8 % SE 52 on Chromosorb W-DCMS. The *Z*/*E*-isomers were analyzed with the Varian Gaschromatograph 3700 combined with the Kipp and Zonen recorder BD 7, the Spectra Physics integrator Minigrator Autolab and the following capillary glass columns; column 4: 45 m, inner Ø 0.2 mm, outer Ø 0.8 mm, 0.3 % SE 52: as 4 but 36 m; nitrogen served as carrier gas. For preparative GLC the Varian instrument P-90 was used, combined with a Kontron recorder and the following columns; column 6: 3 m glass, Ø 8 mm, 15 % SE 30 on Chromosorb W-DMCS; column 7: 3 m, Ø 8 mm, 15 % FFAP on Chromosorb W-DMCS; carrier gas was hydrogen. For gas chromatographic analyses the carboxylic acids were converted to their methyl esters with diazomethane.[14]

The electrolysis cells were 50 and 150 ml double-walled glass vessels with reflux condenser and a Teflon plug with holes for the thermometer and the electrode feeders. The electrodes were 5 cm^2 platinum sheets (0.05 mm). Current was supplied by the Heri potentiostat/galvanostat TNS 300–1500. Cooling was achieved with the Hoco Kryostat Typ HM 60. All solvents were distilled and if necessary dried. The electrolyses were conducted in analytically pure methanol (Merck).

Preparation of Z-3-Methyl-4-octenoic acid (2). 3-Heptin-2-ol (9). In a 1 l three-necked flask with thermometer, dropping funnel, reflux condenser and gas inlet 14.5 g (0.6 mol) magnesium turnings in 50 ml ether were reacted with 64 g (0.59 mol) ethyl bromide, added dropwise in 400 ml ether. To this solution within 2 h and intense stirring 40 g (0.58 mol) 1-pentyne (*8*) were added and after standing overnight excess *8* was removed by distillation. Then with stirring 24 g (0.55 mol) acetaldehyde were added dropwise. After 1 h reflux it was hydrolyzed with ice and an NH_4Cl-solution, extracted with ether, the ether washed with an $NaHSO_3$- and an $NaHCO_3$-solution and dried (Na_2SO_4). Distillation afforded 41.4 g (64 %) *9*, b.p. 75–78 °C, 19 Torr, n_D^{25}=1.4455 (lit.[15] 1.4454). ^{1}H NMR ($CDCl_3$): δ 4.45 (q, 1H), 2.15 (s, 1H), 2.1 (t, 2H), 1.5 (m, 2H), 1.35 (d, 3H), 0.9 (t, 3H). MS: [*m*/*e* (%, rel. int.)]: 112 (4), 97 (98), 43 (100).

Diethyl-2-(3-heptynyl)-malonate (10). 30 g (0.27 mol) *9* in 50 ml ether were added slowly to 3.5 ml dry pyridine and 24.4 g (0.09 mol) PBr_3 in 300 ml dry ether cooled to −20 °C. After warming to room temperature, the ether was separated and distilled to yield 35 g (74 %) bromide; b.p. 62–64 °C, 15 Torr, n_D^{25}=1.4826 (lit.[15] n_D^{25}=1.4825). To a solution of 4.8 g (0.21 mol) sodium and 33 g (0.2 mol) diethyl malonate in 80 ml dry ethanol, 35 g (0.2 mol) of the bromide were added slowly under stirring and reflux. After 2 h ethanol is distilled off, water added and extracted with ether. Distillation yielded 23.5 g (46 %) *10*, b.p. 81–86 °C/0.1 Torr IR (film): 2960–2820, 2220, 1750–1720 cm^{-1}. ^{1}H NMR ($CDCl_3$); δ 4.2 (q, 4H), 3.3 (m, 1H), 2.1 (m, 1H), 1.7–1.2 (m, 4H), 1.2 (t, 6H), 1.15 (d, 3H), 0.9 (t, 3H). MS [*m*/*e* (%, rel. int.)]: 255 (0.5), 226 (3), 181 (88), 153 (100). Anal.

$C_{14}H_{22}O_4$: C, H.

2. 23.5 g (93 mmol) *10* and 18 g (0.32 mol) KOH in 22 ml water and 44 ml ethanol are refluxed for 4 h. Then ethanol was distilled off, acidified to pH 2 with HCl and extracted with ether (5×), the ether dried and evaporated to yield 15.6 g (85 %) malonic acid, which was heated for 4 h to 180 °C. Subsequent distillation afforded 10.0 g (82 %) ynoic acid, b.p. 149–151 °C, 15 Torr. IR (film): 3200–2500, 2960–2870, 2240 (weak), 1730–1700 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.8 (s, 1H), 2.45 (m, 2H), 1.4 (m, 4H), 1.15 (d, 3H), 0.9 (t, 3H). MS as ethylester [*m/e* (%, rel. int.)]: 182 (7), 167 (22), 154 (62), 95 (100).

2.0 g (12.6 mmol) ynoic acid and 50 mg Lindlar catalyst [16] in 20 ml heptane, to which three drops freshly distilled quinoline were added, were hydrogenated for 6 h at 0 °C. Bulb-to-bulb distillation afforded, after separation of the catalyst, at 110 °C, 14 Torr, 1.93 g (97 %) *2*, portion of *E*-isomer 0.3 % (column 4). IR (film): 3200–2500, 2960–2850, 1710 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.15 (s, 1H, exchangeable with D_2O), 5.25 (m, 2H), 3.02 (m, 1H), 2.25 (d, 2H), 2.05 (m, 2H), 1.32 (m, 2H), 1.03 (d, 3H), 0.9 (t, 3H). MS, as methylester [*m/e* (%, rel. int.)]: 170 (2), 139 (5), 96 (65, McLafferty), 55 (100). Anal. $C_9H_{16}O_2$: C, H.

Electrolyses. 1.6–8.3 mmol *2* and a 10-fold excess of acetic acid, dissolved in 30 ml methanol and neutralized as indicated in Table 1 with KOH were electrolyzed until pH 8 was reached. The electrolyte was then extracted with pentane, the pentane extract washed with K_2CO_3-solution, then water and dried ($MgSO_4$). GLC analysis and GLC-MS combination was done with column 4. By preparative GLC *Z/E- 6, 7* could be separated from *11–13*.

Z/E-3-Methyl-4-octene (6). IR (film): 2980–2850, 1690, 1645, 1450, 1370, 960, 725 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.25 (m, 2H), 2.05 (m, 3H), 1.25 (m, 4H), 0.9 (m, 9H). MS [*m/e* (%, rel. int.)]: 126 (12, M), 111 (1), 97 (32), 55 (100). Anal. C_9H_{18}: C, H.

Z/E-2-Methyl-4-octene (7). MS [*m/e* (% rel. int.)]: 126 (15, M), 111 (13), 98 (7), 97 (6), 55 (100).

2-Methyl-1-methoxy-3-heptene (12). MS [*m/e* (%, rel. int.)]: 142 (2, M), 127 (50), 113 (8), 111 (7), 32 (100).

1-(1-Methoxybutyl)-2-methylcyclopropane (11). MS [*m/e* (%, rel. int.)]: 142 (0.5, M), 127 (8), 110 (4), 99 (100).

3-Methoxy-4-octene (13). MS [*m/e* (%, rel. int.)]: 142 (0.5, M), 113 (65), 110 (5), 99 (12), 32 (100). Anal. for the *11–13* mixture $C_9H_{18}O$: C, H.

Hydrogenation of the electrolysis products. The pentane extract was hydrogenated (10 mg 10 % Palladium on charcoal) 2 h at 0 °C and analyzed by GLC-MS (column 4, 80 °C).

3-Methyloctane from Z/E-6. MS [*m/e* (%, rel. int.)]: 128 (2, M), 113 (1), 99 (12), 57 (100).

2-Methyloctane from Z/E-7. MS [*m/e*, (%, rel. int.)]: 128 (1, M), 113 (2), 85 (13), 43 (100).

2-Methyl-1-methoxyheptane from 12. MS [*m/e* (%, rel. int.)]: 144 (0.1, M), 129 (4), 101 (20), 87 (30), 73 (100).

3-Methoxyoctane from 13. MS [*m/e* (%, rel. int.)]: 144 (0.1, M), 115 (20), 83 (38), 73 (100).

11 remained unchanged.

Preparation of Z-2,2-dideutero-4-octenoic acid (16). *Ethyl 3-heptynoate (19)*. To a solution of lithium-1-pentyne (from 0.25 mol 1-pentyne and 0.25 mol butyllithium in 100 ml tetrahydrofuran) held at −20 °C were added, gradually, 0.33 mol boron trifluoride etherate. The resulting mixture was stirred for 0.5 h at −20 °C and then 12.16 g (0.107 mol) ethyl diazoazetate [18] were slowly added. After stirring for 1.5 h water was added, the mixture stirred (*ca.* 20 min) then poured into ice water (200 ml). It was extracted with ether (3×100 ml), the extracts dried (Na_2SO_4), the solvent evaporated and distilled to afford 4.0 g (0.026 mol, 24 %) ethyl 3-heptynoate,[19] b.p. 83–84 °C/14 Torr. (lit.[19] 83–85 °C/15 Torr), n_D^{25}=1.4465 (lit.[19] n_D^{25}=1.4470). IR (film): 2950–2850, 2220, 1780–1720 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.2 (q, 2H), 3.2 (s, 2H), 2.17 (m, 2H), 1.5 (m, 2H), 1.25 (t, 3H), 0.9 (t, 3H). MS [*m/e* (%, rel. int.)]: 154 (10, M), 126 (80, McLafferty), 125 (42), 109 (25), 79 (100).

1,1-Dideutero-3-heptyn-1-ol. To a solution of 200 mg (4.76 mmol) of lithium aluminium deuteride in 30 ml of ether were introduced at a rate such as to produce gentle reflux 1 g (6.5 mmol) *19*. Then water was added dropwise, the mixture was poured into 100 ml of ice water acidified with 50 ml of 10 % sulfuric acid. The usual work-up yielded 0.741 g (89 %) 1,1-dideutero-3-heptyn-1-ol, b.p. 85–90 °C/14 Torr. IR (film): 3600–3200, 2960–2800, 2200, 2100, 1150–1040 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.2 (s, 1H), 2.4 (s, 2H), 2.15 (m, 2H), 1.5 (m, 2H), 0.95 (t, 3H). MS [*m/e* (%, rel. int.)]: 114 (7, M), 99 (4), 95 (3), 67 (100).

1,1-Dideutero-1-cyano-3-heptyne (20). To a mixture of 10.5 g (0.161 mol) potassium cyanide and 1.7 g (6.5 mmol) 18-crown-6 in 40 ml acetonitrile 3.68 g (32.3 mmol) 1,1-dideutero-3-heptyn-1-ol and 7.18 g (35.5 mmol) tributylphosphine in 20 ml acetonitrile are added, followed under cooling in dry ice methanol by the dropwise addition of a solution of 5.5 g (35.5 mmol) carbon tetrachloride in 10 ml acetonitrile. The mixture is stirred at room temperature for 24 h,

diluted with diethyl ether (300 ml) and washed with 70 ml aqueous 10 % nitric acid. After the addition of 10 ml carbon tetrachloride, the mixture is washed with water (2×100 ml), saturated aqueous sodium chloride (100 ml) and dried (Na_2SO_4). The crude product is purified by TLC on silica gel (Merck, CH_2Cl_2/CH_3CCO_2Et, 8:1) to yield 1.9 g (48 %) *20*. IR (film): 2960–2850, 2220, 2120 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.5 (s, 2H), 2.17 (m, 2H), 1.5 (m, 2H), 0.9 (t, 3H). MS [*m/e* (%, rel. int.)]: 124 (2, M), 123 (20), 122 (40), 108 (48), 79 (100).

2,2-Dideutero-4-octynoic acid. A mixture of 1.9 g (15,4 mmol) *20*, 4.2 g of 30 % sodium hydroxide-d_1 and 5 ml of methanol-d_4 was refluxed for 96 h. Then the solvent was evaporated to dryness, the residue dissolved in 20 % aqueous ethanol and twice extracted with ether. After acidification of the aqueous layer with dilute hydrochloric acid the precipitated organic acid was extracted with ether. Distillation afforded 1.53 g (70 %) 2,2-dideutero-4-octynoic acid, b.p. 138 °C/14 Torr. IR (film): 3500–2500, 2960–2850, 2200 2130–2070, 1740–1670 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.5 (s, 1H), 2.48 (s, 2H), 2.05 (m, 2H), 1.5 (m, 2H), 0.9 (t, 3H). MS [*m/e* (%, rel. int.)]: 156 (0.6, M), 141 (3), 128 (33), 125 (10), 99 (100). Anal. $C_8H_{12}D_2O_2$: C, H+D.

Z-2,2-Dideutero-4-octenoic acid (Z-16). 720 mg (5 mmol) 2,2-dideutero-4-octynoic acid and 30 mg Lindlar catalyst [16] in 15 ml heptane, to which three drops freshly distilled quinoline were added, were hydrogenated for 2 h at 0 °C. Bulb-to-bulb distillation afforded after separation of the catalyst at 130 °C, 14 Torr, 710 mg (97 %) *Z-16*, portion of *E*-isomer *ca.* 0.1 % (column 4). IR (film): 3200–2500, 2960–2850, 1720–1690, 1680–1650 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.1 (s, 1H), 5.35 (m, 2H), 2.35 (d, 2H), 2.05 (q, 2H), 1.4 (m, 2H), 0.9 (t, 3H). MS, as methylester [*m/e* (%, rel. int.)]: 158 (3, M), 127 (20), 126 (56), 82 (65, McLafferty), 76 (100).

Electrolyses of Z-16. 1.2–2.1 mmol *Z-16* and 10-fold excess of acetic acid, dissolved in 25 ml methanol and to 5 % neutralized with KOH were electrolyzed until pH 8 was reached. The electrolyte was then extracted with pentane, the pentane extract washed with K_2CO_3-solution, water and dried ($MgSO_4$). GLC analysis and GLC-MS combination was done with column 4. By preparative GLC the mixture of *17* and *18* was purified for ^1H NMR. ^1H NMR ($CDCl_3$): δ 5.35 (m, 2H), 2.0 (m, 3.88 H, portion of D 6 %), 1.36 (m, 2.12 H, portion of D 94 %), 0.9 (t, 6H). MS [*m/e* (%, rel. int.)]: 114 (10, M), 99 (1), 85 (14), 83 (9), 41 (100).

Ozonization of Z/E-17, 18. The *Z/E*-mixture was treated in 5 ml pentane and 5 ml methanol at −40 °C with 2 mmol ozone. Work-up with CrO_3 in acetone afforded butyric acids, which were esterified with diazomethane. The methyl butyrates were analyzed with the capillary GLC-MS combination. From the ratio of the peaks *m/e*=-74/74 (McLafferty) the ratio *17/18* was calculated to be 95/5.

Control experiment for deuterium-exchange of Z-16. A mixture of 20 mg (0.14 mmol) *Z*-16 and 84 mg acetic acid (1.4 mmol) in 15 ml methanol, neutralized to 5 % with KOH, was stirred for 1.5 h. The GLC-MS analysis showed for the reisolated *Z-16* no reduced deuterium content.

Preparation of Z-21 (n=*3–7*). Z-21, n=*3,4*. To a solution of 9.6 g (0.1 mol) 1-heptyne in 100 ml dry tetrahydrofuran and 10 ml HMPT, cooled to −20 °C, 0.1 mol *n*-butyllithium in hexane were injected under stirring. After 40 min 0.1 mol 1-bromo-4-chlorobutane (*n*=3) or 1-bromo-5-chloropentane (*n*=4), respectively, were added dropwise, hydrolyzed (150 ml water) after 12 h and extracted with ether (3×100 ml). Distillation at 78 °C/0.4 Torr afforded for *n*=3 8.5 g (65 %) chloride and at 81 °C/0.3 Torr for *n*=4 13.3 g (71 %) chloride, respectively. The products contained *ca.* 8 % of bromide. MS for *n*=3 [*m/e* (%, rel. int.)]: 174/172 (0.5 M), 159/157 (3), 145/143 (10), 81 (100). MS for *n*=4 [*m/e*, (%, rel. int.)]: 188/186 (0.5, M), 173/171 (2), 159/157 (18), 67 (100).

A mixture of 49 mmol chloride (*n*=3) and 8 g sodium cyanide or 60 mmol chloride (*n*=4) and 9.8 g sodium cyanide in 20 ml of water and 120 ml ethanol was stirred and refluxed for 48 h. The residue was filtered off and 100 ml solvent evaporated. To the solution of 1-cyano-4-decyne (*n*=3) or 1-cyano-5-undecyne (*n*=4) in 50 ml ethanol was added 0.15 mol sodium hydroxide and refluxed for 96 h. The usual work-up and distillation afforded 6.9 g (78 %) ynoic acid (*n*=3) at 138 °C/0.3 Torr or 9.8 g (83 %) ynoic acid (*n*=4) at 145 °C/0.3 Torr.

Ynoic acid, *n*=3: IR (film): 3200–2500, 2950–2820, 1720–1690 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.1 (s, 1H), 2.15 (m, 6H), 1.4 (m, 8H), 0.9 (t, 3H). MS, as methylester, [*m/e* (%, rel. int.)]: 196 (3, M), 181 (4), 165 (4), 164 (3), 122 (18), 80 (100).

Ynoic acid, *n*=4: IR (film): 3200–2500, 2950–2820, 1720–1690 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.6 (s, 1H), 2.15 (m, 6H), 1.4 (m, 10H), 0.9 (t, 3H). MS, as methylester, [*m/e* (%, rel. int.)]: 210 (0.5, M), 195 (1), 179 (3), 178 (4), 136 (15), 80 (100). The hydrogenation with Lindlar catalyst afforded 91 % *Z-21 n*=3 and 97 % *Z-21 n*=4.

Z-21, n=3: B.p. 130 °C/0.3 Torr. IR (film): 3200–2500, 2950–2850, 1725–1690, 730–675 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.8 (s, 1H), 5.35 (m,

2H), 2.35 (t, 2H), 2.05 (m, 4H), 1.7 (m, 2H), 1.25 (m, 6H), 0.9 (t, 3H). MS, as methylester, [*m/e* (%, rel. int.)]: 198 (2, M), 166 (11), 124 (28), 74 (100). Anal. $C_{11}H_{20}O_2$: C, H.

Z-21, n=4: B.p. 138 °C/0.3 Torr. IR (film): 3200–2500, 2950–2850, 1725–1690, 730–675 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.0 (s, 1H), 5.25 (m, 2H), 2.35 (t, 2H), 2.05 (m, 4H), 1.65 (m, 2H), 1.4–1.2 (m, 8H), 0.9 (t, 3H). MS, as methylester, [*m/e* (%, rel. int.)]: 212 (4, M), 181 (10), 180 (18), 138 (28), 74 (100). Anal. $C_{12}H_{22}O_2$: C, H.

Z-21, n=*5*: 3.84 g (40 mmol) 1-heptyne was added to a stirred suspension of 40 mmol lithiumamide in 100 ml liquid ammonia and the mixture was stirred under reflux for 1 h. After addition of 10 mmol 6-bromohexanoic acid in 30 ml tetrahydrofuran, the mixture was refluxed for 8 h and then allowed to evaporate. Dilute hydrochloric acid was added and the mixture was extracted with ether. The washed and dried (Na_2SO_4) solution was evaporated under reduced pressure and the residue distilled at b.p. 160 °C/0.3 Torr to afford 1.47 g (70 %) ynoic acid.

IR (film) 3200–2500, 2950–2820, 1720–1690. ^1H NMR ($CDCl_3$): δ 10.0 (s, 1H), 2.37 (t, 2H), 2.15 (m, 4H), 1.68 (m, 2H), 1.40 (m, 10H), 0.9 (t, 3H). MS, as methylester [*m/e* (%, rel. int.)]: 224 (2, M), 193 (6), 192 (6), 150 (27), 94 (100).

The hydrogenation with Lindlar catalyst [16] afforded 99 % *Z-7*-tridecenoic acid-(*Z-21, n=5*), b.p. 150–154 °C/0.1 Torr, n_D^{27}=1.4522 (lit.[20] n_D^{27}=1.4526). IR (film): 3200–2500, 2960–2850, 1730–1670. ^1H NMR ($CDCl_3$) δ=11.0 (s, 1H), 5.35 (m, 2H), 2.35 (t, 2H), 2.05 (m, 4H), 1.65 (m, 2H), 1.4–1.2 (m, 10H), 0.9 (t, 3H). MS, as methylesters [*m/e* (%, rel. int.)]: 226 (1, M), 195 (5), 194 (7), 152 (15), 55 (100).

Z-21, n=*6*. *7-Bromoheptan-1-ol*. A mixture of 10 g heptane-1,7-diol and 50 ml 48 % HBr in 15 ml water was kept at 70–80 °C and extracted continuously with toluene for 16 h. From the extract 10.9 g (74 %) of 7-bromoheptan-1-ol, b.p. 75 °C/0.05 Torr (lit.[21] 111–112 °C/4 Torr) were yielded. IR (film) 3600–3100, 2930–2860, 1455–1425, 1245, 1050, 750–725 cm^{-1}. ^1H NMR ($CDCl_3$): δ=3.6 (t, 2H), 3.4 (t, 2H), 2.55 (s, 2H), 1.85 (m, 2H), 1.6–1.36 (m, 8H). MS [*m/e* (%, rel. int.)]: 178/176 (0.1, M), 150/148 (20), 55 (100).

8-Tetradecyn-1-ol. 5.2 g (60 mmol) 1-heptyne in 30 ml tetrahydrofuran were added to 0.12 mol lithiumamide in 100 ml liquid ammonia. After stirring for 1 h 5.85 g (30 mmol) 7-bromoheptan-1-ol in 30 ml tetrahydrofuran were added. The mixture was stirred under reflux for 8 h and then allowed to evaporate. Addition of dilute hydrochloric acid and extraction with ether gave by bulb-to-bulb distillation 4.07 g (65 %) alcohol, b.p. 120 °C/0.03 Torr. IR (film) 3600–3100, 2960–2850, 2200 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.6 (t, 2H), 2.06 (m, 4H), 1.8 (s, 1H), 1.6–1.2 (m, 16 H), 0.9 (t, 3H). MS [*m/e* (%, rel. int.)]: 210 (0.5, M), 192 (1), 67 (100).

8-Tetradecynoic acid. 4 g (19 mmol) 8-tetradecyn-1-ol in 57 mol acetic acid were stirred at 15–20 °C while a chromic acid solution (5.7 g, CrO_3 in 50 ml acetic acid) was gradually added. The acetic acid was evaporated under reduced pressure and water (100 ml) was added. The ether extracts were extracted with a sodium hydrogen carbonate solution. Acidification and distillation gave 2.26 g (53 %) ynoic acid, b.p. 155 °C/0.05 Torr. IR (film) 3200–2500, 2960–2850, 1730–1680. ^1H NMR ($CDCl_3$): δ=11.00 (s, 1H), 2.35 (t, 2H), 2.16 (m, 4H), 1.65 (m, 2H), 1.5–1.2 (m, 12H), 0.9 (t, 3H). MS, as methylester [*m/e* (%, rel. int.)]: 238 (0.5 M), 207 (2), 206 (2), 164 (17), 81 (100).

The hydrogenation with Lindlar [16] catalyst afforded 99 % (*Z*)-8-tetradecenoic acid (*Z-21, n=6*), b.p. 160 °C/0.1 Torr. IR (film) 3200–2500, 2960–2850, 1730–1670. ^1H NMR ($CDCl_3$): δ=11.0 (s, 1H), 5.35 (m, 2H), 2.37 (t, 2H), 2.05 (m, 4H), 1.62 (m, 2H), 1.4–1.2 (m, 12H), 0.9 (t, 3H). MS, as methylester, [*m/e* (%, rel. int.)]: 240 (3, M), 209 (18), 208 (23), 166 (27), 55 (100). Anal. $C_{14}H_{26}O_2$: C, H.

Z-21, n=*7*, oleic acid was purchased from Fa. Fluka.

Electrolyses of 21. 0.5 to 6.0 mmol *21* and a 10-fold excess of acetic acid, dissolved in 30 ml methanol and neutralized to 5 % with KOH were electrolyzed until pH 8 was reached. The electrolyte was then extracted with ether, the ether extract washed with K_2CO_3-solution and water and dried (Na_2SO_4). GLC analysis and GLC-MS combination was done with column 4, preparative GLC with column 6.

5-Undecene (Z-22, n=*3*). n_D^{20}=1.4296 (lit.[22] n_D^{20}=1.4285). IR (film): 2980–2830, 1640, 1450, 720–670 cm^{-1}. ^1H NMR ($CDCl_3$) δ=5.35 (m, 2H), 2.0 (m, 4H), 1.3 (m, 10H), 0.9 (t, 6H). MS [*m/e* (%, rel. int.)]: 154 (6, M), 125 (5), 111 (12), 55 (100).

Products of Z-21, n=*4*. 6-Dodecene (*Z-22, n=4*): n_D^{20}=1.4329 (lit.[23] n_D^{20}=1.4334). IR (film): 2980–2830, 1450, 720–670. ^1H NMR ($CDCl_3$): δ=5.37 (2H, m), 2.05 (m, 4H), 1.30 (m, 12H), 0.9 (t, 6H). MS [*m/e* (%, rel. int.)]: 168 (28, M), 140 (2), 139 (1), 125 (4), 55 (100).

2-Cyclopentylheptane (23, n=*4*). MS [*m/e* (%, rel. int.)]: 168 (4 M), 153 (1), 97 (100).

1-Cyclopentyl-1-methoxyhexane (25, n=*4*). MS [*m/e*, (%, rel. int.)]: 184 (6, M), 153 (7), 152 (18), 115 (100), 113 (75).

Products of 21, n=*5*. *6-Tridecene* (Z-22, n=*5*).

n_D^{20}=1.4368 (lit.[24] n_D^{20}=1.4367). IR (film): 2980–2830, 1640, 720–670 cm^{-1}. ^1H NMR ($CDCl_3$): δ=5.35 (m, 2H), 2.03 (m, 4H), 1.3 (m, 14H), 0.9 (t, 6H). MS [*m/e* (%, rel. int.)]: 182 (40, M), 154 (7), 139 (2), 74 (100).

2-Cyclohexylheptane (23, n=*5*). MS [*m/e* (%, rel. int.)]: 182 (25, M), 167 (1), 111 (37), 83 (70), 82 (100).

1-Methoxy-6-dodecene (24, n=*5*). MS [*m/e* (%, rel. int.)]: 198 (2, M), 169 (10), 167 (8), 166 (40), 73 (100).

6-Tetradecene (Z-22, n=*6*). IR (film): 2980–2830, 1720–1690, 1460–1430, 720 cm^{-1}. ^1H NMR ($CDCl_3$): δ=5.35 (m, 2H), 2.05 (m, 4H), 1.3 (m, 16H), 0.9 (t, 6H). MS [*m/e* (%, rel. int.)]: 196 (8, M), 168 (1), 153 (1), 55 (100). Anal. $C_{14}H_{28}$: C, H.

9-Octadecene (Z-22, n=*7*). IR (film): 2980–2830, 1460–1430, 720 cm^{-1}. ^1H NMR ($CDCl_3$): δ=5.35 (m, 2H), 2.05 (m, 4H), 1.3 (m, 24 H), 0.9 (t, 6H). MS [*m/e* (%, rel. int.)]: 252 (9, M), 224 (2), 209 (1), 83 (100). Anal. $C_{18}H_{36}$: C, H.

Preparation of E-*4-octenoic acid* (E-21, n=*2*). A mixture of 4.88 g (48.8 mmol) 1-hexen-3-ol (prepared from 0.2 mol 2-propenone-1 and 0.2 mol 1-bromopropane under Grignard conditions) and 0.13 ml propanoic acid in 60 ml triethyl orthoacetate was heated at 120 °C for 12 h in a water separator. After evaporation of the triethylorthoacetate, the residue was hydrolyzed with 0.15 mol potassium hydroxide in 100 ml methanol. The bulb-to-bulb distillation afforded 4.7 g (67 %) *E*-4-octenoic acid. B.p. 132 °C/15 Torr (lit.[25] 93 °C/1.5 Torr). n_D^{20}=1.4440 (lit.[25] n_D^{20}=1.4441). IR (film): 3300–2500, 2960–2840, 1720–1690, 960 cm^{-1}. ^1H NMR: δ 11.5 (s, 1H), 5.47 (m, 2H), 2.40 (m, 2H), 2.32 (t, 2H), 1.96 (m, 2H), 1.38 (m, 2H), 0.9 (t, 3H). MS [*m/e* (%, rel. int.)]: 156 (3, M), 141 (1), 125 (15), 124 (41), 82 (58), 74 (100).

Electrolysis of E-21, n=*2*. *E-21* (*n*=2) was electrolyzed as *Z-16*.

Products were analyzed by GLC-MS and comparison with authentic *E*-4-octene (Fa. Fluka).

Preparation of reference compounds.

Z-2-Methyl-4-octen (*7*). To 4.76 g (0.07 mol) 1-pentyne in 30 ml dry HPMT under stirring 70 mmol *n*-butyllithium in hexane were injected, after 35 min the hexane was distilled off at reduced pressure and the solution cooled to 0 °C. Then 9.59 g (70 mmol) *i*-butyl bromide were added, hydrolyzed (150 ml water) after 12 h and extracted with pentane (4×75 ml). Distillation at b.p. 66 °C/400 Torr afforded 2.94 g 2-methyl-4-octyne (*14*). n_D^{20}=1.4267. IR (film): 2980–2840, 1470–1430 cm^{-1}. ^1H NMR ($CDCl_3$) δ=2.15 (m, 4H), 1.8–1.2 (m, 3H), 0.9 (m, 9H). MS [*m/e* (%, rel. int.)]: 124 (20, M), 109 (11), 95 (22), 81 (52), 57 (100). Anal. C_9H_{16}: C, H).

200 mg (1.6 mmol) *14* are hydrogenated in 20 ml pentane with the Lindlar catalyst, which was deactivated with 2 drops quinoline. After purification by preparative GLC 0.14 g (70 %) *7* were obtained. n_D^{20}=1.4180 (lit.[17] n_D^{20}=1.4181). IR (film): 2980–2850, 1450, 725 cm^{-1}. ^1H NMR ($CDCl_3$): δ=5.4 (m, 2H), 2.05 (m, 3H), 1.7–1.2 (m, 3H), 0.95 (m, 9H). MS [*m/e* (%, rel. int.)]: 126 (22, M), 111 (11), 98 (10), 97 (4), 84 (12), 83 (15), 55 (100). Anal. C_9H_{18}: C, H.

Isomerization of Z-7. 200 mg *Z-7* were added slowly with intense stirring to a mixture of 5 ml 2n HCl and 250 mg $NaNO_2$. After 5 min it was hydrolyzed and extracted with ether. The product consisted of a 1:1-mixture of *Z*/*E-7*.

1-(1-Methoxybutyl)-2-methylcyclopropan (*11*). *2-Methylcyclopropane carboxaldehyde* (*15*). To the zinc-silver-couple (prepared from 17 g zinc and 100 mg silver acetate in 100 ml acetic acid) in ether were first added 7 g (0.1 mol) crotonaldehyde and then dropwise 34 g (0.13 mol) diiodmethane. For work-up ether and pyridine were added, the precipitate removed by filtration and the ether evaporated to yield 4.7 g (56 %) *15* that contained unreacted crotonaldehyde, which could not be removed by distillation or TLC. MS [*m/e* (%, rel. int.)]: 84 (18, M), 83 (68), 70 (5), 55 (100); it corresponded to the MS in lit.[26]

1-(1-Methoxybutyl)-2-methylcyclopropan (*11*). 4 g of impure *15* were added to *n*-propyl magnesiumbromide (55 mmol Mg, 6.76 g (55 mmol) 1-bromopropan) in 50 ml ether. After heating for 2 h under reflux, hydrolysis with NH_4Cl and usual work-up 3 g (49 %) cyclopropylcarbinol containing heptenol, were obtained.

1-(1-Hydroxybutyl)-2-methylcyclopropan: IR (film): 3500–3200, 3050, 2990, 2950–2860 cm^{-1}. ^1H NMR ($CDCl_3$): δ=2.9 (m, 1H), 1.6–1.4 (m, 4H), 1.4 (s, 1H), 1.02 (m, 3H), 0.9 (m, 3H), 0.6 (m, 2H), 0.4–0.2 (m, 2H). MS [*m/e* (%, rel. int.)]: 128 (0.5, M), 110 (6), 85 (100), 81 (12). Anal. $C_8H_{16}O$: C, H.

500 mg (3.9 mmol) of impure cyclopropylcarbinol, after being distilled from sodium, were reacted in 20 ml tetrahydrofuran with 3 g (80 %) sodium hydride. After 1 h 35 g (0.25 mol) methyliodide were added and stirred for 24 h. After the usual work-up *11* was isolated by preparative GLC (column 6, 90 °C iso.). IR (film): 3050, 2990, 2950–2860, 1090–1140 cm^{-1}. ^1H NMR ($CDCl_3$): δ=3.35 (s, 3H), 2.45 (m, 1H), 1.7–1.2 (m, 4H), 1.05 (m, 3H), 0.9 (m, 3H), 0.7–0.2 (m, 4H). MS [*m/e* (%, rel. int.)]: 142

(0.5, M), 127 (1), 110 (2), 99 (100). Anal. $C_9H_{18}O$: C, H.

2-Cyclopentylheptane (23, n=6). 10 g (67 mmol) cyclopentylbromide were reacted under Grignard conditions with 2-heptanone to the tertiary alcohol, which was dehydrated giving two isomers. The hydrogenation with 10 % palladium/charcoal afforded 2-cyclopentylheptane, which was purified by preparative GLC. IR (film): 2960–2840, 1460–1430, 1365 cm^{-1}. ^1H NMR ($CDCl_3$): δ=1.8–1.1 (m, 18H), 0.9 (m, 6H). MS [*m/e* (%, rel. int.)]: 168 (2, M), 153 (1), 97 (100). Anal. $C_{12}H_{24}$: C, H.

1-Cyclopentyl-1-methoxyhexane (24, n=4). The Grignard reaction of 7.5 g (50 mmol) cyclopentylbromide and of 4.0 g (40 mmol) hexanal afforded 5.0 g (74 %) 1-cyclopentylhexanol-1, b.p. 140 °C/20 Torr. IR (film): 3500–3200, 2960–2850 cm^{-1}. ^1H NMR ($CDCl_3$): δ=3.4 (m, 1H), 1.9 (m, 1H), 1.57 (m, 8H), 1.5 (s, 1H), 1.3 (m, 8H), 0.9 (t, 3H). MS [*m/e* (%, rel. int.)]: 169 (1), 152 (3), 81 (100). 200 mg (1.2 mmol) of cyclopentylhexanol-1 in 10 ml ether were added to a suspension of 1 g (30 mmol) sodium hydride in 10 ml dry ether. After 1 h 5 ml methyliodide were added. The suspension was stirred for 6 h, hydrolyzed and extracted with ether. The bulb-to-bulb distillation gave 160 mg (72.5 %) 1-cyclopentyl-1-methoxyhexane, b.p. 138 °C/18 Torr. IR (film): 2960–2830, 2810, 1170–1150 cm^{-1}. ^1H NMR ($CDCl_3$): δ=3.35 (s, 3H), 3.0 (m, 1H), 2.0 (m, 1H), 1.5 (m, 8H), 1.2 (m, 8H), 0.9 (t, 3H), – MS [*m/e* (%, rel. int.)]: 184 (1, M), 153 (1), 152 (2), 115 (100), 113 (82). Anal. $C_{12}H_{24}O$: C, H.

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A High Current Density Electrosynthesis of Amines from Nitro Compounds Using Metal Powders as Intermediates

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Copper, iron, tin and zinc powders may each be formed in very good yield in acidic aqueous solution by electrolysis at high current density (0.5 A cm^{-2}) of a solution of their metal salts (0.4–1.0 M) in an appropriate electrolyte. It is further shown that a range of nitro compounds may be reduced to the corresponding amines by electrolysis at 0.5 A cm^{-2} of an emulsion of the nitro compound, or its solution in an inert solvent, with the aqueous medium for deposition of the metal powder (typical ratio of water to organic 4:1). Tin and zinc are the best metals for this reaction when the organic and current yields can exceed 90 %.

In industry the reduction of aromatic nitro compounds to amines is carried out either catalytically or using metal/acid reductions. The latter are more suited to medium and small scale processes but lead to large volumes of effluent containing heavy metal ions. Both types of reaction have been extensively reviewed.[1,2,3] The electrolytic reduction of nitro compounds has also been extensively discussed as an alternative route [4,5] but, in general, suffers from problems of selectivity and insufficient current density. As a result, a number of indirect cathodic reductions, *e.g.* with titanium ions as redox catalysts,[6] have been proposed. In this paper we discuss the optimisation of a reaction route where the electrode process is the formation of metal powder, *i.e.* the reaction sequence is

$$M^{2+} + 2e \longrightarrow M$$

$$3M + 6H^+ + C_6H_5NO_2 \longrightarrow 3M^{2+} + 2H_2O + C_6H_5NH_2$$

where M^{2+} is Cu^{2+}, Fe^{2+}, Sn^{2+} or Zn^{2+}. An earlier preliminary communication [7] has reported that this reaction route is possible at high current density and gives good selectivity. Moreover, the metal ion is recycled continuously so that the reaction need lead to no effluent containing heavy metal ions.

RESULTS AND DISCUSSION

Selection of the metal intermediate. Four metals, copper, iron, tin and zinc were considered as the intermediate for the high currect density electrochemical, reduction of nitro compounds to amines. The metals cover a spectrum of reducing ability, their standard electrode potentials in non-complexing media spanning the range from +0.03 to −0.78 V *vs.* NHE (see Table 1). In order to be suitable intermediates for the desired reaction, it was necessary to find conditions where the powders could be deposited with good efficiency at high current density from an acid electrolyte since the reduction of the nitro compounds consumed 6 H^+/molecule and requires a low pH for a good yield.

Hence, several electrolytes were investigated for each metal. Those found suitable are listed in Table 1 which further reports the potentials where deposition commences in each electrolyte and also the current efficiencies for metal powder formation at 0.5 A cm^{-2} for three concentrations of the metal ions. Finally, Table 1 reports yields of aniline from nitrobenzene from experiments where an emulsion of 50 cm^3 of aqueous electrolyte and 10 cm^3 of nitrobenzene were electrolysed at a rotating C or Al cylinder; the electrolyses

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Table 1. A comparison of metal powder intermediates for the reduction of nitrobenzene to aniline.

Electrolyte	E_e° *vs.* NHE/V [a]	E_{dep} *vs.* NHE/V [b]	Current efficiency [c] $M^{2+}+2e\rightarrow M$ at various $[M^{2+}]$/% 1 M	0.4 M	0.1 M	Current efficiency [c,d] $C_6H_5NO_2\rightarrow C_6H_5NH_2$
$ZnSO_4+H_2SO_4$ (1 M)	−0.76	−0.90	83	52	14	49 (74 [e,f])
$ZnCl_2$+HCl (0.1 M)	−0.76	−0.78	90	98	21	93 [f]
$SnCl_2$+HCl (1 M)	−0.14	−0.28	92	98	83	96
$CuSO_4+H_2SO_4$ (1 M)	+0.03	+0.03	94	91	23	16
$FeCl_2$+HCl (0.01 M)+ NaCl (0.7 M)	−0.44	−0.75	89	95	94	low (80 [g])

[a] Standard potentials for the M^{2+}/M couple in non-complexing media. [b] Potential where metal deposition commences. [c] Current density 0.5 A cm^{-2}; charge for formation 1 g of M passed. [d] For conditions, see text. [e] With 0.1 M H_2So_4. [f] Acid added in aliquots during electrolysis. [g] Metal powder formed then nitrobenzene and 1 M HCl added and the emulsion refluxed.

were carried out at 0.5 A cm^{-2} until the charge for deposition of 1 g metal had been passed and were carried out at room temperature.

Comparison of the data shows it was possible to find conditions where deposition of each metal powder was possible with high current efficiency even when the metal ion concentration was as low as 0.4 M. Zinc and tin were, however, the better metals for this organic reaction. The conversion of nitrobenzene is low when the intermediate is copper and, indeed, at the end of the electrolysis, considerable metal powder was visible in the electrolyte. It was also not possible to find a very acidic electrolyte where iron powder was formed efficiently (iron is a good hydrogen evolution catalyst) and the electrolyte selected only gave a very low yield of aniline. Iron powder is, however, widely used in industry for the chemical reduction of nitrobenzenes but generally the reaction is carried out under reflux in more acid solution. Hence a further experiment was attempted where the iron powder was first formed by electrolysis and then 1 M HCl and nitrobenzene were added and the emulsion refluxed; the yield then increased to 80 %.

While both zinc and tin were used in later experiments, the latter is clearly the superior choice. Zinc requires the acid concentration to be controlled within a limit and, consequently, during the reduction of nitro compounds, aliquots of acid must be added during electrolysis. In contrast, tin power formation was satisfactory even from 0.1 M $SnCl_2$+1.0 M HCl. Moreover, tin deposition occurs at least 0.5 V less negative than zinc deposition and, in an industrial cell, this would lead to a significant reduction in energy consumption.

Selection of other reaction conditions. A number of other experimental parameters, namely cathode material, cathode current density, stirring of the emulsion, the concentration of metal ion, the use of an inert solvent for the nitro compound (so that solid substrates could be used) and temperature, were considered.

The cathode cylinder must be stable both during electrolysis and on open-circuit and it was thought that a metal with an oxide coating would aid release of the intermediate metal powder. Even so, it was considered initially that several materials would be suitable; aluminium, steel and graphite were selected for trials. Some problems were, however, encountered; for example, an aluminium electrode was found to react chemically with Sn^{2+} ($2\ Al+3\ Sn^{2+}\rightarrow 2\ Al^{3+}+3\ Sn$) in an $SnCl_2$/HCl solution while adhesion of zinc to graphite was good. Hence, in general, a graphite cathode was used for reaction with Sn^{2+} and aluminium for reactions with Zn^{2+}.

Electrolyses were carried out at 0.5, 1.0, 2.0 and 5.0 A cm^{-2} using nitrobenzene as the substrate and the 0.4 M $SnCl_2$+1.0 M HCl solution; in all experiments, the yield of aniline was above 95 %. In most electrolyses 0.5 A cm^{-2} was used for experimental convenience. All electrolyses used a rotating cathode to promote the release of metal powder from the cathode surface into the catholyte and to produce a dispersion of nitrobenzene in the aqueous elec-

trolyte. Most experiments used a rotating cylinder cathode. Although the rate of rotation of the cylinder did not change the current efficiency for metal deposition, it did affect the coupled chemical reaction. In experiments using 0.4 M $SnCl_2$+1 M HCl as the electrolyte, the yield of aniline from nitrobenzene was 17, 76 and 98 % when the rotation rates were 100, 500 and 1000 r.p.m.; the rest of the charge was accounted for by unreacted metal in the electrolyte at the end of the reaction. At the present time, it is not clear whether this effect is due to metal particle size or the state of dispersion of the emulsion.

In general, it was considered that the concentration of metal ion in solution should be as low as possible, both for economic reasons and because it simplifies the extraction of product when this requires neutralisation of the acidic medium. It is clear from the experiments in Table 1 that 0.4 M metal ion is sufficient to give an almost quantitative yield of metal powder. Also the yield of aniline from nitrobenzene was good and hence it can be concluded that the 4 M $ZnCl_2$ used in an earlier report [7] is quite unnecessary. In the next section, the reduction of a range of nitrobenzenes is reported and some of these were solids and needed to be introduced into the cell as solutions. To test the effect of an inert organic solvent, electrolyses were carried out where the organic phase was various mixtures of nitrobenzene and toluene, as the inert solvent, see Table 2. These experiments show that dissolution of the nitro compound in an inert solvent is possible but the concentration of the nitro com-

Table 2. Effect of composition of the organic phase on the efficiency of nitrobenzene reduction. Electrolysis used 40 cm^3 of 0.4 M $SnCl_2$+1 M HCl and 10 cm^3 of organic phase, 0.5 A cm^{-2}, a carbon cathode and room temperature. Electrolysis terminated after charge for formation of 1 g Sn.

Organic phase $C_6H_5CH_3/cm^3$ + $C_6H_5NO_2/cm^3$		Current efficiency $C_6H_5NO_2 \rightarrow C_6H_5NH_2$/%
0	10	96
2.5	7.5	96
5.0	5.0	87
7.5	2.5	68

Table 3. Preparative electrolyses of nitro compounds with Zn and Sn as intermediates. Electrolyses at 0.5 A cm^{-2} to charge for 1 g of metal. Total catholyte volume approximately 50 cm^3.

Nitro compound	Conditions	Isolated yields of corresponding amine/% Current	Organic [c]
(a) 0.4 M $SnCl_2$+1 M HCl			
$C_6H_5NO_2$	liquid (6.6 g)	96	89
2-F−$C_6H_4NO_2$	liquid (6.6 g)	96	89
2-Br−$C_6H_4NO_2$	6 g in 10 cm^3 C_6H_6	Ratio $C_6H_5NH_2$: 2-Br−$C_6H_4NH_2$ 21:79 [b]	
2-Cl−$C_6H_4NO_2$	8 g in 30 cm^3 $CHCl_3$ 70 °C	96	–
2-CH_3O−$C_6H_4NO_2$	liquid (5 g)	99	82
4-HO−$C_6H_4NO_2$	6 g in 10 cm^3 $C_2H_5OC_2H_5$	89	80
4-CN−$C_6H_4NO_2$	7 g in 20 cm^3 C_6H_6	30	80
3-CN−$C_6H_4NO_2$	7 g in 30 cm^3 C_6H_6, 70 °C	75	88
2,5-Cl_2−$C_6H_3NO_2$	9 g in 20 cm^3 C_6H_6 70 °C	31	90
$(CH_3)_3CNO_2$	5 g in 20 cm^3 C_6H_6	48	90
$C_{10}H_7NO_2$	6 g in 20 cm^3 C_6H_6	91	79
(b) 0.4 M $ZnCl_2$+0.1 M HCl [a]			
$C_6H_5NO_2$	liquid (6 g)	93	89
2-F−$C_6H_4NO_2$	liquid (6.6 g)	92	90
2-Br−$C_6H_4NO_2$	6 g in 10 cm^3 C_6H_6	Ratio $C_6H_5NH_2$: 2-Br−$C_6H_4NH_2$ 8:92 [b]	

[a] HCl added in aliquots during electrolysis to maintain constant concentration. [b] GLC yield only. [c] Based on nitrobenzene not recovered.

pound should be high. In later experiments, benzene, diethyl ether and chloroform were used interchangably with toluene as the inert solvent.

The cell was fitted with a steam coil for carrying out electrolyses above room temperature. Experiments were carried out at higher temperature only when reaction did not take place at 20 °C, and in this study, this was only the case for some chlorinated derivatives (see later). The use of high temperature did not, however, present any special problems.

The reduction of substituted nitrobenzenes. Table 3 reports the isolated yields of amines from the reduction with tin of eleven nitro compounds and with zinc of three nitro compounds. It can be seen that it is possible to define conditions where the organic yields for all reactions are very good. In the cases where the current efficiency is poor, this is likely to be due to poor kinetics of the metal–nitrobenzene reaction. Certainly the current efficiency for the two chlorinated derivatives improved on increasing the temperature of electrolysis. Also with the cyanocompounds there were solubility limitations which restricted the maximum concentration of the nitro compounds in the organic phase (see Table 2); there was no reduction of the cyano group. With 2-bromonitrobenzene, and in contrast to the 2-chloro and 2-fluorocompounds, there was some cleavage of the carbon–bromine bond but this side reaction was less important using zinc than where the intermediate was tin.

The procedure reported here would seem to be a useful laboratory method for the conversion of nitro compounds to amines and it also has considerable promise as an industrial process. It gives good yields and works well for a range of structures. Moreover, from the industrial viewpoint, it has the advantages that it is successful at very high current density, in principle leads to no heavy metal effluent and that the cell could be used for a variety of reactions.

Mechanism. It has been suggested [2] that the mechanism for metal/acid reduction of nitro compounds parallels strongly that for the cathodic reaction, *i.e.* the reduction occurs in 2e steps with phenylhydroxylamine as a well-defined intermediate. In these experiments, there was, however, no evidence of intermediate reduction products even though the electrolyses were carried out with a substantial excess of nitro compounds in the catholyte. This would suggest that the initial reduction of the nitro compound is the rate determining step. Moreover, metal powder was commonly present in small amounts at the end of the electrolysis and this would suggest that the reaction is not very fast.

The basic nature of the mechanism in the three phase (water, organic phase and metal powder) system is not clear. It is even uncertain as to whether the reaction occurs at the metal/organic interface, at a three phase interface or by dissolution of the nitro compound in the aqueous solution. Some competitive experiments were carried out; equimolar amounts of two nitro compounds were electrolysed simultaneously. The results are reported in Table 4 but are both surprising and inconclusive. For example, the *o*-fluoro and *o*-methoxy compounds seem to reduce at an identical rate to the parent compound while *o*-chloronitrobenzene is reduced much more slowly than nitrobenzene.

Table 4. 6 g of nitrobenzene and the equivalent weight of substituted compound were dissolved in an organic solvent and the emulsion of the organic phase with 30–40 cm^3 of 0.4 M $SnCl_2$+1 M HCl was electrolysed at 0.5 A cm^{-2}. Charge for 1 g Sn was passed. Room temperature.

Other nitro compound	Solvent	Current yields of amines/%	
		Aniline	Other amine
2-F–$C_6H_4NO_2$	C_6H_6 (10 cm^3)	44 [a]	48 [a]
2-Cl–$C_6H_4NO_2$	C_6H_6 (20 cm^3)	90 [a]	6 [a]
2-CH_3O–$C_6H_4NO_2$	C_6H_6 (10 cm^3)	45 [b]	45 [b]
4-HO–$C_6H_4NO_2$	$C_2H_5OC_2H_5$ (10 cm^3)	47 [b]	46 [b]
4-CN–$C_6H_4NO_2$	C_6H_6 (20 cm^3)	60 [b]	30 [b]
$C_{10}H_7NO_2$	C_6H_6 (20 cm^3)	52 [b]	41 [b]

[a] Isolated yield. [b] GLC yield.

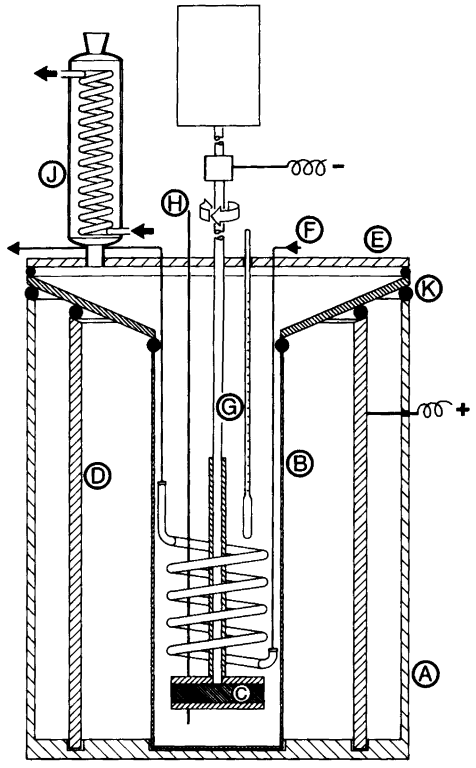

Fig. 1. Design of cell. A, polypropylene cell body; B, porous pot; C, rotating cylinder cathode; D, carbon tube anode; E, cell top; F, steam coil; G, thermometer; H, nitrogen inlet; J, condenser; K, O-rings.

EXPERIMENTAL

The electrolyses were carried out in the cell shown in Fig. 1. The cell body (A) was made of polypropylene and the catholyte compartment is the inside of a porous pot (B), type celloton V10 supplied by Doulton Industrial Products Ltd. and with dimensions 4.5 cm internal diameter and height 15 cm. Moreover the cell was sealed with 0-rings so that the anolyte and catholyte compartments were completely separated. The metals were deposited onto a rotating cylinder of carbon or aluminium (C) whose upper and low surfaces were masked with PTFE. The anode (D) was a carbon tube, internal diameter 8 cm, with holes drilled at the top to allow release of the gas formed at the anode. The cell top (E) was fitted with a steam coil (F), thermometer (G), nitrogen inlet (H) and condenser (J). The catholyte was an emulsion of the aqueous electrolyte and the organic phase stirred by the rotating cathode, while the anolyte was the same aqueous electrolyte. The constant current was supplied by a Sorensen Power Supply, type SRC 60–35 and charge was measured with a laboratory-built integrator.

Electrodeposition of metals. Iron, copper and zinc metals were deposited from their respective salt solutions on an aluminium cathode while tin was deposited on a carbon cathode. Charge was passed to deposit 1 g of the metal, which was washed thoroughly with water and dried in an oven before being weighed.

Reduction of nitro compounds. Appropriate amounts of nitro compounds either dissolved in a minimum amount of solvent or as neat liquids were mixed with aqueous electrolyte in the porous pot. Before electrodeposition of metals, the catholyte was saturated with nitrogen. After the charge had been passed, the stirring was continued for a further 20 mins. The remaining metal in the porous pot, if any, was washed and isolated before any neutral organic products were extracted with ether (30 $cm^3 \times 3$) from the aqueous electrolyte. The aqueous phase was then neutralised with K_2CO_3 and saturated with sodium chloride before further extraction with ether (20 $cm^3 \times 3$). The solution was dried over magnesium sulfate before evaporation of ether. The products were purified by passing them through a short column of silica gel using an appropriate solvent. The purity and identity (by comparison of retention times with authentic samples or by GLC-MS spectroscopy) of the products were checked by GLC and quantitative analyses were by weight following evaporation of the solvent.

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Electrophilic Amidoalkylation of C–H Acidic Compounds with Cyclic *N*-Formylimmonium Precursors

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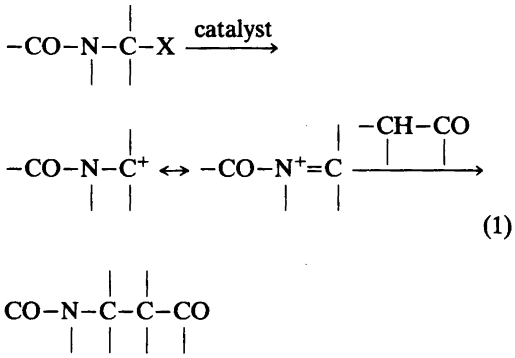
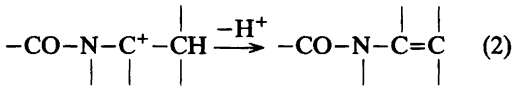
C–H acidic compounds, such as esters of malonic acid, substituted malonic acids, 2-oxocyclopentanecarboxylic acid and acetoacetic acid and in some cases monocarbonyl compounds, *e.g.* acetone and cyclopentanone, react with cyclic *N*-formyl-*N*-α-methoxyamines, easily prepared by the anodic methoxylation of cyclic *N*-formylamines, to form amidoalkylated products in fair to good yields. These compounds are convertible to a large variety of *N*-α-substituted heterocycles by well-established methods. As examples, the hydrolysis and decarboxylation of malonic and acetoacetic ester derivatives and the reaction between amidoalkylated malonic esters and urea to form barbituric acids are described.

The amidoalkylation of C–H acidic compounds is well documented.[1] The reaction has been carried out with various α-substituted amide derivatives, some of which are exemplified in eqn. (1). In the majority of cases the α-substituted amides have been acyclic, mainly because of the lack of expedient methods for the preparation of suitable α-substituted nitrogen heterocycles of the required type. By the introduction of the convenient, high-yield anodic α-methoxylation of cyclic amides a general method for the preparation of cyclic amidoalkylating agents was available. These reactions can be run on a large laboratory scale, the preparation of 10–20 mol samples being a matter of days.[2–5]





We have previously demonstrated the use of the α-methoxylated nitrogen heterocycles *1*–*6* as amidoalkylating agents toward aromatic compounds and dimethyl malonate.[6–8] The reaction proceeds *via* an α-nitrogen stabilized carbocation (eqn. (1)) which can also lose a proton to give an enamide (eqn. (2)). This frequently observed side-reaction does, however, not interfere in synthesis, since enamides usually are at least as reactive as amidoalkylating agents as the corresponding methoxy compounds. The enamides, which often can be used with some advantage over the α-methoxy derivatives, are easily prepared by elimination of methanol from α-methoxyamides.[9] An alternative route, isomerization of β,γ-unsaturated amides by

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Table 1. Yields and conditions for reaction between α-methoxylated amides or enamides and malonic esters. Reactions were run at room temperature, unless otherwise stated.

α-Methoxylated amide or enamide [a]	R in $RCH(COOR')_2$ [b]	Ratio substrate/ ester/$AlCl_3$	Reaction period/h	Yield/ % [c]
1	H	1.0/1.0/1.4	20	70 [d]
2	H	1.0/1.0/1.4	20	73 [d]
3	H	1.0/1.0/1.4	20	73 [d]
5	H	1.0/1.1/1.4	19	48
6	H	1.0/1.1/1.4	2	62
1	CH_3	1.0/1.0/1.0	48	68
2 (E)	CH_3	1.0/2.0/1.4 [e]	120	38
3	CH_3	1.0/1.0/1.0	48	88
6	CH_3	1.0/1.0/1.0	17	38
1	C_4H_9	1.0/1.5/1.0 [e]	24	55
2	C_4H_9	1.0/1.0/1.4		0 [f]
1	C_6H_5	1.5/1.0/1.5 [e]	78+144 [g]	51
3	C_6H_5	2.0/1.0/2.0 [e]	216	24
1	$PhCH_2$	2.0/1.0/1.4 [e]	192	60
2 or 2(E)	$PhCH_2$			0 [f]

[a] An (E) after the compound No. indicates that the corresponding enamide was used. [b] R'=Et in all cases, except for R=H or $PhCH_2$. [c] Isolated yield of purified product. [d] See Ref. 7. [e] Successive addition of reagents in order to shift the equilibrium to the right; ratios refer to total amounts of reagents (see text and Experimental). [f] Product was detectable but the yield was too low for isolation to be meaningful. [g] The second period was run at reflux temperature in dichloromethane.

ruthenium or rhodium catalysts,[10] is far more laborious and applicable on a small scale only.

This paper is a full report on the amidoalkylation of C–H acidic compounds (malonates, acylacetic esters, monocarbonyl compounds) by *1–6*. A few conventional further conversions of the products thus prepared are exemplified. While this work was in progress, similar methods were described by Schmalzl [11] and Shono *et al.*[12] The latter group employed as the key step the anodic α-methoxylation of carbamates, the products of which can be used for amidoalkylation in much the same way as *N*-formyl compounds.

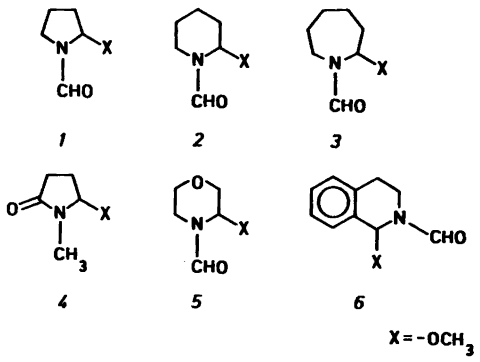
RESULTS

All amidoalkylations described here were carried out with anhydrous $AlCl_3$ as catalyst and dichloromethane as solvent. Other previously used catalysts, such as methanesulfonic acid or BF_3,[6,8] failed, mainly due to competition from the dimerization and polymerization of the enamide formed *via* eqn. (2).[8,11] $TiCl_4$ [12] was found to be a good alternative to $AlCl_3$ whereas $ZnCl_2$ gave inferior yields.

As in Friedel-Crafts acylation, stoicheiometric amounts or an excess of the catalyst must be employed due to the strong tendency of $AlCl_3$ to form complexes with Lewis donors, *i.e.*, with both starting materials and products of the amidoalkylation reaction, thus reducing its catalytic activity.[13,14] This effect was compensated for by keeping the catalyst/substrate ratio ≥1.4 in the appropriate cases. The C–H acidic reaction component was normally used in 10–100 % excess.

Amidoalkylation of malonic esters. Table 1 gives yields and reaction conditions for the amidoalkylation of malonic esters by *1–6*. In general, fair to good yields were obtained with unsubstituted or moderately hindered alkylmalonic esters, whereas more sterically hindered ones, such as diethyl cyclohexyl- or neopentylmalonate, failed to react.

The least reactive compound among *1–6* was *2*, presumably due to the known tendency of sp^2-hybridized atoms being more energetically favourable in a six-membered ring. This effect stabilizes the carbocation and/or the enamide, and thus the reactivity of the cation is low and/or the equilibrium becomes unfavourable for product formation. This situation could be slightly



improved by adding an excess of catalyst and malonic ester. By equilibrating pure diethyl (1-formyl-2-piperidyl)-methylpropanedioate with an equimolar amount of $AlCl_3$ in dichloromethane for 120 h, a value of the equilibrium constant $=1.5\ M^{-1}$ could be estimated. Reversibility has also been observed in the amidoalkylation of aromatics.[6]

In principle, *5* might eliminate methanol during the reaction to give an enamide with the possibility of reprotonation or coordination with the catalyst at two sites, *α* to nitrogen or oxygen. The product isolated was however only the 3-isomer, *i.e.* attack *α* to nitrogen was preferred. No reaction was observed when 5 was replaced by its enamide. The phenyl- and benzylmalonic esters might in principle be attacked at ring positions as well, but no such products were detected. The low yield in some of the reactions in Table 1 are due to competing polymerization reactions, especially with *1*, *3* and *6*. In contrast, the enamide from *2* turned out to be stable for several weeks in the presence of $AlCl_3$.

Amidoalkylation of acylacetic esters. Table 2 lists reaction conditions and yields for the amidoalkylation of a few acylacetic esters, including a cyclic one, ethyl 2-oxocyclopentanecarboxylate, by *1*, *3* and *6*. Good yields were obtained in most cases. Somewhat surprisingly, acetylacetone did not give any amidoalkylation product, neither by using $AlCl_3$ nor $TiCl_4$ or BF_3 as a catalyst.

Amidoalkylation of monocarbonyl compounds. Table 3 lists reaction conditions and yields for the amidoalkylation of monocarbonyl compounds by *6* and, in one case, *1* (*2* and *3* were also found to react with cyclopentanone but the products were not isolated due to poor yields). Compound *6* reacted with a number of monocarbonyl compounds, albeit in low to moderate yields. Other C–H acidic compounds (acetonitrile, nitromethane, ethyl phenylacetate, ethyl acetate and malononitrile) did not react with *6* under the conditions employed, 3,4-dihydroisoquinoline being the only detectable product.

Further conversions of amidoalkylated products. The amidoalkylated products obtained *via* the reactions of Tables 1–3 can be converted to a large number of other derivatives by conventional synthesis methodology. To exemplify, alkaline hydrolysis and subsequent decarboxylation of a few malonates (see Experimental) afforded the corresponding amino acids,[15] whereas reaction with urea in the classical manner [16] gave the corresponding barbituric acids (see Experimental).

Table 2. Yields and conditions for reaction between *α*-methoxylated amides and acylacetic esters. All reactions were run at room temperature.

α-Methoxylated amide	R and R′ in RCOCH(R′)COOMe	Ratio substrate–methylene compound–$AlCl_3$	Reaction period/h	Yield/ % [a]
1	CH_3,H	1.0:1.1:1.4	1	66
2	CH_3,H	1.0:1.1:1.4	2.5	71 [b]
3	CH_3,H	1.0:1.1:1.4	1.5	57
6	CH_3,H	1.0:1.1:1.4	1	58
2	Ph,H	1.0:1.0:1.0	2	58 [b]
1	$-(CH_2)_3-$ [c]	1.0:1.1:1.4	72	55
3	$-(CH_2)_3-$ [c]	1.0:1.1:1.4	48	45

[a] Isolated yield of purified product. [b] Mixture of two diastereomers. [c] A mixture of the methyl and ethyl ester was used.

Table 3 . Yields and conditions for reaction between α-methoxylated amides and monocarbonyl compounds. All reactions were run at room temperature.

α-Methoxylated amide	Carbonyl compound	Ratio substrate–carbonyl compound–$AlCl_3$	Reaction period/h	Yield/ % [a]
1	Cyclopentanone	1.0:2.0:1.0 [b]	168	37
6	Cyclopentanone	1.0:1.4:1.4	2	17
6	Cyclohexanone	1.0:1.4:1.4	17	33
6	Cycloheptanone	1.0:1.1:1.4	2	31
6	Acetone	1.0:1.0:1.4	2	70
6	3-Pentanone	1.0:2.0:1.4	2	42
6	Acetophenone	1.0:1.1:1.4	24	43
6	Phenylacetone	1.0:1.0:1.4	20	8 [c]
6	Acetaldehyde	1.0:1.4:1.4	1	11

[a] Isolated yield of purified product. [b] Successive addition of reagents in order to shift the equilibrium to the right; ratios refer to total amounts of reagents (see text and Experimental). [c] GLC analysis of the crude product mixture indicated a 70 % yield of four products (3:5:1.5:1); MS analysis showed that the major point of attack was the benzylic carbon.

The *N*-formyl compounds obtained from the amidoalkylation reactions reported here have not been described earlier. Among the malonic ester derivatives a few compounds with similar structure have been reported. Noteworthy are the preparations of 2-pyrrolidinylidene-, 2-piperidinylidene- and 2-azepinylidenemalonic esters. These compounds have been prepared by condensation of cyclic imino ethers or cyclic thioimino ethers with malonic ester,[17,18] condensation of thiolactams with bromomalonic esters,[19,20] and condensation of cyclic 2,2-diethoxyamines with malonic ester.[21–24] These and a few other methods have also been useful in the preparation of analogous derivatives from other carbonyl compounds, such as acetoacetic ester,[17,18,20,25–27] acetylacetone [17,25,27–29] and acetophenone.[25]

To our knowledge none of the heterocyclic systems *1–6*, substituted in the α-position with a barbituric acid moiety, has been reported. Among the *N*-formyl analogues of 2-acetonyl substituted compounds only 2-(2-oxopropyl)-1-piperidinecarboxaldehyde has been previously prepared for subsequent use in the preparation of myrtine.[30] However, some of the nonformylated analogues together with *N*-acetyl and *N*-methyl derivatives are well known alkaloids and have been thoroughly studied. This is also the case for the acetic acid derivatives described here.

Related work involving anodic acetoxylation and/or methoxylation of acylaminomalonic acid monoesters, *N*-acylprolines and *N*-acylpipecolic acids and their subsequent use as amidoalkylating agents has been carried out by Iwasaki *et al.*[31–33] Ben Ishai *et al.* have reported the amidoalkylation of β-dicarbonyl compounds with glyoxylic acid derivatives.[34] Amidoalkylation of malonic acid ester and acetoacetic acid ester with ω-alkoxylactams was the key step in the approach to pyrrolizidine alkaloids reported by Kraus and Neuenschwander.[35] A different approach to the subject of this paper has recently been made by Gugelchuk *et al.* in their preparation of vinylogous amides from *N*-alkyllactams.[36]

Concluding, the combination of fast and efficient anodic *N*-α methoxylation of cyclic amides and the subsequent use of the α-methoxyamides (or the corresponding enamides) as amidoalkylating agents toward enolizable carbonyl compounds offers a convenient approach to a large number of functionalized nitrogen heterocycles.

EXPERIMENTAL

In most cases reaction conditions and work-up methods were almost identical. In the case of significant deviations from the general procedure described below, these are detailed in connection with the actual reaction.

General procedure for amidoalkylation. The methoxylated amide or the corresponding ena-

mide, together with the carbonyl compound, usually in a slight excess, was dissolved in dichloromethane and added slowly to a stirred mixture of $AlCl_3$ in dichloromethane. As a general example 0.1 mol of the amide together with the carbonyl compound was dissolved in 20 ml of dichloromethane and added to 0.14 mol of $AlCl_3$ in 80 ml of dichloromethane. The course of reaction was checked by GLC analysis and, when necessary, additional catalyst and/or substrate were added.

After the appropriate time of reaction, water (same amount as the total volume of dichloromethane used as solvent) was added or the reaction mixture was poured into water. The organic layer was separated and the aqueous phase extracted with two more portions of dichloromethane. The combined dichloromethane solutions were washed with water, sodium hydrogen carbonate solution and finally with water. After drying over magnesium sulfate the solvent was removed by evaporation *in vacuo* and the product isolated by recrystallization or distillation at reduced pressure using a Claisen apparatus equipped with a Vigreux column.

The course of the reactions and the purity of the products were checked using a Hewlett-Packard HP-5830 gas chromatograph fitted with a 5 % OV 17 column (3 m×3 mm) or a Dexsil 300 column (0.5 m×3 mm). ^1H NMR spectra were recorded on a Jeol 100 MHz instrument with $CDCl_3$ as solvent and MS analyses were carried out on a Finnigan 4021 spectrometer at 70 eV using GLC inlet unless otherwise indicated.

In some cases unreacted starting material was removed by distillation using a "Kugelrohr" apparatus (Aldrich Co.).

The methoxylated amides [2,3] and the enamides [9] were prepared according to previously published methods whereas other chemicals where of commercial quality.

Dimethyl (4-formyl-3-morpholinyl)propanedioate. Compound *5* (0.1 mol) and dimethyl malonate in CH_2Cl_2 (20 ml) were added to $AlCl_3$ in CH_2Cl_2 (80 ml). The product was isolated by distillation at reduced pressure and later solidified. Yield 11.7 g (48 %), b.p. 159–165 °C/1.0–1.3 mmHg, m.p. 54–58 °C.

MS *m/e* (% rel. int.): 245 (2, M), 217 (3), 186 (29, M–$COOCH_3$), 174 (5), 156 (6), 132 (8), 114 (100, M–$CH(COOCH_3)_2$), 100 (18), 86 (35, C_4H_8NO), 56 (37).

^1H NMR: δ 2.80–3.16 (1 H, t with further splitting, J 12.8 and 3.8 Hz), 3.24–4.42 (7 H, several m), 3.72 and 3.77 (6 H, 2 s). 8.01 and 8.07 (1 H, 2 s); the close shifts made a more accurate evaluation uncertain.

Dimethyl (2-formyl-1,2,3,4-tetrahydro-1-isoquinolinyl)propanedioate. Compound *6* (0.025 mol) and dimethyl malonate dissolved in CH_2Cl_2 (5 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (20 ml). After the usual work-up procedure the crude product was recrystallized from diethyl ether (5 ml). Yield 4.5 g (62 %), m.p. 76–79 °C.

MS *m/e* (% rel. int., direct inlet): 291 (3, M), 262 (10, M–CHO), 172 (5), 160 (100, M–$CH(COOCH_3)_2$), 132 (59, $C_9H_{10}N$), 130 (20), 117 (20), 115 (21), 105 (15), 77 (14).

^1H NMR: δ 2.95 (2 H, t, J 6.8 Hz), 3.22–3.52 (1 H, 2 t, J 6.8 Hz), 3.62, 3.64, 3.71 and 3.76 (6 H, 4 s), 3.92 and 3.94 (1 H, 2 d, J 10.2 and 7.4 Hz), 4.01–4.49 (1 H, 2 t, J 6.8 Hz), 5.39 and 6.14 (1 H, 2 d, J 10.2 and 7.4 Hz), 7.10–7.38 (4 H, m), 8.18 and 8.35 (1 H, 2 s).

Diethyl (1-formyl-2-pyrrolidinyl)methylpropanedioate. Compound *1* (0.2 mol) and diethyl methylmalonate were dissolved in CH_2Cl_2 (40 ml) and added to $AlCl_3$ in CH_2Cl_2 (160 ml). Yield 37.0 g (68 %), b.p. 165–167 °C/1.6 mmHg.

MS *m/e* (% rel. int.): 271 (2, M), 242 (3, M–CHO or M–CH_2CH_3), 226 (3, M–OCH_2CH_3), 152 (5), 98 (100, C_5H_8NO), 70 (58, C_4H_8N).

^1H NMR: δ 1.27 (6 H, t, J 7 Hz), 1,41 and 1.43 (3 H, 2 s), 1.70–2.47 (4 H, m), 2.92–4.04 (2 H, m), 4.18 and 4.19 (4 H, 2 q, J 7 Hz), 4.55–4.80 (1 H, m), 8.21 and 8.33 (1 H, 2 s).

Diethyl (1-formyl-2-piperidyl)methylpropanedioate. 3,4-Dihydro-1(2*H*)-pyridinecarboxaldehyde (0.2 mol) and diethyl methylmalonate were dissolved in CH_2Cl_2 (40 ml) and added to $AlCl_3$ in CH_2Cl_2 (160 ml). After 72 h GLC analysis showed the reaction to be incomplete and 0.08 mol of $AlCl_3$ was added. In order to improve the yield of product an additional 0.2 mol of the malonic ester was introduced and in 48 h most of the enamide was consumed. Work-up was performed as usual. Yield 21.8 g (38 %), b.p. 163–165 °C/0.9 mmHg.

MS *m/e* (% rel. int.): 285 (1, M), 256 (2, M–CHO or M–CH_2CH_3), 240 (2, M–OCH_2CH_3), 174 (3), 166 (3), 147 (4), 129 (17), 112 (100, $C_6H_{10}NO$), 102 (10), 84 (35, $C_5H_{10}N$), 80 (27) 74 (33).

^1H NMR: δ 1.16–1.38 (6 H, 4 t, J 7 Hz), 1.46 and 1.54 (3 H, 2 s), 1.30–2.30 approx. (6 H, m), 2.58–2.91 and 3.25–3.47 (2 H, m), 4.01–4.34 (4 H, 4 q, J 7 Hz), 4.25–4.50 and 5.05–5.20 (1 H, m), 8.10 and 8.13 (1 H, 2 s).

Diethyl (1-formyl-hexahydro-1H-azepin-2-yl)-methylpropanedioate. Compound *3* (0.1 mol) and diethyl methylmalonate in CH_2Cl_2 (20 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (80 ml). Yield 20.2 g (88 %), b.p. 163–170 °C/0.7–0.9 mmHg.

MS *m/e* (% rel. int.): 300 (1, M+1), 299 (1, M) 270 (2, M−CHO or M−CH_2CH_3), 254 (2, M−OCH_2CH_3), 180 (3) 152 (3), 126 (100, $C_7H_{12}NO$), 98 (25, $C_6H_{12}N$), 55 (28).

^{1}H NMR: δ 1.16−1.39 (6 H, 4 t, J 7.5 Hz), 1.44 and 1.46 (3 H, 2 s), 1.10−2.35 approx. (8 H, m), 2.55−2.87 and 3.09−4.4 and 4.75−5.01 (3 H, several coincident m), 4.04−4.33, (4 H, 4 q, J 7.5 Hz), 8.21 and 8.24 (1 H, 2 s).

Diethyl (2-formyl-1,2,3,4-tetrahydro-1-isoquinolinyl)methylpropanedioate. Compound *6* (0.025 mol) and diethyl methylmalonate in CH_2Cl_2 (10 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (20 ml). The crude product was recrystallized from ether (6 ml). Yield 3.16 g (38 %), m.p. 85−88 °C.

MS *m/e* (% rel. int.): 333 (very small, M), 304 (2, M−CH_2CH_3 or M−CHO), 214 (3), 197 (3), 186 (3), 174 (4), 160 (100, M−CCH_3 $(COOCH_2CH_3)_2$), 132 (30, $C_9H_{10}N$), 130 (30), 117 (10), 103 (15).

^{1}H NMR: δ 0.92, 1.18 and 1.26 (6 H, 3 t, J 6.9 Hz), 1.30 and 1.40 (3 H, 2 s, 2.76−2.99, 3.09−3.40 and 3.64−4.53 (4 H, m), 4.14 and 4.18 (4 H, 2 q, J 6.9 Hz), 5.77 and 6.33 (1 H, 2 s), 7.03−7.48 (4 H, m), 8.16 and 8.28 (1 H, 2 s).

Diethyl butyl(1-formyl-2-pyrrolidinyl)propanedioate. Compound *1* (0.1 mol) and diethyl butylmalonate (0.1 mol) in CH_2Cl_2 (20 ml) were added to $AlCl_3$ (0.1 mol) in CH_2Cl_2 (80 ml). After 24 h *1* was completely consumed while still large amounts of the malonic ester remained unreacted. Another portion of *1* (0.05 mol) was added and the reaction mixture was worked up as general after 72 h. Yield 17.3 g (55 %), b.p. 166−171 °C/0.8 mmHg.

MS *m/e* (% rel. int.): 313 (very small, M), 284 (2, M−CHO or M−CH_2CH_3), 268 (1, M−OCH_2CH_3), 194 (3), 98 (100, C_5H_8NO), 70 (38, C_4H_8N).

^{1}H NMR: δ 0.77−1.00 (3 H, t, J 7.5 Hz), 1.10−1.37 (6 H, 2 t, J 7.5 Hz), 1.0−1.4 approx. (4 H, m), 1.51−2.39 (6 H, m and multiple t, J 7.5 Hz), 2.82−4.05 (2 H, m), 4.10−4.35 (4 H, 3 t, J 7.5 Hz), 4.54 and 4.77 (1 H, 2 t, J 6.5 Hz), 8.22 and 8.44 (1 H, 2 s).

Diethyl (1-formyl-2-pyrrolidinyl)phenylpropanedioate. Compound *1* (0.1 mol) and diethyl phenylmalonate (0.1 mol) in CH_2Cl_2 (20 ml) were added to a mixture of $AlCl_3$ (0.1 mol). After 78 h at room temperature most of *1* was consumed while substantial amounts of diethyl malonate still remained. Compound *1* (0.05 mol) was added and the mixture was refluxed for 72 h. GLC analyses showed a slight increase in the ratio of product/unreacted malonic ester and an additional 0.05 mol of $AlCl_3$ was introduced. The reaction mixture equilibrated within 72 h at reflux temperature and was then worked up as general. The crude product was dissolved in ether (20 ml) and chilled. The solid product was filtered off and washed with ether (yield 39 %) and the remaining ether solutions were concentrated. The resulting oil was heated at reduced pressure (160 °C/1 mmHg), in order to remove most of the remaining starting materials, and the residue was recrystallized from ethanol. Yield 17.2 g (51 %), m.p. 77−80 °C.

MS *m/e* (% rel. int.): 333 (very small, M), 236 (3, M−C_5H_7NO), 190 (4), 164 (4), 163 (4), 136 (4), 135 (4), 118 (7), 98 (100, C_5H_8NO), 91 (8), 77 (7), 70 (43, C_4H_8N), 68 (18).

^{1}H NMR: δ 1.23 and 1.26 (6 H, 2 t, J 7 Hz), 1.95−2.89 (4 H, m), 3.41−3.47 and 4.09−4.43 (2 H, m), 4.23, 4.26 and 4.28 (4 H, 3 q, J 7 Hz), 4.90−5.05 (1 H, dd, J 4.5 and 7.2 Hz), 7.28−7.38 (5 H, m), 8.22 and 8.36 (1 H, 2 s).

Diethyl (1-formyl-hexahydro-1H-azepin-2-yl)-phenylpropanedioate. Compound *3* (0.1 mol) and diethyl phenylmalonate (0.1 mol) dissolved in CH_2Cl_2 (20 ml) were added to $AlCl_3$ (0.1 mol) in CH_2Cl_2 (80 ml). GLC analyses showed the equilibrium to be in favour of the starting materials and successive additions of *3* and catalyst were made with periodic checking of the composition of the reaction mixture. Totally 0.1 mol of *3* and $AlCl_3$ were added during 216 h before work-up. The residue after evaporation of the solvent was purified by removing most of the unreacted starting materials by distillation at reduced pressure (135 °C/0.8 mmHg). The crude product was dissolved in ether (25 ml) and temporarily chilled to −78 °C to promote crystallization. The crystals were filtered off and washed with ether and from the concentrated mother liquors additional product was retained by repeated crystallization from ether. Yield 8.7 g (24 %), m.p. 72−78 °C.

MS *m/e* (% rel. int., direct inlet): 361 (very small, M), 316 (1, M−OCH_2CH_3), 236 (2, M−$C_7H_{11}NO$), 190 (3), 127 (8), 126 (100, $C_7H_{12}NO$), 105 (4), 98 (14, $C_6H_{12}N$), 55 (13).

^{1}H NMR: δ 1.10−2.42 (8 H, m), 1.22 and 1.24 (6 H, 2 t, J 7 Hz), 3.34−3.83 and 4.09−4.36 (2 H, m), 4.23 and 4.24 (4 H, 2 q, J 7 Hz), 4.40−4.61 (1 H, dd, J 5.7 and 11.7 Hz), 7.28−7.56 (5 H, m), 8.33 (1 H, 2 s).

Diethyl (1-formyl-2-pyrrolidinyl)phenylmethylpropanedioate. Compound *1* (0.1 mol) and diethyl benzylmalonate (0.1 mol) in CH_2Cl_2 (20 ml) were added to a mixture of $AlCl_3$ (0.1 mol) in CH_2Cl_2 (80 ml). Substantial amounts of starting materials were still present after 48 h and another portion of *1* (0.05 mol) was introduced. A slightly increased yield was observed after another 48 h

and was further improved by subsequent additions of catalyst (0.04 mol) and *1* (0.05 mol). After a total reaction period of 192 h most of the starting materials were consumed and the mixture was worked up as general. The product solidified after removal of the starting materials on a Kugelrohr apparatus at reduced pressure and was recrystallized from ethyl acetate (15 ml). Yield 19.0 g (60 %), m.p. 101–103 °C. MS *m/e* (% rel. int., direct inlet): 319 (1, M), 290 (1, M–CHO), 221 (3, M–C_5H_8NO), 99 (79), 98 (100, C_5H_8NO), 91 (19), 77 (6), 70 (71, C_4H_8N).

^{1}H NMR: δ 1.66–1.94 (2 H, q, *J* 7 Hz), 2.02–2.35 (2 H, m), 2.84–3.2 and 3.7–4.02 approx. shifts due to complex signal pattern (2 H, several t, *J* 7 Hz), 3.24, (1 H, d, *J* 9 Hz), 3.47 (1 H, d, *J* 9 Hz), 3.57 (3 H, s), 3.68 (3 H, s), 4.51 and 4.85 (1 H, 2 t, *J* 6 and 7 Hz resp.), 7.00–7.32 (5 H, m), 8.31 and 8.71 (1 H, s).

Methyl 2-(1-formyl-2-pyrrolidinyl)-3-oxobutanoate. Compound *1* (0.2 mol) together with methyl acetoacetate in CH_2Cl_2 (40 ml) was added to $AlCl_3$ in CH_2Cl_2 (160 ml). Yield 28.3 g (66 %), b.p. 155–161 °C/1.1–1.5 mmHg.

MS *m/e* (% rel. int.): 213 (1, M), 184 (3, M–CHO), 170 (23, M–$COCH_3$), 138 (30), 110 (29), 98 (76, C_5H_8NO), 70 (100, C_4H_8N).

^{1}H NMR: δ 1.57–2.45 (4 H, m), 2.24 and 2.26 (3 H, 2 s), 3.09–3.95 and 4.27–4.76 (4 H, m and multiple d *J* 9 Hz), 3.72–3.76 (3 H, 4 s), 8.26 (1 H, 2 s).

Methyl 2-(1-formyl-2-piperidyl)-3-oxobutanoate. Compound *2* (0.2 mol) and methyl acetoacetate dissolved in CH_2Cl_2 (40 ml) were added to $AlCl_3$ in CH_2Cl_2 (160 ml). Yield 32.2 g (71 %), b.p. 154–161 °C/1.0–1.4 mmHg.

MS *m/e* (% rel. int.): 227 (2, M), 212 (2, M–CH_3), 198 (3, M–CHO), 184 (6, M–$COOH_3$), 152 (13), 124 (13), 112 (100, $C_6H_{10}NO$), 84 (62, $C_5H_{10}N$).

^{1}H NMR: δ 1.23–1.89 (6 H, m), 2.16, 2.20, 2.23 and 2.26 (3 H, 4 s), 3.06–4.55 and 5.13–5.43 (4 H, m), 3.65, 3.70, 3.72 and 3.76 (3 H, 4 s), 7.94, 7.96, 8.04 and 8.06 (1 H, 4 s).

Methyl 2-(1-formyl-hexahydro-1H-azepin-2-yl)-3-oxobutanate. Compound *3* (0.2 mol) and methyl acetoacetate in CH_2Cl_2 (40 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (160 ml). The crude oil was dissolved in ether (50 ml) and the solid product was filtered off and washed with ether. Additional product was recovered from the concentrated mother liquor by repeated treatment with ether. Yield 27.6 g (57 %), m.p. 87–91 °C.

MS *m/e* (% rel. int., direct inlet): 241 (3, M), 226 (1, M–CH_3), 212 (1, M–CHO), 198 (8, M–$COCH_3$), 166 (16), 138 (14), 126 (100, $C_7H_{12}NO$), 98 (34, $C_6H_{12}N$).

^{1}H NMR: δ 1.05–2.77 (9 H, several m), 2.19 and 2.22 (3 H, 2 s), 3.65–4.39 (3 H, m), 3.74 and 3.82 (3 H, 2 s), 8.07 and 8.11 (1 H, 2 s).

Methyl 2-(2-formyl-1,2,3,4-tetrahydro-1-isoquinolinyl)-3-oxobutanoate. Compound *6* (0.05 mol) and methyl acetoacetate in CH_2Cl_2 (10 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (40 ml). The pure product was obtained by recrystallization from ether (10 ml). Yield 8.0 g (58 %), m.p. 93–102 °C; the product decomposed at GLC analysis.

MS *m/e* (% rel. int., direct inlet). 275 (4, M), 246 (4, M–CHO), 243 (7), 232 (3, M–$COCH_3$), 204 (4), 200 (7), 172 (11), 160 (100, M–$CH_3COCHCOCH_3$), 132 (68, $C_9H_{10}N$), 117 (23), 115 (20), 105 (20), 77 (23).

^{1}H NMR: δ 2.22 and 2.30 (3 H, 2 s), 2.94 (2 H, t, *J* 6.5 Hz), 3.15–3.47 (1 H, multiplet, *J* 6.5 Hz), 3.59 and 3.61, (3 H, 2 s), 3.59–4.35 (2 H, m and 2 d corresponding to approx. 1 H and centered at 3.86 and 4.16, *J* 9 and 9.5 Hz), 5.47 and 6.22 (1 H, 2 d, *J* 9.5 and 9 Hz), 7.01–7.37 (4 H, m), 8.12 and 8.32 (1 H, 2 s).

2-(2-Oxocyclopentyl)-1-pyrrolidinecarboxaldehyde. Compound *1* (0.1 mol) together with cyclopentanone (0.1 mol) dissolved in CH_2Cl_2 (20 ml) was added to $AlCl_3$ (0.1 mol) in CH_2Cl_2 (80 ml). After 48 h GLC analysis showed substantial amounts of unreacted starting materials and 0.1 mol of cyclopentanone was added. The reaction mixture, still containing some unreacted amide, was worked up after totally 168 h. The diastereomers were detected at GLC analysis in a ratio of 1:6.5. Yield 6.8 g (37 %), b.p. 134–150 °C/0.6–1.0 mmHg.

MS *m/e* (% rel. int.): 181 (3, M); 151 (3), 136 (3), 125 (3), 110 (3), 98 (100, C_5H_8NO), 70 (97, C_4H_8N).

^{1}H NMR: δ 1.51–2.69 (10 H, approx., m), 2.79 (1 H approx., t, *J* 6.8 Hz), 3.05–3.83 (3 H approx., m), 4.07–4.49 (1 H approx., m), 8.11, 8.24 and 8.31 (1 H, 3 s).

3,4-Dihydro-1-(2-oxocyclopentyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.05 mol) and cyclopentanone in CH_2Cl_2 (10 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (40 ml). Yield 2.12 g (17 %), m.p. 81–83 °C.

MS *m/e* (% rel. int.): 243 (3, M), 212 (7), 160 (100, M–C_5H_7O), 132 (39, $C_9H_{10}N$), 117 (13), 105 (10), 77 (13).

^{1}H NMR: δ 1.19–3.30 and 4.28–4.55 (9 H, m), 2.91 and 3.67 (2 H, 2 t, *J* 6 Hz), 5.24 and 5.81 (1 H, 2 d, *J* 3.3 and 4.1 Hz), 6.84–7.37 (4 H, m), 8.21 and 8,42 (1 H, 2 s).

3,4-Dihydro-1-(2-oxocyclohexyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.025 mol) and cyclohexanone in CH_2Cl_2 (5 ml) were added to $AlCl_3$ in CH_2Cl_2 (20 ml). The crude

product was dissolved in ether and the slowly crystallizing product was filtered off and washed with ether. The mother liquors were concentrated and chromatographed on silica gel with CH_2Cl_2 as eluent in order to remove polymeric material. The eluate was concentrated and additional product was recovered by repeated treatment with ether. Yield 2.10 g (33 %), m.p. 102–104 °C.

MS *m/e* (% rel. int.): 257 (4, M), 228 (5, M–CHO), 185 (5), 160 (100, M–C_6H_9O), 132 (42, $C_9H_{10}N$), 117 (14), 105 (11), 77 (12).

^{1}H NMR: δ 1.32–2.20 approx. (6 H, m), 2.20–2.60 (2 H, m), 2.60–3.80 and 4.10–4.44 (7 H, m), 5.30 and 6.00 (1 H, 2 d, *J* 7 and 9 Hz), 7.00–7.37 (4 H, m), 8.17 and 8.30 (1 H, 2 s).

3,4-Dihydro-1-(2-oxocycloheptyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.05 mol) and cycloheptanone dissolved in CH_2Cl_2 (10 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (40 ml). The crude oil was dissolved in ether (10 ml) and the solid product was filtered off and washed with ether. Additional product was obtained by repeated crystallization of the concentrated mother liquors from ether. Yield 4.2 g (31 %), m.p. 108–111 °C.

MS *m/e* (% rel. int.): 271 (3, M), 242 (4, M–CHO), 161 (12), 160 (100, M–$C_7H_{11}O$), 132 (41, $C_9H_{10}N$), 117 (15), 105 (11), 77 (11), 55 (15).

^{1}H NMR: δ 1.05–2.01 (8 H, m), 2.32–2.56 (2 H, m), 2.80–3.30, 3.40–3.81 and 4.18–4.54 (5 H, m), 5.13 and 5.73 (1 H, 2 d, *J* 8.2 and 8 Hz), 6.95–7.22 (4 H, m), 8.23 (1 H, s).

Ethyl 1-(1-formyl-2-pyrrolidinyl)-2-oxocyclopentanecarboxylate. Compound *1* (0.1 mol) and ethyl 2-oxocyclopentanecarboxylate (the commercially purchased ethyl ester later turned out to contain substantial amounts of the methyl ester. This was also reflected in the composition of the isolated product) dissolved in CH_2Cl_2 (20 ml) was added to a mixture of $AlCl_3$ in CH_2Cl_2 (80 ml). Yield 13.9 g (55 %), b.p. 162–166 °C/0.6–0.8 mmHg.

MS *m/e* (% rel. int.), M=ethyl ester, M′=methyl ester: 253 (3, M), 239 (2, M′), 224 (3, M–CHO or M=CH_2CH_3 or M′–CH_3), 210 (3, M′–CHO), 180 (5, M and M′–COOR), 152 (9, M and M′–(COOR and CHO)), 98 (100, C_5H_8NO), 70 (79, C_4H_8N). MS of the two diastereomers were almost identical.

^{1}H NMR; as mentioned above the product was a mixture of methyl and ethyl ester in the ratio 1:1.4: δ 1.13–1.37 (2 H, approx., 2 t, *J* 7 Hz), 1.52–2.76 (10 H, m), 2.92–4.40 (2 H, m) 3.73 and 3.79 (1 H, approx., 2 s), 4.08–4.33 (1.2 H approx., 2 q, *J* 7 Hz), 4.43–4.88 (1 H, m), 8.11, 8.23, 8.28 and 8.32 (1 H, 4 s).

Ethyl 1(1-formyl-hexahydro-1H-azepin-2-yl)-2-oxocyclopentanecarboxylate. Compound *3* (0.1 mol) and ethyl 2-oxocyclopentanecarboxylate (a mixture of methyl and ethyl ester as in the preceding experiment) dissolved in CH_2Cl_2 (20 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 80 ml). Yield 12.6 g (45 %), b.p. 187–190 °C/1.3 mmHg; after distillation a minor portion of the product slowly crystallized, m.p. 119–122 °C.

MS *m/e* (% rel. int.), 281 (2, M, ethyl ester), 267 (1, M, methyl ester), 253 (1), 236 (1), 197 (4), 180 (3, M–(CHO+COOR)), 126 (45, $C_7H_{12}NO$), 125 (33), 110 (33), 98 (10, $C_6H_{12}N$), 96 (32), 82 (43), 68 (80), 55 (100).

^{1}H NMR; δ 0.97–2.97 (14 H, m), 1.11–1.37 (2 H approx., 2 t, *J* 7 Hz), 3.12–4.75 (3 H. m), 3.70 and 3.72 (1 H, 2 s), 4.07–4.37 (1.3 H approx., 2 q, *J* 7 Hz), 8.01, 8.07, 8.17 and 8.23 (1 H, 4 s).

Ethyl α-benzoyl-1-formyl-2-piperidineacetate. Compound *2* (0.1 mol) and ethyl benzoylacetate were dissolved in CH_2Cl_2 (20 ml) and added to a mixture of $AlCl_3$ in CH_2Cl_2 (80 ml). Since the crude oil, after evaporation of the solvent, resisted all attempts to achieve crystallization the residual starting materials were removed by distillation on a Kugelrohr apparatus at 0.1–0.2 mmHg. According to GLC analysis the resulting light brown oil was 100 % pure. Attempts to distill the product resulted in decomposition. Yield 17.6 g (58 %).

MS *m/e* (% rel. int., direct inlet): 304 (1, M+1), 303 (2, M), 285 (2), 274 (2, M–CHO or M–CH_2CH_3), 258 (2), 257 (2), 230 (7, M–$COOCH_2CH_3$), 212 (4), 202 (7), 152 (11), 124 (8), 112 (100, $C_6H_{10}NO$), 111 (60), 105 (58, COC_6H_5), 84 (61, $C_5H_{10}N$), 77 (61, C_6H_5), 56 (40).

^{1}H NMR: δ0.96–1.30 (3 H, 3 t, *J* 7 Hz), 1.30–3.42 (7 H approx., m), 3.81–5.20 (3 H, m and multiple d at 4.58–4.79 and 5.01–5.20, *J* 11 Hz), 3.90–4.29 (2 H, 3 q, *J* 7 Hz), 7.29–7.65 (3 H, m), 7.93–8.12 (2 H, t with additional splitting, *J* 7 and 2 Hz). 8.02 and 8.11 (1 H, 2 s).

3,4-Dihydro-1-(2-oxopropyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.025 mol) was dissolved in acetone and $AlCl_3$ was added in portions. After 2 h reaction was complete and excess acetone was removed by evaporation *in vacuo*. The residue was dissolved in CH_2Cl_2 (25 ml) and after addition of water (25 ml) work-up was performed as usual. The solid product was recrystallized from ethyl acetate. Yield 3.8 g (70 %), m.p. 106–107.5 °C.

MS *m/e* (% rel. int.): 217 (36, M), 188 (56, M–CHO), 174 (20, M–$COOH_3$), 160 (100, M–CH_2COCH_3), 146 (24), 132 (85, $C_9H_{10}N$), 130 (22), 117 (24), 115 (18), 105 (17).

^{1}H NMR; δ 2.13 and 2.22 (3 H, 2 s), 2.65–3.34, 3.56–3.74 and 4.32–4.60 (6 H, m),

5.17–5.34 and 5.76–5.93 (1 H, 2 dd, *J* 3.6 and 9.5 and 5.9 and 8.2), 7.02–7.37 (4 H, m), 8.11 and 8.29 (1 H, 2 s).

3,4-Dihydro-1-(3-oxo-2-pentyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.025 mol) and 3-pentanone in CH_2Cl_2 (5 ml) were added to a mixture of $AlCl_3$ in CH_2Cl_2 (20 ml). Ether (5 ml) was added to the crude oil and the product precipitated. The crystals were filtered off and washed with ether. From the concentrated mother liquors additional product was recovered by repeated crystallization from ether. Yield 2.56 g (42 %), m.p. 74–79 °C.

MS *m/e* (% rel. int.): 245 (1, M), 216 (3, M–CHO), 160 (100, M–C_5H_9O), 132 (39, $C_9H_{10}N$), 130 (32).

^{1}H NMR: δ 0.80–1.20 (6 H, multiple d and t, *J* 7 Hz), 1.68–2.62 (2 H, 4 q, *J* 7 Hz), 2.82–4.45 (5 H, several m), 4.81, 5.62 and 5.78 (1 H, 3 d, *J* 9.5, 7 and 8 Hz), 6.84–7.38 (4 H, m), 8.13, 8.18 and 8.26 (1 H, 3 s).

3,4-Dihydro-1-(2-oxo-2-phenylethyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.05 mol) and acetophenone in CH_2Cl_2 (10 ml) were added to $AlCl_3$ in CH_2Cl_2 (40 ml). The product was purified by recrystallization from ethanol (6 ml). Yield 6.0 g (43 %), m.p. 104–107 °C.

MS *m/e* (% rel.int.): 279 (4, M), 251 (7), 250 (20, M–CHO), 207 (3), 174 (13, M–COC_6H_5), 160 (100, M–$CH_2COC_6H_5$), 132 (72, C_9H_{10}), 130 (33), 117 (31), 105 (71), 91 (17), 77 (82).

^{1}H NMR: δ 2.73–3.92 and 4.31–4.60 (6 H, m), 5.37–5.54 and 5.88–6.04 (1 H, dd and t, *J* 3 and 9.6 and 6.3 Hz), 7.03–7.31 (4 H, m), 7.31–7.67 (3 H, m), 7.82–8.08 (2 H, m), 8.10 and 8.42 (1 H, 2 s).

3,4-Dihydro-1-(2-oxo-1-phenylpropyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.05 mol) and phenylacetone in CH_2Cl_2 (10 ml) were added to $AlCl_3$ in CH_2Cl_2 (40 ml). GLC analysis indicated the presence of four products in the proportions 3:5:1.5:1 in a total yield of 70 %. Phenylacetone was still present while the methoxy compound was completely consumed. The two minor products gave mass spectra in agreement with formation of the 1-(3-phenyl-2-oxopropyl)- and 1-(2-oxopropylphenyl) derivatives, respectively. The identical mass spectra of the two major products only gave clear evidence for the presence of the *N*-formyltetrahydroisoquinoline moiety, whereas heavier fragments were of low intensity. From these results we conclude that alkylation takes place preferably at the benzylic carbon, giving a mixture of diastereomers. The less satisfactory mass spectrum might be explained by a strong tendency of the molecular ion to fragment into an *N*-formyltetrahydroisoquinoline cation and a relatively stable benzyl radical. The ^{1}H NMR spectrum, displaying 2+2 doublets (due to the hindered rotation around the CO–N bond) with identical coupling constants, and with chemical shifts in agreement with expectations, indicated alkylation at the benzylic carbon.

The crude oil was dissolved in ethyl acetate. However, only minor amounts of the main products (probably only one of the diastereomers) were collected at recrystallization and considerable amounts were retained in the mother liquors. No additional product could be isolated by repeated recrystallization from various solvents. Proper isolation may probably be accomplished by preparative GLC. Yield 1.18 g (8 %), m.p. 175–176 °C.

MS *m/e* (% rel. int.); the spectra of the diastereomers proved to be identical: The spectra showed numerous peaks of low intensity between 264 (0.5, M–CHO) and 160. No molecular ion was recorded. 160 (100, M–$C_6H_5CHCOCH_3$), 132 (44, $C_9H_{10}N$), 117 (16), 105 (13), 91 (8), 77 (12).

^{1}H NMR: δ 1.95 and 1.97 (3 H, 2 s), 2.80–3.15, 3.30–3.47 and 3.87–4.08 (4 H, m), 4.18 and 4.12 (1 H, 2 d, *J* 9.5 Hz), 4.49 and 6.47 (1 H, 2 d, *J* 9.5 Hz), 7.11–7.42 (9 H, m), 7.72 and 7.91 (1 H, 2 s).

3,4-Dihydro-1-(2-oxoethyl)-2(1H)-isoquinolinecarboxaldehyde. Compound *6* (0.05 mol) and acetaldehyde in CH_2Cl_2 (25 ml) were added to a stirred mixture of $AlCl_3$ in CH_2Cl_2 (25 ml).

The crude oil, after evaporation of the solvent, was dissolved in ethyl acetate (6 ml) and the solution was chilled. The solid product was filtered off and washed with ethyl acetate. Yield 1.15 g (11 %), m.p. 84–86 °C.

MS *m/e* (% rel. int., direct inlet): 203 (18, M), 186 (6, M–OH), 174 (17, M–CHO), 160 (94, M–CH_2CHO), 132 (100, $C_9H_{10}N$), 117 (43), 105 (34), 91 (18), 77 (41).

^{1}H NMR: δ 2.61–3.88 and 4.29–4.58 (6 H, m), 5.19–5.38 and 5.81–5.99 (1 H, 2 dd, *J* 3.8, 8.7, 6.0 and 8.0 Hz), 6.96–7.26 (4 H, m), 8.14 and 8.34 (1 H, 2 s), 9.73–9.82 (1 H, m).

Preparation of barbituric acid derivatives. Sodium (1 eq.) was dissolved in the appropriate alcohol to give a 1 M solution and the amidoalkylated malonic ester was added (1 eq.). Urea (1 eq., dried *in vacuo* at 110 °C) was added and the mixture refluxed for 2–24 h. The time of reaction was roughly estimated from the rate of precipitation of the product. Finally the reaction mixture was chilled (0 °C) and the solid product was filtered off and washed with alcohol. The sodium barbiturate was converted to the acid by dissolving it in water and adding a slight excess of hydrochloric acid. The product precipitated and

was filtered off and washed with water. Only the acid was subjected to MS and NMR analyses.

5-(1-Formyl-2-piperidyl)-2,4,6-(1H,3H,5H)-pyrimidinetrione. The reaction was carried out with 0.02 mol of each reactant and was worked up after 17 h at reflux temperature. Yield 4.63 g (89 %), m.p. (partial decomposition) 240–250 °C. Some of the sodium salt (2 g) was treated with diluted hydrochloric acid and the acid could be collected. Yield 1.04 g (57 %), m.p. 162–165 °C.

MS *m/e* (% rel. int., direct inlet): 239 (small, M), 222 (6, M–OH), 204 (16), 193 (4), 150 (6), 129 (6), 128 (100, $C_4H_4N_2O_3$), 112 (7, $C_6H_{10}NO$), 111 (24, C_6H_9NO), 85 (29, $C_5H_{11}N$), 82 (19), 68 (7), 69 (9), 70 (8).

^{1}H NMR (DMSO-d_6 as solvent): δ 1.08–2.25 (5 H, m), 2.57–3.17 (1 H, m), 3.24–3.52 (1 H, m), 3.65 and 3.68 (1 H, 2 d, J 8.5 and 10 Hz), 3.91–4.57 (2 H, m), 7.91 and 7.93 (1 H, 2 s), 10.95, 11.07. 11.10 and 11.16 (2 H, 4 s).

5-(1-Formyl-hexahydro-1H-azepin-2-yl)-2,4,6-(1H,3H,5H)-pyrimidinetrione. Starting from 0.01 mol of each reactant the resulting mixture was refluxed for 5 h before subsequent isolation of the product. Yield 1.24 g (45 %), m.p. 230–235 °C. 1 g of the sodium salt was dissolved in water (20 ml) and the solution was acidified. Yield 0.70 g (76 %), m.p. 124–127 °C.

MS *m/e* (% rel. int., direct inlet): The spectra showed a large number of peaks between 128 and 251, all of low intensity (less than 5 %). The peak of the molecular ion (253) was absent. 128 (82, $C_4H_4N_2O_3$), 112 (17), 98 (32, $C_6H_{12}N$), 85 (59), 70 (57), 69 (68), 58 (100), 55 (100), 45 (100).

^{1}H NMR (DMSO-d_6 as solvent): δ 0.90–2.26 (8 H, m), 2.44–4.62 (3 H approx., m), 3.49 (br. s, presumably originating from water), 3.61 (1 H approx., d J 3.5 Hz), 7.85 and 8.16 (1 H, 2 s), 11.13, 11.16. 11.29 and 11.32 (2 H, 4 s).

5-(2-Formyl-1,2,3,4-tetrahydro-1-isoquinolinyl)-2,4,6-(1H,3H,5H-pyrimidinetrione. The reaction was carried out by refluxing a mixture of 0.015 mol of each reactant for 24 h. Yield 4.40 (95 %), m.p. >325 °C. 0.5 g of the sodium barbiturate was dissolved in water (20 ml at 100 °C) and the solution was acidified. Yield 0.25 g (54 %), m.p. (simultaneous decomposition) 145–195 °C.

MS *m/e* (% rel. int., direct inlet): 287 (3, M), 258 (12, M–CHO), 242 (7), 172 (7), 160 (68, M–$C_4H_3N_2O_3$), 132 (68, $C_9H_{10}N$), 131 (68), 130 (100, C_9H_8N), 128 (53, $C_4H_4N_2O_3$), 117 (31), 115 (41), 103 (53), 85 (28), 77 (73).

^{1}H NMR (DMSO-d_6): δ 2.62–2.98 (2 H, m), 3.60–3.88 (1 H, m), 4.00–4.66 and 4.51–5.93 (3 H, m), 6.92–7.28 (4 H, m), 8.15, (1 H, apparently br s), 10.59–11.57 (2 H, 2 very br s).

5-(1-Formyl-2-pyrrolidinyl)-5-methyl-2,4,6-(1H)-pyrimidinetrione. The reaction mixture –0.05 mol of each reactant – was refluxed for 2 h before work-up. The solid product proved to be hygroscopic. Yield 8.5 g (65 %). The sodium salt was dissolved in water (20 ml) and the acid precipitated as the solution was acidified. Yield 4.1 g (53 %), m.p. (involving slight decomposition) 235–237 °C. MS *m/e* (% rel. int., direct inlet): 239 (5, M), 210 (18, M–CHO), 142 (3, $C_5H_6N_2O_3$), 124 (7), 98 (100, C_5H_8NO), 97 (22), 70 (96, C_4H_8N), 68 (32). ^{1}H NMR (DMSO-d_6 as solvent: δ 1.35 and 1.38 (3 H, 2 s), 1.51–2.26 (4 H, m), 2.79–3.79 (4 H, m), 3.32 (br s, presumably due to water), 4.13–4.46 (1 H, m), 7.95 and 8.10 (1 H, 2 s), 11.05, 11.08, 11.32 and 11.40 (2 H, 4 s).

2-Piperidineacetic acid hydrochloride. Dimethyl 1-formyl-2-piperidylmalonate (0.05 mol) was dissolved in sodium hydroxide solution (100 ml, 2.5 M) and the mixture refluxed for 20 h. The solution was cooled off and acidified with a slight excess of hydroxhloric acid. The solvent was removed by evaporation *in vacuo* and the residual solid was dried and decarboxylated at 100 °C at 1 mmHg. Anhydrous ethanol (100 ml) was added and the sodium chloride was filtered off and washed several times with anhydrous ethanol. The alcohol was removed by evaporation *in vacuo* and the product was collected as an oil. The product solidified after addition of ethanol and was, after cooling (9 °C), sucked off and washed with ethanol. Yield 5.22 g (58 %), m.p. 181–185 °C (reported 185 °C). MS *m/e* (% rel. int., direct inlet): 144 (2, M–Cl), 143 (5, M–HCl), 85 (7), 84 (100, $C_5H_{10}N$), 82 (9), 60 (10), 56 (17), 55 (11). ^{1}H NMR (DMSO-d_6 as solvent): δ 1.26–2.05 (6 H, m), 2.42–3.48 (5 H, m), 8.8–10.4 (2–3 H, very br s).

1-Formyl-hexahydro-α-phenyl-1H-azepine-2-acetic acid. Diethyl (1-formyl-hexahydro-1*H*-azepin-2-yl)phenylpropanedioate (0.0084 mol) was added to sodium hydroxide solution (35 ml, 1 M) and ethanol (20 ml) was added. Most of the starting material was dissolved and the mixture was stirred for 24 h at room temperature. As considerable amount of the starting material still remained unreacted (undissolved) the reaction mixture was refluxed for 1 h and a homogeneous solution was obtained. The solution was made acidic with hydrochloric acid and evaporated *in vacuo* at 100 °C. This procedure has earlier been observed to bring about decarboxylation of similar compounds. The residual dry solid was washed several times with anhydrous ethanol and the solution was filtered and evaporated *in vacuo*. The product was recrystallized from ethanol (10 ml). According to MS and NMR analyses,

the formyl group was still present in the isolated product despite exposure to high temperature under hydrolytic conditions. Yield 0.81 g (37 %), m.p. 214–215 °C.

MS *m/e* (% rel. int., direct inlet): 262 (1, M+1); the spectrum showed several peaks of low intensity between 262 and 126, 127 (9), 126 (100, $C_7H_{12}NO$), 98 (20, $C_6H_{12}N$), 91 (9), 77 (7).

^{1}H NMR δ 0.97–1.91 (6 H, m), 2.20–2.90 (2 H, m), 3.06–3.26 and 3.39–4.21 (3 H, m), 3.68 and 3.81 (1 H, 2 d, *J* 10.5 Hz), 7.19–7.32 (5 H, m), 7.41 and 7.79 (1 H, 2 s).

α-Phenyl-2-pyrrolidinylacetic acid hydrochloride. Diethyl (1-formyl-2-pyrrolidinyl)phenylmalonate (0.02 mol) was added to sodium hydroxide solution (40 ml, 2.5 M) and the mixture was heated. The starting material was found to be sparingly soluble in sodium hydroxide solution even at reflux temperature and after 30 min, without any indication of reaction, ethanol (5 ml) was added. After 15 min a homogeneous solution was obtained and reflux was continued for 12 h. The reaction mixture was allowed to cool down and was acidified with hydrochloric acid, causing evolution of CO_2. The acidic solution was refluxed for a few min to assure complete decarboxylation and filtered, in order to remove a small amount of precipitates, before evaporation of the solvent. In order to obtain a dry product anhydrous ethanol (50 ml) was added to the residual solid and then evaporated *in vacuo*. The procedure was repeated twice.

Finally anhydrous ethanol was added to the dry product and the sodium chloride was filtered off and washed with ethanol. The alcohol was evaporated *in vacuo* and the solid product was recrystallized from ethanol (5 ml). Yield 2.82 g (58 %), m.p. (simultaneous decomposition) ≥214 °C (reported 223 °C (decomposition). MS *m/e* (% rel. int., direct inlet): No molecular ion was observed; small peaks between 224 and 136, 136 (6, $C_6H_5-CH_2-COOH$), 91 (33), 77 (6), 70 (100, C_4H_8N).

^{1}H NMR (DMSO-d_6 as solvent): δ 1.35–2.43 (4 H m), 2.92–3.34 (2 H, m), 3.78–4.30 (2 H, m and d (centered at 4.20), *J* 11 Hz), 8.9–9.2 and 9.3–9.8 (2 H approx., 2 very br s).

(1-Formyl-2-piperidyl)propanedioic acid. Dimethyl (1-formyl-2-piperidyl)-malonate (0.03 mol) was dissolved in sodium hydroxide solution (50 ml, 2.5 M) and allowed to stand for 20 h at room temperature. The solution was acidified with hydrochloric acid and the solvent was removed by evaporation *in vacuo*. Dry acetone was added to the residue and subsequently removed by evaporation *in vacuo*. The procedure was repeated twice and the mixture of product and NaCl was dried for several h at 1 mmHg. The product was dissolved in acetone and the solution was filtered. After evaporation of the solvent the product crystallized after being dissolved in 8 ml of acetone. During the work-up care was taken not to exceed 60 °C. Yield 5.0 g (78 %), .p. (involving simultaneous decarboxylation) >140 °C.

^{1}H NMR (DMSO-d_6 as solvent): δ 0.98–1.83 (6 H, m), 2.62 and 3.14 (1 H, 2 t, *J* 12 Hz), 3.36–4.34 and 4.84–5.05 (2 H, m), 3.83 and 3.93 (1 H, 2 d, *J* 11 Hz), 8.07 and 8.11 (1 H, 2 s), the carboxylic acid protons were scarcely discernible as a broad signal at δ 10–14.

1-Formyl-2-piperidineacetic acid. (1-Formyl-2-piperidyl)propanedioic acid (0.0139 mol) was kept at 140–150 °C for 4 h. Acetone (2 ml) was added and the solution was chilled. The solid product was filtered off and washed with acetone. Yield 1.25 g (53 %), m.p. 89–94 °C.

MS *m/e* (% rel. int., direct inlet): 171 (13, M), 142 (24, M–CHO), 125 (7), 112 (100, $C_6H_{10}NO$), 97 (13), 84 (82, $C_5H_{10}N$), 56 (75).

^{1}H NMR (DMSO-d_6 as solvent): δ 1.02–1.77 (6 H, m), 2.24–3.25 (3 H, several coincident dd, *J* 9 and 15 Hz, and m), 3.34–3.60, 3.88–4.22 and 4.60–4.85 (2 H, m), 7.93 and 7.98 (1 H, 2 s).

2-(2-Oxopropyl)-1-piperidinecarboxaldehyde. Methyl 2-(1-formyl-2-piperidyl)-3-oxobutanoate (0.056 mol) was dissolved in sodium hydroxide solution (110 ml, 1 M) and was allowed to react for 24 h at room temperature. The solution was acidified with hydrochloric acid and evaporated *in vacuo* at 100 °C. Evolution of carbon dioxide was observed during the acidification. Several portions of anhydrous ethanol were added and the sodium chloride was filtered off. The solvent was removed by evaporation *in vacuo* and the residual oil was distilled at reduced pressure. The isolated product was found to be contaminated by 10 % of 1-formyl-2-piperidineacetic acid ethyl ester. Yield (including the by-product) 3.53 g (37 %), b.p. 140–143 °C/1.2–1.6 mmHg.

MS *m/e* (% rel. int.): 169 (17, M), 152 (17), 140 (22, M–CHO), 126 (70, M–$COCH_3$), 112 (87, $C_6H_{10}NO$), 98 (36), 84 (100, $C_5H_{10}N$), 56 (98), and (carboxylic acid ester): 199 (4, M), 170 (25, M–CHO or M–C_2H_5), 154 (7, M–OC_2H_5), 126 (7, M–$COOC_2H_5$), 125 (10), 122 (100, $C_6H_{10}NO$), 84 (88, $C_5H_{10}N$), 56 (49).

^{1}H NMR: δ 1.40–1.85 (6 H, m), 2.16 and 2.19 (3 H, 2 s), 2.51–4.37 and 4.86–5.11 (3 H, several coincident m), 2.70 and 2.90 (2 H, 2 d, *J* 8 Hz), 8.09 and 8.43 (1 H, 2 s). The main part of the spectrum was poorly resolved.

Hexahydro-2-(2-oxopropyl)-1H-azepine-1-carboxaldehyde. 2-(1-Formyl-hexahydro-1*H*-azepin-2-yl)-3-oxobutanoate (0.047 mol) was dissolved in sodium hydroxide solution (110 ml, 1 M) and

allowed to stand for 120 h at room temperature before acidification with hydrochloric acid. During the addition of hydrochloric acid evolution of carbon dioxide was observed. The solution was evaporated *in vacuo* at 100 °C and the residue was washed several times with anhydrous ethanol. The ethanol solutions were filtered and the solvent was removed by evaporation *in vacuo*. The resulting oil was distilled at reduced pressure. According to MS and NMR analyses the isolated product was contaminated by 10 % of 1-formyl-hexahydro-1*H*-azepine-2-acetic acid ethyl ester. Yield (including the by-product) 5.96 g (69 %), b.p. 140–143 °C/0.8–1.2 mmHg.

MS *m/e* (% rel. int.): 183 (7, M+1), 183 (12, M), 166 (5), 154 (17, M−CHO), 140 (72, M−$COCH_3$), 126 (87, M−CH_2COCH_3), 112 (41), 98 (72, $C_6H_{12}N$), 70 (40), 56 (100), and (carboxylic acid ester): 213 (4, M), 184 (21, M−CHO or M−CH_2CH_3), 168 (7, M−OCH_2CH_3), 140 (10, M−$COOCH_2CH_3$), 126 (100, M−$CH_2COOCH_2CH_3$), 112 (12), 98 (57, $C_6H_{12}N$), 70 (18), 56 (42), 55 (43).

^{1}H NMR: δ 1.07–2.89 (8 H, m), 2.11 and 2.15 (3 H, 2 s), 2.53–2.74 (2 H, m), 3.03–3.65, 3.83–4.24 and 4.34–4.64 (3 H, m), 8.08 (1 H, s).

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Electron Transfer from Alkylmagnesium Compounds to Organic Substrates

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The present article gives a survey over some mechanistic problems concerning the reactions of Grignard reagents, which have had the interest of the author since 1965. It is perceived from a rather personal point of view and does not pretend to be comprehensive in covering the many very recent contributions, which have appeared in the field.

Alkylmagnesium compounds are the most important representatives of organometallics; they are in everyday use in any chemical institution concerned with organic chemistry. Apart from their synthetic usefulness, the compounds are mechanistically interesting because of the wide spectrum of "normal", "abnormal" or even "paradoxical" products, which are found among the reaction products.[1]

Because of the polar nature of the carbon–magnesium bond the reactions of the Grignard reagents have usually been considered those of potential carbanions, but for many years the mechanistic suggestions in the area were not given a thorough kinetic foundation.[1] Many good reasons may be given for this situation. The reagents are difficult to handle, because contact with air and moisture must be rigorously avoided. The purity of the reagents is difficult to ascertain, since crystallization and sublimation and other separation procedures are usually not feasible. Metallic impurities in the magnesium are almost impossible to avoid and may alter the course of the reactions when present even in trace amounts. The reaction rates to be measured are often very high with half lives of the order of ms.

Even when kinetic measurements have been obtained in spite of the practical obstacles, their interpretation presents many problems. Since magnesium is a Lewis acid the reagents are at the same time electron rich and electron deficient. For this reason Grignard reagents enter into various coordination equilibria by, for example, self-association, reagent-solvent association, and reagent-substrate association. Because of the Schlenk equilibrium:

$$2\,RMgX \rightleftharpoons R_2Mg + MgX_2,$$

at least three types of electrophilic magnesium species may enter into all the coordination equilibria mentioned. All the equilibria are of importance for the kinetics and should be studied.

Progress in the understanding of the self-association of Grignard reagents has been obtained mainly by ebullioscopic measurements of the vapour pressure of the solvent.[2,3] Reagent-solvent equilibria were studied by the use of optically active solvents [4] and by calorimetric and spectrographic procedures.[5] The Schlenk equilibrium was investigated by thermometric titration [6] and by the NMR technique,[7] and the reagent-substrate equilibria were studied by UV and IR spectroscopy [8,9] as well as kinetic methods.[10]

Because of the complicated nature of Grignard reagent-substrate mixtures the most useful and reliable method to obtain kinetic results has been to use pseudo first-order conditions with the reagent present in very large excess. In this way the reagent is virtually unchanged during the

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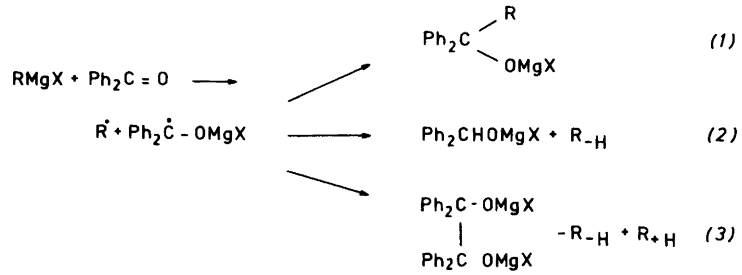


Scheme 1.

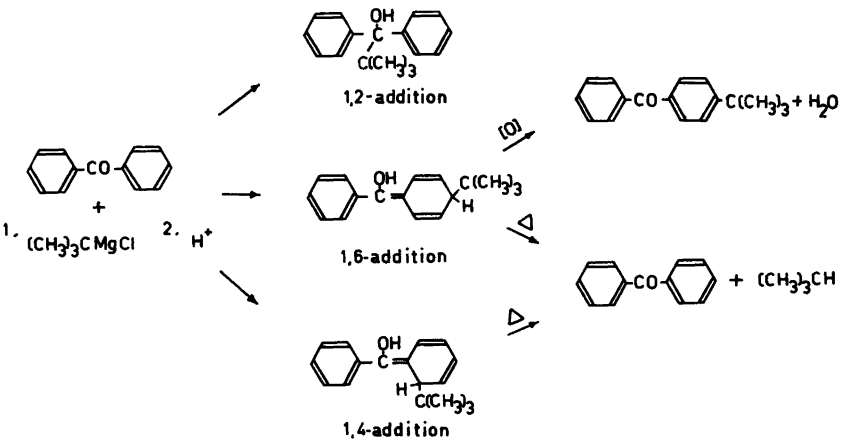
reaction. The kinetic procedures have been spectroscopic or thermographic, and flow techniques have often been required. The results obtained have given a few answers, while many questions are left open.

Grignard reagents+benzophenone. Electron transfer or polar mechanism? The problem which will be dealt with in the following is whether Grignard reagents behave primarily as anion precursors and react by concerted reaction mechanisms, or whether they are electron donors and therefore react by stepwise mechanisms. It is not to be expected that a simple answer may be given, which would cover all reagents reacting with any substrate. It seems, however, that if the question is limited to Grignard reagents reacting with benzophenone the answer is that the reactions are stepwise and are always initiated by the transfer of a single electron (ET) to benzophenone.

Blicke and Powers [11] in 1929 proposed that Grignard reagents are homolyzed in the reaction with benzophenone and loose magnesium subhalide to form an alkyl radical and the magnesium salt of benzophenone ketyl; Scheme 1.

The two radicals might combine to form the 1,2-addition product (1) or possibly, the ketyl would abstract hydrogen from the β-position of the alkyl radical, which would lead to the reduction product, benzhydrol and alkene (2). The authors also suggested that two ketyl radicals might combine to form benzopinacol and that the alkyl in this case would disproportionate to form alkene and alkane (3). At the time of its proposal, the theory was purely hypothetical, but today, after half a century, it seems that the mechanism in Scheme 1 is very close to being correct.

Evidence from by-products and ESR. Benzopinacol, which should be expected as a by-product according to the radical mechanism, was not observed by Blicke and Powers, but it was reported a few years later by Arbuzov and Arbuzova [12] in the reaction of cyclohexylmagne-



Scheme 2.

sium bromide with benzophenone. It would seem that the radical mechanism could then be established, but it was argued that metallic magnesium present in the Grignard reagent could be the reducing agent. Another serious problem arose when Kharash and Lambert [13] showed that trace amounts of transition metals added to methylmagnesium bromide would prevent the formation of addition product and lead to high yields of benzopinacol. The interest in the radical mechanism was renewed in 1968, when Blomberg and Mosher [14] using filtered solutions of neopentylmagnesium chloride prepared from sublimed magnesium obtained a 20 % yield of benzopinacol in the reaction of benzophenone. Also the ESR technique had shown the formation of ketyl during Grignard reactions with benzophenone.[14,15] A radical mechanism was no doubt in operation, but there were no means by which one could tell whether the benzopinacol was a by-product produced in an ET mechanism in competition with a polar mechanism, which might be alone responsible for the addition product.

Evidence from reaction rates and products distributions. The answer had to come from kinetic work. A study was made of the reaction of benzophenone with *t*-butylmagnesium chloride. This reaction was reported to give *ca.* 60 % 1,2-addition product and no reduction product.[16] It was found that when the reaction mixture was worked up cold and in the absence of air, NMR showed the presence of large amounts of the 1,6-addition product, a dihydrobenzophenone enol, Scheme 2.[17] The reaction mixture also contained the 1,2-addition product and benzopinacol. A series of substituted benzophenones was now tested for reactivity toward *t*-butylmagnesium chloride and for product distribution in the reaction. It showed that the overall reaction, *i.e.*, the rate at which the substituted benzophenone was consumed, followed the Hammett rate law, Fig. 1, but the product distribution was 0–55 % for the 1,2-addition product, 0–39 % for the 1,4-addition product, 0–100 % for the 1,6-addition product and 0–21 % for the benzopinacol, Table 1. It was obvious that the product distributions were extremely sensitive to steric factors, but the overall rate was not. This allowed the conclusion that the reaction had a discrete rate-determining step, and that the product distribution was determined in fast consecutive steps. ET was most likely as the rate-determining

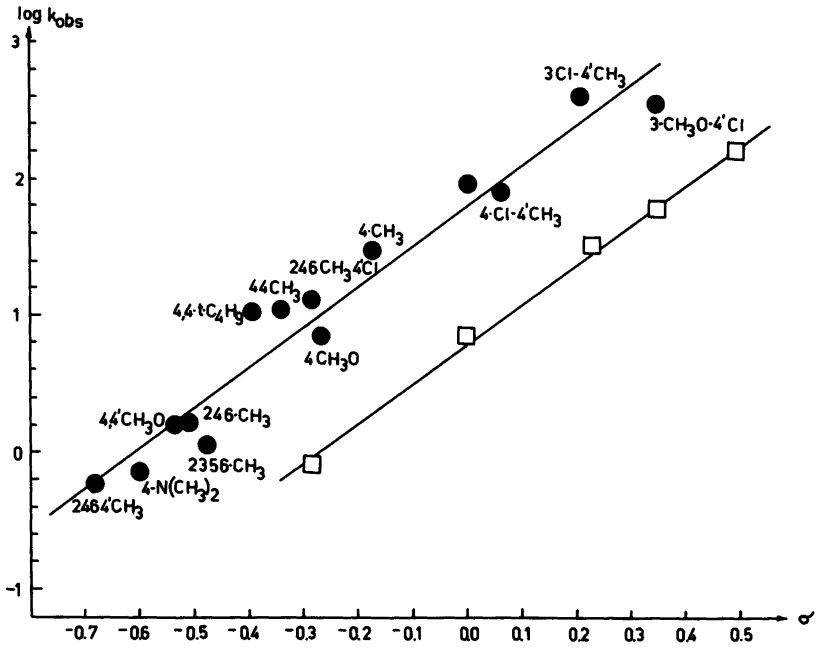


Fig. 1. Hammett plot for the reaction of *t*-butylmagnesium chloride 0.5 M with 0.02 M of substituted benzophenones. Temperature 20 °C (●) and −30 °C (□).

Table 1. Product distributions in the reaction of *t*-butylmagnesium chloride in excess with substituted benzophenones in diethyl ether.

Benzophenone	% Benzopinacol	% Addition		
		1,2-	1,4-	1,6-
Unsubstituted	6	44	0	50
4,4'-dimethyl-	12	55	0	33
4,4'-di-*t*-butyl-	21	40	39	0
4,4'-dichloro-	0	50	21	29
2,4,6-trimethyl-	0	0	0	100
2,3,5,6-tetramethyl-	0	0	0	100

step and the *t*-butyl radical would combine with sterically accessible carbon atoms of the ketyl molecule, which shared the spin density by resonance.

Evidence from the correlation of reaction rates with the anodic oxidation potentials for Grignard reagents. The establishment of ET as the only important reaction mechanism for a typical tertiary Grignard reagent reacting with benzophenone in diethyl ether under normal conditions was very welcome after several decades of suggestive, but non-conclusive evidence. It obviously created an urge to reach conclusions also concerning the reaction mechanisms for primary and secondary Grignard reagents with benzophenone. Several differences in the reaction pattern were *a priori* to be expected. Secondary and especially primary radicals are much less stable than tertiary, and the lifetime of a radical pair (alkyl and ketyl, after ET and homolysis) would be expected to be extremely short. If ET and radical combination follow each other with almost no separation it would seem impossible to distinguish between the two-step ET mechanism and the one-step concerted anionic mechanism. One thing would, however, be different: The predicted relative rates for reaction of alkylmagnesium reagents with the ketone. For a concerted reaction a reactivity series tertiary<secondary<primary would be expected for steric reasons, while for ET the series would be reversed. Since ET involves the transfer of an electron from the different reagents to one and the same acceptor, *e.g.*, benzophenone, it should be possible to relate the reaction rate to the anodic oxidation potential of the individual Grignard reagents.

As early as 1935, Evans, Lee and Lee determined values for the "decomposition potentials" of Grignard reagents using an electrolysis set-up with large platinum electrodes and rather concentrated ethereal solutions of Grignard reagents.[18] From the simple plots of current *versus* potential was chosen a value "where the curve showed a distinct break" and this potential was given as the decomposition potential.

In an attempt to repeat the work of Evans *et al.*, it was found that the plots of current density *versus* potential are, as would be expected, exponential, and do not show any distinct breaks.[19] If, however, corrections were made for ohmic potential drops and for concentration

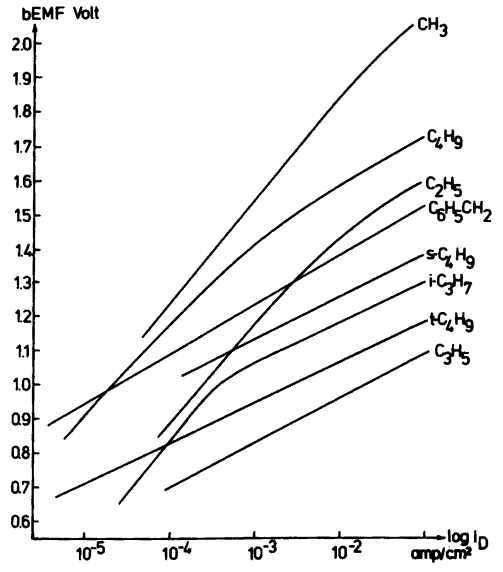


Fig. 2. Tafel plots for the electrolysis of *ca.* 1 M ethereal alkylmagnesium bromides showing the anodic overvoltage at platinum relative to the Pt|Mg|MgBr cathode.

potentials, *etc.* linear Tafel plots were obtained for many Grignard reagents, Fig. 2. The Tafel plots for some of the alkylmagnesium bromides show a change in slope as the current density is varied. While the plots for *t*-butyl, allyl and benzyl (which form stable radicals) have a constant slope of 0.15, plots for reagents, which form more reactive radicals show a break from a slope of ~0.30 at low current densities of ~0.15 at high values of I_D. The break occurs at higher currents the more reactive the corresponding radical is in the sequence $CH_3>C_2H_5>C_4H_9>i\text{-}C_3H_7$. A comparison of the electrochemical reactivity is only possible at a current density at which the slopes are identical and for this reason the overpotentials at $I_D=0.06$ A cm^{-2} were taken as being representative for the anodic oxidation potential and given in Table 2 as $\eta_{0.06}$.

The values of $\eta_{0.06}$ are not standard oxidation potentials, since they are measured at a working electrode and relative to the Pt|Mg|$MgBr_2$ electrode. Relative to this standard a Pt|H_2|HBr electrode is found at 1.92 V and a Pt|*t*-Bu·|*t*-BuMgBr electrode at 0.85 V. On this basis the $\eta_{0.06}$ values may be tentatively converted to standard oxidation potentials relative to SHE by substraction of 2.23 V. In order to see if the electron transfer process at the anode is related to the reaction of Grignard reagents with benzophenone, a correlation was made between log *k* for the chemical reaction and $\eta_{0.06}$ for the electrode process, Fig. 3. The correlation was found to be linear provided the reaction constants were obtained for the reaction of the

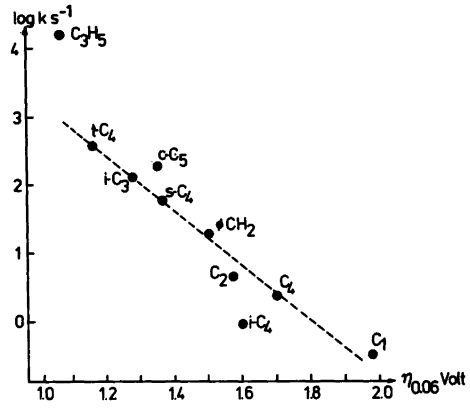


Fig. 3. Log pseudo first-order rate constants for the reaction at 20 °C of alkylmagnesium bromide 0.02 M in diethyl ether with 0.25 M benzophenone.

Grignard reagent with an excess of benzophenone, Table 3. When the reaction takes place in this way the kinetics is first order in benzophenone and first order in Grignard reagent, which is not the case if the relative concentrations are reversed. *t*-Butylmagnesium chloride in ether is, for example, just as reactive toward 0.02 M benzophenone at 0.1 M as when the concentration is 1.0 M. This rate-levelling effect is well known for any Grignard reagent reacting in ether solution with aliphatic ketones like acetone, [20] while with aromatic ketones it only applies to tertiary and secondary reagents. The effect probably is steric and is a consequence of complex

Table 2. Anodic overvoltage at a platinum electrode relative to the reference electrode Pt|Mg|$MgBr_2$ at a current density $I_D=0.06$ amp cm^{-2} for Grignard reagents in diethyl ether at the concentration given, with an excess magnesium bromide given as % Br^-. Also shown are tentative values for E_o for alkylmagnesium bromides relative to SHE.

R	% Br^-	[RMgBr] M	$\eta_{0.06}$ V	E_o V
CH_3	0.8	1.00	1.98	−0.25
C_2H_5	2.8	0.87	1.57	−0.66
i-C_3H_7	13.0	0.99	1.28	−0.95
C_4H_9	4.4	1.09	1.70	−0.53
i-C_4H_9	9.5	1.00	1.60	−0.63
s-C_4H_9	19.6	0.98	1.36	−0.87
t-C_4H_9	30.0	0.69	1.16	−1.07
C_3H_5	19.7	0.68	1.07	−1.16
$C_6H_5CH_2$	15.0	1.02	1.50	−0.73
c-C_5H_9	9.9	0.98	1.35	−0.88

Table 3. Pseudo first-order rate constants, k_{obs}, in s^{-1} for the reaction of 0.02 M alkylmagnesium bromide with 0.25 M benzophenone in diethyl ether at 20 °C. Anodic overvoltage $\eta_{0.06}$ (see text) is given in V.

Grignard reagent	k_{obs}	$\eta_{0.06}$
CH_3MgBr	0.3	1.98
C_2H_5MgBr	2.8	1.57
i-C_3H_7MgBr	133	1.28
C_4H_9MgBr	2.6	1.70
i-C_4H_9MgBr	0.9	1.60
s-C_4H_9MgBr	64	1.36
t-C_4H_9MgBr	400	1.16
$CH_2=CHCH_2MgBr$	16 000	1.07
$C_6H_5CH_2MgBr$	21	1.50
cyclo-C_5H_9MgBr	214	1.35

formation in ether solution between the electrophilic magnesium compounds and ketones with donor properties. The rate constants obtained under the two sets of conditions cannot be compared because the Grignard reagents are different: The ligands bound to magnesium are in the first case (large excess of Grignard reagent) predominantly ether, but in the second case (large excess of benzophenone) supposedly ether and benzophenone.

That the possibility exists to correlate the reactivity of the Grignard reagents with the anodic oxidation potential is very good evidence for a rate-limiting electron transfer. At the same time it indicates that with excess benzophenone the ET mechanism is not sensitive to steric hindrance since *t*-butylmagnesium bromide is 1330 times more reactive than methylmagnesium bromide.

Evidence from kinetic isotope effects, etc. The possibility of discerning a rate-limiting step different from the product-determining step, which was used to prove the stepwise nature of the reaction of *t*-butylmagnesium chloride with benzophenone is also present, for primary and secondary reagents, although the picture is a little less clear-cut. Secondary and primary Grignard reagents react with benzophenone to form the 1,2-addition product and large amounts of the reduction product benzhydrol formed by transfer of a β-hydrogen from the Grignard reagent to the carbonyl carbon, Scheme 1. The product distribution was changed when the β-hydrogens were exchanged with deuterium so that more addition and less reduction took place. If the partial rates for the addition and the reduction were calculated on the basis of the overall rate and the product distributions, it was found that the reduction was slowed down, while the addition was accelerated.[21] The simplest way to interpret the result is to assume rate-limiting ET, which has a rather low deuterium isotope effect and a product-determining radical combination step. Since the abstraction of a β-deuterium is rather much slower than abstraction of hydrogen, the recombination to form addition product is favoured leading to an increase in the rate of formation of addition product.

Substituents in the benzophenone also affect the ratio of addition/reduction in the reaction with primary Grignard reagents. With isobutylmagnesium bromide the ratio is 100 times lower with *p,p'*-dichloro substituted benzophenone than with *p,p'*-dimethylamino substitution and an irregular Hammett plot, Fig. 4, is obtained for $\log(k_{red}/k_{add})$ *versus* σ. The overall reaction also obeys the Hammett rate law although somewhat irregularly, Fig. 5. A comparison of *p,p'*-dimethylbenzophenone (3) with *p,p'*-di-*t*-butylbenzophenone (4) is interesting. These ketones have the same overall reactivity toward isobutylmagnesium bromide. This is in keeping with the almost identical σ-values for methyl and *t*-butyl. The ratio reduction/addition is, however, 4.26 for methyl and 1.44 for *t*-butyl in the para positions, which means that the addition taken as a partial reaction is much faster for *t*-butyl- than for methyl substituted benzophenone.[22] It is impossi-

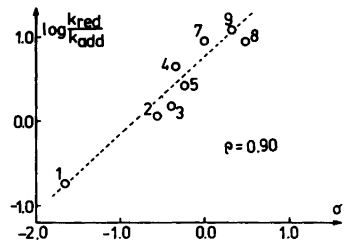


Fig. 4. Logarithm to the ratio reduction: addition in the reaction of isobutylmagnesium bromide in diethyl ether with substituted benzophenones *versus* Hammett σ constants. 1=*p,p'*-dimethylamino, 2=*p,p'*-dimethoxy, 3=*p,p'*-di-*t*-butyl, 4=*p,p'*-dimethyl, 5=*p*-methoxy, 7=unsubstituted, 8=*m*-methoxy-*m'*-chloro, 9=*m*-fluoro.

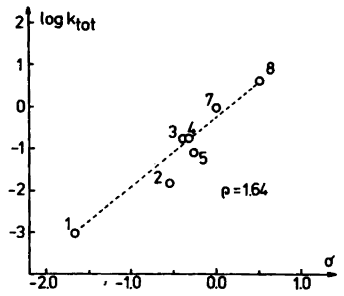


Fig. 5. Log relative rate for the overall reaction of isobutylmagnesium bromide in diethyl ether with substituted benzophenones (see Fig. 4) *versus* Hammett σ constants.

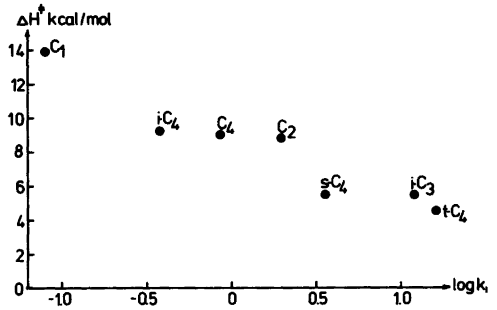


Fig. 6. Enthalpy of activation for the reaction of 0.1 M alkylmagnesium bromide with 0.02 M benzophenone in diethyl ether *versus* log pseudo first-order rate constant for the reaction at 20 °C.

ble that the substitution of methyl with the bulkier *t*-butyl should cause a significant increase in the rate of an independent addition reaction of whichever mechanism, and the only possible explanation is, therefore, that the addition is not independent but that an SET with low steric requirements is rate-limiting for the overall reaction, and that the bulk of the *t*-butyl groups impedes the facile collapse to reduction product and a larger fraction of the radical pairs, therefore, collapses in the 1,2-fashion.

Evidence from an isokinetic relation between activation parameters. In the search for a reaction mechanism for primary and secondary Grignard reagents reacting with benzophenone, it was found of interest to compare the activation parameters. Rate measurements for the reaction of seven alkylmagnesium bromides (0.1 M) with 0.02 M benzophenone in ether were performed at 20 °C and at 40 °C, which allowed estimates of the enthalpy of activation and the entropy of activation.[23] It was found that low reactivity of a reagent like methylmagnesium bromide was the result of a high enthalpy of activation (13.7 kcal mol^{-1}) with a rather favourable entropy of activation (−12.2 e.u.), while a highly reactive reagent like *t*-butylmagnesium bromide had a low enthalpy of activation (4.5 kcal mol^{-1}) combined with an unfavourable $\Delta S^{\ddagger}$ (−33.3 e.u.). The relation between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ tended to be linear. This isokinetic relationship is an indication to similarity in mechanism within the reaction series.[24]

It has been shown that because of the way in which the parameters are calculated they are not quite independent, but if there is linear correlation between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ the same should apply to $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ and therefore to log *k* and $\Delta H^{\ddagger}$, which are derived independently.[25] In Fig. 6 the values of log *k* *versus* $\Delta H^{\ddagger}$ for the seven alkylmagnesium bromides are shown. While the isokinetic plot of $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ (not shown) yields a linear correlation with a correlation coefficient of 0.986, the plot of $\Delta H^{\ddagger}$ *versus* log k_{20} has a correlation coefficient of 0.94.

As mentioned in the previous sections, the observed rate of a Grignard reaction is dependent on equilibria of several types between reagent and substrate and within the reagent itself. The measured enthalpy of activation therefore includes *e.g.* the enthalpy of the Schlenk equilibrium and of the equilibrium reagent-substrate. To make the true test for isokinetic correlation, it will be necessary to determine all the relevant contributions since one or more of them may not be isokinetically correlated.

Concerted electrophilic displacement of magnesium. (a) Reactivity series. As pointed out above, Grignard reagents are both electron donors and potential anions, and toward benzophenone or a platinum anode they play the role as electron donors. It would be of interest to find substrates toward which Grignard reagents do not react by electron transfer, but rather in a truly concerted fashion. The property in the substrate, which would be of importance would be the relative ease with which a single electron is accepted, as compared with the ease with which a nucleophile is accepted. The reduction potential of Grignard substrates may be measured as the polarographic

half wave potential, $E_{1/2}$ (*versus* SCE), and half wave potentials for substituted benzophenones have been correlated with the Hammett σ constants, but no direct correlation has been obtained between reaction rates for Grignard substrates of different types of compounds and the measured reduction potentials. This shows that even if the electron transfer reaction is simple in principle, its mechanism is nevertheless highly individual for each type of substrate, and this fact will be discussed later.

True nucleophilic behaviour of Grignard reagents should be expected toward substrates, which have a polar carbonyl group but a relatively high reduction potential, since they lack the ability to stabilize a radical anion by resonance. Purely aliphatic ketones like acetone fill this description, and a strong indication of a concerted course of the reactions of acetone with Grignard reagents is the reactivity series, Table 4, found by flow stream thermographic kinetics

Table 4. Pseudo first-order rate constants for the overall reaction of 0.05 M of ketone with 0.5 M of alkylmagnesium bromide in diethyl ether at 20 °C.

RMgX	k_1 s^{-1} (acetone)	k_1 s^{-1} (benzophenone)
CH_3MgBr	3.8	0.30
C_2H_5MgBr	7.5	7.2
C_3H_7MgBr		3.2
$(CH_3)_2CHMgBr$	1.6	21
C_4H_9MgBr	2.2	3.2
i-C_4H_9MgBr	0.1	27
C_3H_5MgBr	momentary	momentary
$C_6H_5CH_2MgBr$	150	91
p-$CH_3C_6H_4MgBr$	69	1.0
C_6H_6MgBr	42	0.3
p-ClC_6H_4MgBr	14	0.17
c-C_5H_9MgBr		2.6
C_4H_9MgCl	36	
t-C_4H_9MgCl		94
p-$CH_3C_6H_4MgCl$		700
$C_6H_5CH_2MgCl$		350
p-ClC_6H_4MgCl		200
$(CH_3)_2Mg$	15	1.9
$(C_2H_5)_2Mg$	110	
$(i\text{-}C_4H_9)_2Mg$		30
$(s\text{-}C_4H_9)_2Mg$		630
$(t\text{-}C_4H_9)_2Mg$		110
$(C_4H_9)_2Mg$	120	64
C_4H_9MgI	0.6	

and showing phenyl ≫ethyl>methyl>i-propyl>*t*-butyl.[26] As seen from Table 4, this is the reverse order which is valid for the ET reaction with benzophenone. The reactivity series is not a direct proof for a concerted mechanism, but it clearly shows that a mechanism is operating in which the steric effects are extremely important.

The high rates obtained with acetone reacting with aromatic Grignard reagents deserve comment. In diethyl ether Grignard reagents and ketones momentarily form complexes and the coordinated ketone is much less reactive than is the small amount of free uncoordinated ketone, which is present according to the equilibrium. This "self inhibition" of the reaction is common for all aliphatic Grignard reagents and is responsible for the fact that Grignard reagents have an extremely high reactivity toward an excess of aliphatic ketone, while the reaction of ketone of a low concentration with an excess of Grignard reagent tends to be very low order or even zero order with respect to Grignard reagent.[20] The conclusion was reached that ketone-Grignard reagent complexes may represent "blind alleys" in the reaction. The complexes do not rearrange to products, but as the reaction goes on they supply by dissociation reactive uncoordinated ketone in a very fast equilibrium.

In contrast to the aliphatic Grignard reagents, aromatic reagents seem to form complexes with acetone which are capable of rearrangement and this may be one of the reasons for the high reactivities observed. The normal reactivity series for nucleophilic substitution is methyl>ethyl>i-propyl>*t*-butyl≫phenyl. That phenyl is at the top instead of at the bottom, when the substitution is electrophilic, is explainable from the fact that S_N2 requires attack from the rear (impossible in bromobenzene), while S_E2 requires frontal attack, which is very facile in phenylmagnesium bromide.

A further example of an electrophilic substrate, which reacts with Grignard reagents by a concerted electrophilic substitution, is carbon dioxide, for which the same reactivity series applies as was found for acetone.[22]

(b) Stereochemistry. The investigation of the steric course of nucleophilic substitution has been of crucial importance for the distinction between the concerted and the stepwise mechanisms S_N2 and S_N1. Grignard reagents usually have a very low barrier for inversion and the stereochemical

course is therefore impossible to follow. Only one example is known of a Grignard reagent which is chiral and has magnesium at the asymmetric carbon. The reagent is 1-methyl-2,2-diphenylcyclopropylmagnesium bromide and was prepared by Walborsky and Young.[27] With this reagent the reaction with carbon dioxide was found to take place with full retention at carbon. The reaction with benzophenone, however, was found to take place predominantly with retention, but with a 20 % loss of optical activity.[22] While a concerted reaction is indicated with electrophilic CO_2, the partial loss of optical activity in the reaction with benzophenone indicates a finite lifetime of a cyclopropyl radical on the reaction path. It should be remembered that the strained cyclopropyl radical has a rather high barrier for inversion.

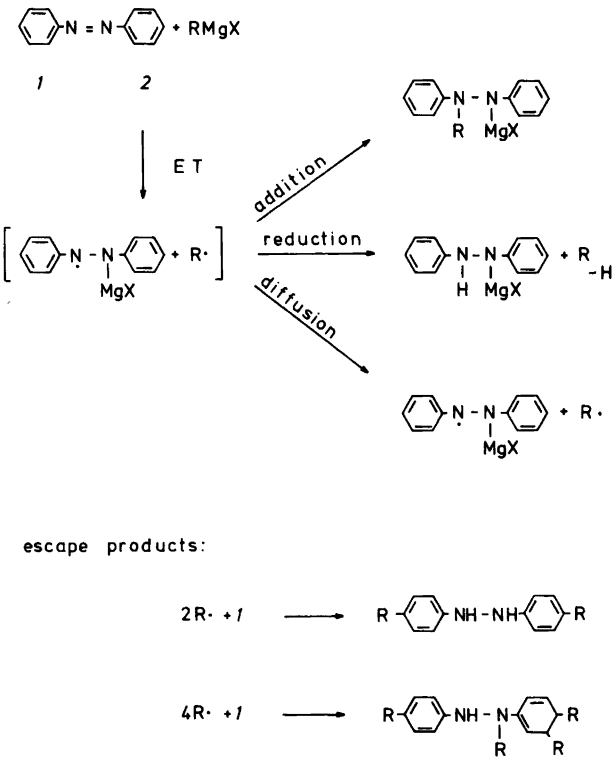
Carbon dioxide and acetone are then obviously electrophiles toward Grignard reagents, while benzophenone, azobenzene and other highly conjugated substrates are electron acceptors. No doubt it will be possible to find substrates in which the concerted mechanism and the stepwise are of comparable efficiency and will therefore compete. One might, for example, expect to find substrates reacting by ET with *t*-butylmagnesium halide, but by concerted mechanism with methyl- or phenylmagnesium reagents. Most likely, however, the two competing mechanisms would lead to the same product(s) and only special kinetic investigations would reveal how large the individual contributions had been.

The evidence for concerted electrophilic displacement of magnesium which has been presented, *e.g.*, the reactivity series and the steric outcome, although very persuasive is not definitive, since it would be indistinguishable from a very "tight" ET mechanism. The subject of the review is, however, ET reactions in which the two steps are clearly distinguishable. It seems that more than one type of ET from a Grignard reagent is possible, dependent on which type of substrate is the acceptor.

Four-center and six-center ET. Primary and secondary cage product. For the ET reaction of benzophenone with Grignard reagents several examples have been given of changes in the product distribution without changes in the overall reaction rate. This may have left the impression, that ET is an extremely unselective process with no steric or conformational restrictions and requirements. This is probably an incorrect picture, and it is likely that a number of ET types may be distinguished, each characterized by a specific geometry of the transition state. Often the radical recombination will occur extremely fast to form a product with a structure closely related to the ET transition state. This product may be named the primary cage product. Sometimes, when the immediate collapse of the radicals fails to take place, an alternative secondary cage product is formed after a reorientation of the radicals. The third possibility is that the radicals diffuse from each other to form escape products. The concept of different types of ET emerged from work with azobenzene as the substrate for reactions with Grignard reagents.

Reactions of azobenzene with Grignard reagents. Azobenzene has been known to react with Grignard reagents with formation of hydrazobenzene and the hydrocarbons, which result from coupling or disproportionation of the alkyl of the Grignard reagent.[28] During a reinvestigation of the reaction it was found, however, that addition to form monoalkylhydrazobenzene was important; in the 1,2- as well as the 1,6-fashion, and that also *p,p'*-dialkylated hydrazobenzenes, and even tetraalkylated products, were sometimes formed,[29] Scheme 3.

A study of the reaction rates as well as the product distributions when using different Grignard reagents revealed that allylic reagents reacted very fast to give addition product exclusively; benzyl and *t*-butyl both gave more than 50 % addition, but benzyl was 100 times slower than *t*-butyl. Primary and secondary reagents all gave between 10 and 20 % addition, but the rates increased by a factor 10 for each hydrogen in the β-position, Table 5. Isopropylmagnesium bromide therefore reacted 10 000 times faster than isobutylmagnesium bromide. In spite of these exceedingly large variations in rate, the fraction of addition product was the same within narrow limits. This indicates that the factors which determine the rate are common for the two reactions, which must therefore have closely related reaction mechanisms. Because of the analogy with benzophenone, this mechanism was assumed to be ET. To test this theory the plot of log rate *versus* the anodic oxidation potential was made and a nice linear relation was observed except for *t*-butyl and benzyl, which were both too slow, Fig. 7.



Scheme 3.

The best interpretation must be that the rate controlling step is ET. Since benzyl reacts several hundred times more slowly than expected from the value of its anodic oxidation potential, an important role has to be assigned to the β-hydrogens. A six-center transition state seems indicated in which the β-hydrogen is very close to an azo-nitrogen, Fig. 8a. After the ET the tendency is high for the hydrazyl radical to abstract the close-by hydrogen atom and thereby

Table 5. Pseudo first-order rate constants and product distributions (see text) for the reaction of 0.1 M azobenzene with 0.5 M alkylmagnesium bromide in diethyl ether at 20 °C. Number of experiments given in parantheses.

Alkyl	% Addition product	k_{obs} s^{-1}
CH_3	14	0.5×10^{-5} (3)
C_2H_5	10	1×10^{-2} (3)
i-C_3H_7	14	1 (1)
C_4H_9	15	2×10^{-3} (3)
i-C_4H_9	22	1×10^{-4} (3)
s-C_4H_9	12	6×10^{-1} (1)
t-C_4H_9	55	5×10^{-2} (1)
C_3H_5 (allyl)	100	120 (1)
$C_6H_5CH_2$	57	3×10^{-4} (3)
C_6H_5		1×10^{-5} (1)
$CH_3CH{=}CHCH_2MgBr$	100	13 (1)
$CH_3CH{=}CH{-}CH(MgBr)CH_3$	100	50 (1)

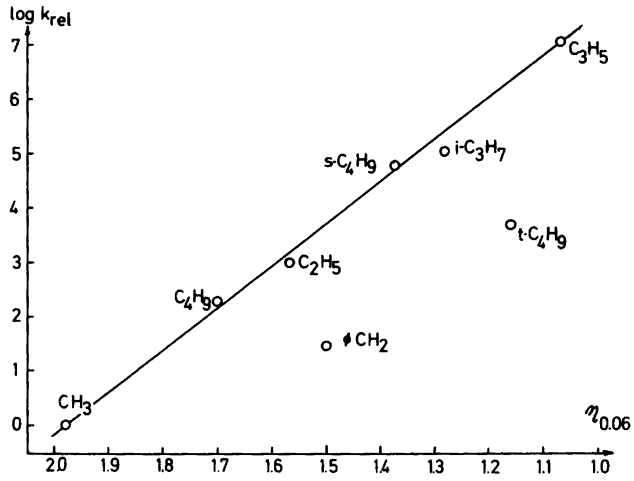


Fig. 7. Log (k_{RMgBr}/k_{CH_3MgBr}) for the reaction of Grignard reagents with azobenzene (Table 5) *versus* the anodic oxidation potential of the Grignard reagent. See text.

producing the primary cage product. But in spite of the proximity of the hydrogen, the immediate collapse may fail and a collapse to form addition product is then possible, leading to a secondary cage product. Because of a rather low affinity of the hydrazyl radical for alkyl radicals, the latter may also diffuse out of the cage and attack unreacted azobenzene, which may lead to the various escape products.

The geometry for the transition state for the ET taking place with benzophenone and Grignard reagents must be different from that of the azobenzene-ET, since benzyl has the expected reactivity in the first-mentioned reaction. A four-center transition state is therefore indicated, in which case the 1,2-addition product is the primary cage product, Fig. 8b. A study of the kinetic isotope effect of deuterium in the β-positions of the Grignard reagent, however, seems to indicate that reactions in which reduction product is formed have a higher value of k_H/k_D than reactions in which addition predominates, Table 6. It seems that six-center and four-center ET are of almost the same efficiency toward benzophenone and that one may substitute the other according to the steric require-

Table 6. Relative yield (%) of benzhydrol in the reaction of Grignard reagents with benzophenone in diethyl ether at 20 °C. Kinetic isotope effect k_H/k_D for the overall reaction by the introduction of deuterium in the β-position(s) of the Grignard reagent. The effect given is the estimated value for 100 % substitution based on rate measurements with a reagent of the stated degree of deuteration.

Grignard reagent	[Benzophenone] M	k_H/k_D	% benzhydrol	% deuteration
0.50 M C_2H_5MgBr	0.02	1.01	6	95
0.20 M C_4H_9MgBr	0.02	1.28	55	97
0.50 i-C_3H_7MgBr	0.02	1.16	20	92
0.25 i-C_4H_9MgBr	0.02	1.46	91	95
0.26 M *t*-C_4H_9MgCl	0.02	1.0	0	64
0.02 M i-C_3H_7MgBr	0.25	2.5		92
0.02 M i-C_4H_9MgBr	0.25	2.5	95.5	95
0.02 M *t*-C_4H_9MgCl	0.25	1.0		64
0.10 M c-C_5H_9MgBr	0.02	1.67	100	75
0.02 M c-C_5H_9MgBr	0.25	2.8	100	75

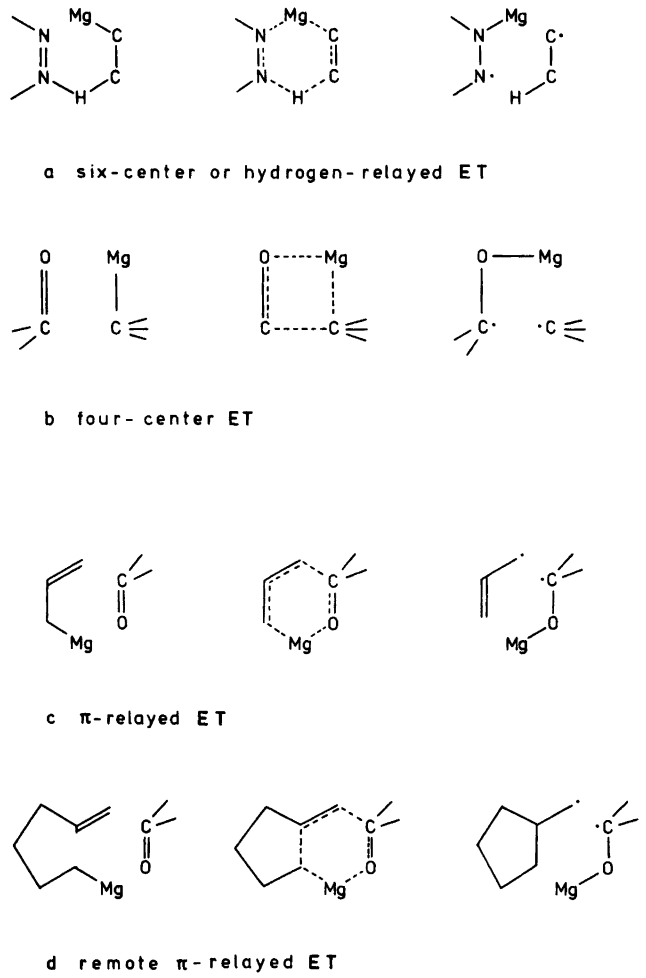


Fig. 8. Geometry of the transition states for electron transfer from Grignard reagents to various substrates.

ments without any significant effect on the rate. The six-center ET is not possible in the case of methyl- benzyl-, or *t*-butylmagnesium halide reacting with either benzophenone or azobenzene. For this reason methyl, benzyl, and *t*-butyl are out of line in the reactions with azobenzene, which is not well-suited for the four-center ET. With benzophenone these reagents have the expected reactivities reacting by a four-center ET. Both with azobenzene and benzophenone the primary cage products for methyl, benzyl and *t*-butyl reagent are 1,2-addition products. Isobutylmagnesium bromide is not suited for a four-center ET, but has a β-hydrogen. It is therefore well-behaved in the reaction with azobenzene (six-center ET) and the six-center ET in the reaction with benzophenone is only slightly less effective than required according to its oxidation potential. A less hindered primary Grignard reagent reacts by four-center ET with benzophenone and by six-center ET with azobenzene yielding 1,2-addition product and reduction product, respectively, as the primary cage product.

Four-center and six-center reaction mechanisms for Grignard reagents are not new. Especially a six-center concerted mechanism for the Grignard reduction, Fig. 9, has been widely

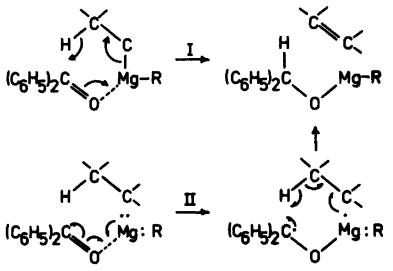


Fig. 9. I. Cyclic concerted mechanism for the reduction of benzophenone with isobutylmagnesium bromide. II. Stepwise electron transfer mechanism for the same reaction.

accepted after being suggested by Whitmore.[30] Many observations of asymmetric induction, when reduction of ketones is performed using an asymmetric reagent have been rationalized on the basis of the Whitmore mechanism.[31] Also rather refined experiments have shown that a *cis* planar arrangement of magnesium, α- and β-carbon, and β-hydrogen of the reducing Grignard reagent is favourable, or even necessary for the reaction.[32] It seems, however, that the *cis* planar arrangement may just as well be a requirement for the six-center ET, Fig. 8a, as for the Whitmore mechanism.

One may wonder why a stepwise ET should be preferred to a six-center concerted reaction. The question probably should be answered by a consideration of orbital symmetry rules for compounds with highly polar bonds. The simple Whitmore reduction mechanism may just not be allowed. A closely related question would be: why do we so often get secondary cage products as *e.g.* 1,6-addition with benzophenone or 1,2-addition with azobenzene? It might be a result of the geometry of the transition state not being optimal for bonding. Possibly the one electron transfer does not require an approach nearly as close as does actual C–C or C–H bonding.

The possibility that spin correlation in the radical pair is a factor of importance for the product distribution has been considered. According to the theory developed for the CIDNP phenomena it has been argued that combination of radical pairs may be under the influence of magnetic fields,[33] and that the product distribution in the reaction of benzyl chloride and butyllithium may be changed by the application of a magnetic field.[34] The reaction between azobenzene and butylmagnesium bromide was performed in a 15 000 G magnetic field with no clear-cut effect on the product distribution.[29] Attempts to reproduce the results obtained with benzyl chloride and butyllithium were, however, also unsuccessful, since the magnetic field did not change the product distribution significantly.[29]

Reaction of ethyl cinnamate with t-butylmagnesium chloride. Escape products. The "normal" reaction products from the addition of a Grignard reagent to an α,β-unsaturated ester are the 1,4- and the 1,2-adducts. When reacting ethyl cinnamate with *t*-butylmagnesium chloride, Crossland,[35] however, obtained the 1,3-adduct in very significant yields, Fig. 10.

In a study of the mechanism of this reaction, free *t*-butyl radicals were found to play an important role.[36] It was found possible to suppress the 1,3-addition by the addition of α-methylstyrene, which acted as a free radical scavenger. The formation of 1,4-adduct was not influenced by the addition of scavenger. It was concluded that the reaction is initiated by ET from the Grignard reagent to the ester and that because of low affinity between the *t*-butyl radical and the ester anion radical, a large fraction of the radicals diffuses out of the cage, Scheme 4. The affinity of the *t*-butyl radical toward ethyl cinnamate is higher than that toward other species in the reaction mixture, and addition takes place with formation of a neutral benzylic radical. This radical accepts an electron from the ester anion radical, and after transfer of the magnesium ion, the 1,3-adduct is formed. Since the adduct is an ester and a Grignard reagent, it undergoes cyclization and a cyclopropanone hemiketal salt is produced.

Factors which influence the ratios between primary/secondary cage products and escape products. From the experience obtained with

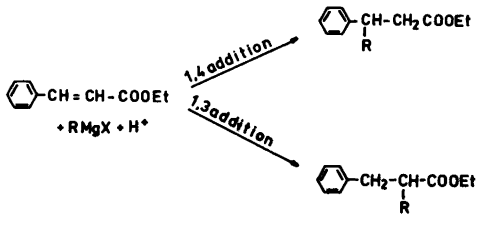
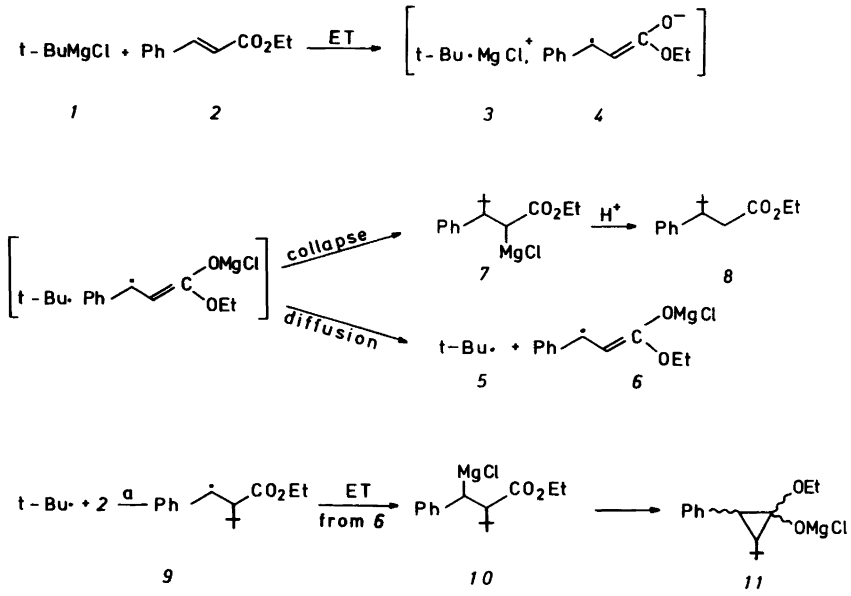


Fig. 10. Products obtained in the reaction of *t*-butylmagnesium chloride with ethyl cinnamate.



Scheme 4.

benzophenone, azobenzene and ethyl cinnamate, one is left with the impression that the most important factor ruling the distribution of products in an ET reaction of a Grignard reagent is the affinity of the substrate anion radical toward the alkyl radical. The benzophenone ketyl has a rather high affinity for the free radicals and the amount of escape products is usually below 10 % for *t*-butyl reagents, and much lower for less hindered alkylmagnesium halides. On the other hand, with varying substitution in the benzophenone the ratio between the primary and the secondary cage product may vary almost between 0 and 100 %, Table 1.

Anion radicals of ethyl cinnamate and azobenzene are much less reactive and escape products account for large fractions of the reaction products. At the same time secondary cage products have been of little importance in the examples investigated.

Important for which type of escape products is formed is, for instance, the affinity of the unreacted substrate toward alkyl radicals. The reactivity series is here α-methylstyrene>ethyl cinnamate>azobenzene≫benzophenone. Anion radical dimerization is fast in benzophenone ketyl, and very slow with the anion radicals from azobenzene and ethyl cinnamate.

Dimesityl ketone is highly hindered and the primary cage product, the 1,2-adduct, is not formed. The caged radical pairs, however, predominantly collapse in a 1,6-fashion to form the secondary cage product when the Grignard reagent is secondary or tertiary, or the reduction product if the alkyl is primary.[37] A smaller fraction diffuses out of the cage and since the ketyl is sterically hindered toward pinacol formation, the stable magnesium ketyl free radical is the escape product.

In a recent publication,[38] dimesityl ketone was reported to yield with Grignard reagents stable pairs of anion radical/cation radical. The evidence was ESR spectra and reactivity series based on observation of the rate of formation of absorption at 579 mm. It was shown, however, that this absorbtion is due to the ketyl and that if transition metal impurities are avoided no colour is developed in the reaction with primary Grignard reagents.[37]

Radical probes in electron reactions of Grignard reagents. The ET mechanism requires a finite lifetime as a free radical for the alkyl of the Grignard reagent. ESR experiments have shown beyond any doubt the presence of ketyl radicals, while only very vague indications have been obtained by this method as evidence for the

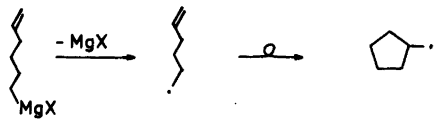


Fig. 11. Cyclization of the 5-hexenyl radical as obtained by homolysis of 5-hexenylmagnesium halide.

existence of alkyl radicals in the reaction mixture. Although the evidence from free radical by-products and from rate correlations proves that Grignard reactions with benzophenone and azobenzene run *via* ET, an analysis is required of the details of the ET and the fate of the ion radicals, which are the immediate products of the electron transfer.

A promising approach to the problem seems to be the use of radical probes in the form of Grignard reagents, in which the alkyl is capable of very fast rearrangement, if it is released in its free state with an unpaired electron. The 5-hexenyl radical has been shown to cyclize to cyclopentylmethyl radical with a rate constant of 10^5 s^{-1},[39] Fig. 11. In the reaction of the stable 5-hexenylmagnesium bromide with oxygen, Lamb *et al.*[40] obtained significant amounts of cyclopentanemethanol, which indicated a free radical chain mechanism. Ashby and Bowers [41] used this probe in the reaction with benzophenone. With the simple 5-hexenylmagnesium bromide they obtained equal amounts of benzhydrol and uncyclized, 1,2-addition product, but with a tertiary reagent, 1,1-dimethyl-5-hexenylmagnesium bromide, they obtained a cyclized 1,6-addition product and a 1,2-addition product, which was practically uncyclized, when ether was the solvent, but which was up to 40 % cyclized in THF, Fig. 12. With azobenzene in ether, even the primary 5-hexenylmagnesium bromide gave a cyclized 1,2-addition product and also a cyclized 1,6-addition product.[29] Ashby and Bowers also prepared a neopentyl-like reagent, 2,2-dimethyl-5-hexenylmagnesium bromide, and with this primary Grignard reagent they obtained a 10 % yield of cyclized 1,2-addition product in the reaction with benzophenone in ether.

In order to interpret these results, Ashby and Bowers suggest that the alkyl radicals when formed by ET are still bound to the magnesium ion. These magnesium cation radicals are supposed to be stable in the open chain state. They may in time either collapse to form uncyclized 1,2-addition product or they may by dissociation from magnesium form genuine free radicals, which rearrange and add to the ketyl in the 1,6- or the 1,2-fashion.

A serious objection may be raised against this interpretation. It is known that the rate constant of cyclization of the hexenyl radical is $\simeq 10^5$ s^{-1}. This does not leave time for the radical to rearrange while in the cage. In reactions, which like the autoxidation of Grignard reagents have a chain mechanism, the radicals live long enough for the rearrangement to take place, especially if the concentration of oxygen is kept low, but in the reaction with benzophenone both 1,2- and 1,6-addition products are formed within the cage, since scavengers like α-methylstyrene or fluorenone ketyl fail to interfere with the normal product distribution or reaction rate. It is difficult to give an explanation why the cage in this reaction should live several thousands times longer than normal caged radical pairs.[42]

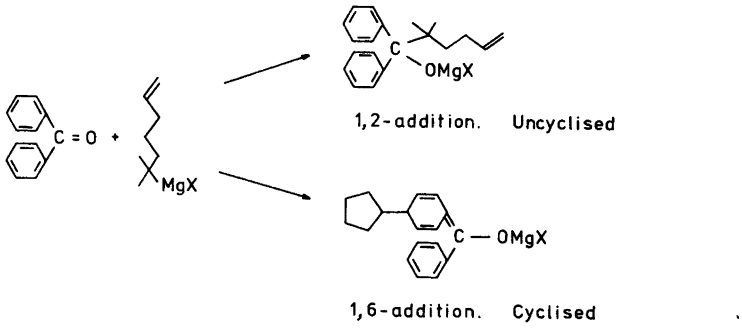


Fig. 12. Products obtained in the reaction of benzophenone with 1,1-dimethyl-5-hexenylmagnesium halide.

Since an alternative interpretation seems needed, one might imagine a "ring closing ET", Fig. 8d, which becomes a possible alternative when the more 'normal' ET types are blocked by steric hindrance. The electron donating ability of the Grignard reagent is caused by the high electron density at the α-carbon. By means of resonance this excess charge may be relayed either to the β-hydrogen, Fig. 8a, or to the γ-carbon of an allylic reagent, Fig. 8c. It seems possible that the electron pressure might also be relayed to the π bond of a remote double bond if a suitable conformation is obtainable, Fig. 8d.

A factor which would favour the relayed ET mechanisms is the low steric requirements, since the voluminous magnesium atom does not have to approach the substrate very closely. The collapse of the ketyl and the cyclized radical would be possible in a 1,2- or 1,6-fashion within the cage.

Transfer coefficients for ET from Grignard reagents. In the preceeding sections several examples have been described in which $\Delta G^{\neq}$ for the reaction of a Grignard reagent has been linearly correlated with ΔG for the reaction, the last quantity being measured as the anodic oxidation potential. The slope of the plot:

$$\Delta G^{\neq} = \alpha \Delta G + \text{constant}$$

is the symmetry or transfer coefficient, which for processes at electrodes is often between 0.3 and 0.7.

For oxidation of Grignard reagents at a platinum anode the Tafel plots [19] showed two different values for α since at low current densities and with alkyls, corresponding to unstable radicals, the value was 0.20, while at higher current densities and with alkyls forming stable radicals (benzylic, tertiary, secondary), the value was 0.45. The changeover from the lower value to the higher was seen with isopropyl at 5×10^{-4} amp cm^{-2} and with ethyl at 10^{-2} amp cm^{-2}. The change in the value of the transfer coefficient indicates a change in the mechanism of the electrode process.

For the reaction of benzophenone and azobenzene [29] the plots of log rate *versus* anodic oxidation potential for the various Grignard reagents have very different slopes, since the transfer coefficient for the reaction of azobenzene is 0.45, Fig. 7, while for the reaction of benzophenone, the transfer coefficient is only 0.23. Fig. 3. That the two values result from dissimilar reaction mechanisms seems obvious. A more difficult problem is whether the finding can give any support to the assignment of the six-center SET to the reaction with azobenzene and the four-center SET to the reaction with benzophenone. Electron transfer in organic chemistry and organometallic chemistry has been the object of much activity and theories have developed which allow predictions concerning the relations between the activation energy for a reaction and the difference in electrochemical potentials of the reactants.

The Marcus theory of ET reactions. The theory developed by Marcus and others for reactions having an ET as the rate determining step has in recent years successfully been applied to organic reactions and organometallic reactions.[44] The theory relates the reaction rate to two variables of which λ is the reorganization free energy on going from the ground state to the transition state and $\Delta G^{\circ\prime}$ is the driving force for the reaction. In a simplified form the theory requires that:

$$\Delta G^{\neq} = \frac{\lambda}{4}\left(1+\frac{\Delta G^{\circ\prime}}{\lambda}\right) \qquad (1)$$

$\Delta G^{\circ\prime}$ is available from measurements of standard oxidation potentials of Grignard reagents as described earlier in this review combined with the standard reduction potentials for the substrates,

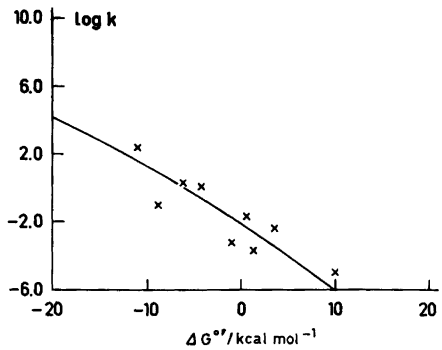


Fig. 13. Marcus plot (eqn. (1)) of rate data for the reaction between azobenzene and alkylmagnesium bromides (Table 5), using an electrostatic term of -10 kcal mol^{-1} to correct ΔG° values. The reduction potential of azobenzene was taken to be -1.12 V and approximate E° values of alkylmagnesium bromides were from Table 2.

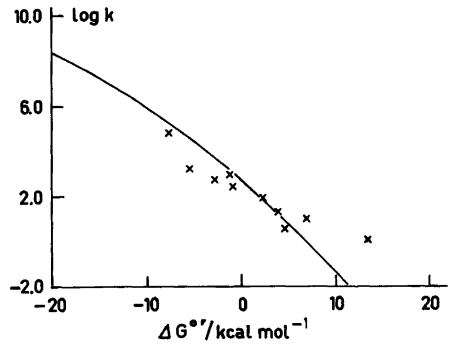


Fig. 14. Marcus plot (eqn. (1)) of rate data for the reaction between benzophenone and alkylmagnesium bromides (Table 3), using an electrostatic term of -15 kcal mol^{-1} to correct $\Delta G°$ values. The reduction potential of benzophenone was set equal to -1.48 V and approximate $E°$ values of alkylmagnesium bromides were from Table 2.

e.g. $E_o = -1.48$ V for benzophenone and -1.12 V for azobenzene. Using the rate data in Table 3 and Table 5, Professor L. Eberson has kindly performed a series of calculations to find the λ values which give the best possible fit of the rate data to the Marcus parabolae (1). Because of the low dielectric constant of the ether solvent, a correction in $\Delta G°'$ had to be added for the change in electrostatic energy at the moment of electron transfer. For azobenzene the correction used was -10 kcal mol^{-1} and the best fit of the data was obtained for a λ value of 70(5) kcal mol^{-1}, Fig. 13. For benzophenone an electrostatic term of -15 kcal mol^{-1} was used and the best fit was obtained for a λ of 44(5) kcal mol^{-1}, Fig. 14.

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Synthesis of the Heteroaromatic Selenatriazole Ring System. 5-Amino-1,2,3,4-selenatriazoles

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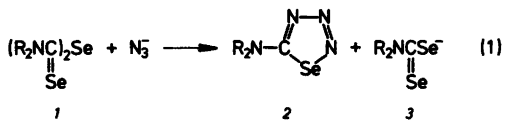
The synthesis of 5-amino-1,2,3,4-selenatriazoles, derivatives of the hitherto unknown selenatriazole ring system, is described. Reaction between bis(*N,N*-disubstituted selenocarbamoyl)selenides and azide ion gives the title compounds in good yield. The reactions of 4,4-dialkylselenosemicarbazides with nitrous acid or an aza-transfer reagent also lead to aminoselenatriazoles. Disubstitued aminoselenatriazoles are thermally unstable decomposing with formation of disubstituted cyanamides, nitrogen and selenium. 5-(Diethylamino)selenatriazole (half-life *ca.* 180 h in $CHCl_3$ at 20 °C) is thermally more stable than 5-(methylphenylamino)selenatriazole. 5-(Alkylamino)selenatriazoles decompose to hydrazoic acid and an isoselenocyanate, and evidence for their formation was only obtained indirectly.

5-Substituted 1,2,3,4-selenatriazoles are hitherto unknown. A report in the literature indicates that they may be highly unstable.[1] Thus stable salts of 1,2,3,4-thiatriazole-5-thiol are formed by reaction of inorganic azides with carbon disulfide while it is found that carbon diselenide reacts with sodium azide in aqueous or aqueous–alcoholic solution with immediate precipitation of red selenium even at −20 °C. It was concluded that *if* a selenatriazole is formed in this reaction it must be extremely unstable.

Introduction of an amino group in the 5-position of the thiatriazole ring causes a significant stabilization decreasing the rate of thermal decomposition leading to nitrogen, sulfur and an organic fragment.[1,2] A similar approach toward the selenatriazole system thus appeared promising. We now report that certain 5-amino-1,2,3,4-selenatriazoles actually can be prepared although they are thermally less stable than the sulfur analogues.

The formation of *N,N*-disubstituted 5-amino-1,2,3,4-selenatriazoles has been investigated by three different reactions. *a.* Reaction between bis(*N,N*-disubstituted selenocarbamoyl)selenides [3] (*1*) and azide ion. *b.* Reaction between 4,4-dialkylselenosemicarbazides and an aza-transfer reagent. *c.* Direct nitrosation of 4,4-dialkylselenosemicarbazide.

Path *a.* The reaction between ammonium azide and bis(*N,N*-diethylselenocarbamoyl)selenide (*1*, R=Et) in methanol proceeds smoothly at room temperature within 24 h according to eqn. 1.



(1)

The diselenocarbamate (*3*) undergoes a facile oxidation by air to give monoselenide *1* and bis(*N,N*-diethyl diselenocarbamato)selenium-(II) [3] (*4*) (eqn. 2). The monoselenide reacts to give additional selenatriazole (*2*) while the triselenide *4* is inactive towards azide ion. Thus 2/3 of the selenocarbamoyl units of *1* may theoretically be converted into *2*. Essentially pure 5-diethylamino-1,2,3,4-selenatriazole has been obtained in 85 % of the theoretical yield.

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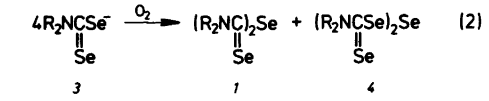
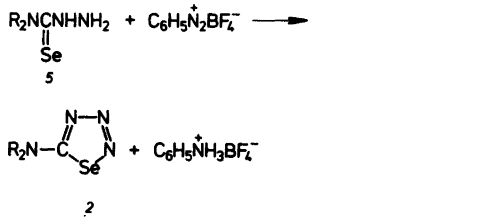


(2)

5-(Methylphenylamino)-1,2,3-selenatriazole was prepared in the same manner but it is apparently less thermally stable and was isolated in mixture with its decomposition product, methylphenylcyanamide (ratio 1:3).

Path *b*. The aza-transfer reaction, with benzenediazonium tetrafluoroborate or diazotized sulfanilic acid, has recently been investigated with thiosemicarbazides and found to be an alternative method for the preparation of 5-amino-1,2,3,4-thiatriazoles.[4] In the present investigation the reaction between benzenediazonium tetrafluoroborate and 4,4-diethylselenosemicarbazide (*5*) was investigated (eqn. 3) and found to give *2* in a slightly lower yield (70 %) and in less pure state than obtained *via* path *a*.

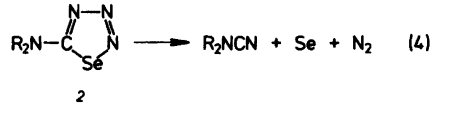


(3)

MS showed the presence of diphenyldiselenide and triselenide as well. The process by which the diphenyl selenides is formed has not been investigated.

Direct nitrosation (path *c*) of 4,4-diethylselenosemicarbazide proceeds with immediate evolution of gases indicating undesired side-reactions and *2* was formed in a low yield (20–30 %) as calculated from ^{1}H NMR spectra.

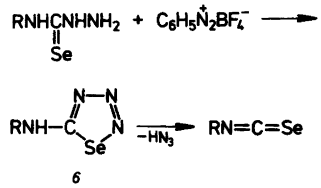
5-Diethylamino-1,2,3,4-selenatriazole is an oil decomposing slowly at room temperature with formation of diethylcyanamide and elemental selenium (eqn. 4). An approximate half-life of 8 days in chloroform at room temperature has been estimated.



(4)

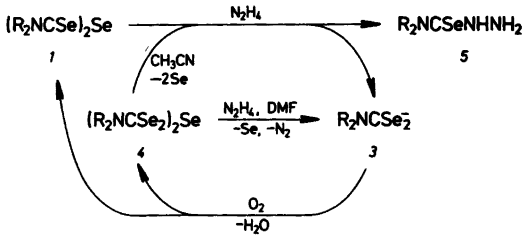
Compound *2* (R=Et) is sufficiently stable to be purified by means of column chromatography but an elemental analysis could not be performed because of its explosive decomposition when heated in oxygen. The structure assignment of *2* is in agreement with MS, ^{1}H NMR and IR analysis (experimental section). The thermal decomposition is similar to that of aminothiatriazoles which gives rise to cyanamides, sulfur and nitrogen although thiatriazoles decompose at a lower rate.[2,3]

The formation of *N*-monosubstituted 5-amino-1,2,3,4-selenatriazoles was investigated by the reaction between 4-benzyl- and 4-cyclohexylselenosemicarbazides and benzenediazonium tetrafluoroborate in methanol (path *b*). With 4-cyclohexylselenosemicarbazide at 0 °C immediate gas evolution and formation of red selenium was observed. An oil was isolated from the reaction mixture which according to GLC/MS consists of a mixture of cyclohexylisoselenocyanate and diphenylselenide. This result indicates formation of 5-cyclohexylamino-1,2,3,4-selenatriazole (*6*, R=cyclohexyl) decomposing with formation of hydrazoic acid and isoselenocyanate (eqn. 5) although attempts to isolate the selenatriazole or observe it by means of ^{1}H NMR were unsuccessful. Monosubstituted 5-aminothiatriazoles undergo a similar decomposition in the presence of base with formation of azide ion and an isothiocyanate.[1] 4-Benzylselenosemicarbazide reacted with benzenediazonium tetrafluoroborate to give a mixture of products among which benzylisoselenocyanate was shown to be present. Again attempts to isolate the selenatriazole were unsuccessful.



(5)

The 4,4-disubstituted selenosemicarbazides were prepared from bis(*N,N*-disubstituted selenocarbamoyl)selenides, *1* [3] and hydrazine in the presence of air. Both selenocarbamoyl moieties of *1* may be utilized when an excess of hydrazine is employed. Thus yields up to 1.4 mol of *5* per mol of *1* have been realized. The reaction is rather complex and can involve two independent reaction cycles as outlined in Scheme 1.



Scheme 1.

The initial step appears to be a nucleophilic substitution on *1* giving one mol of *5* and one mol of diselenocarbamate ion *3*. The subsequent air oxidation of *3* to give a 1:1 mixture of *1* and bis-(*N,N*-dialkyldiselenocarbamato)selenium(II) (*4*) is well documented (see Ref. 3 and refs. cited). The two routes from *4* to *3* were demonstrated when *4* in the absence of oxygen was treated with hydrazine. In DMF as solvent rapid evolution of nitrogen was noted and 1 mmol of *4* (R=Et) yielded 1 mmol of Se together with *3* which was trapped as the *Se*-benzyl ester ($Et_2N-CSe_2-CH_2Ph$, 1.64 mmol). When the same reaction was performed in acetonitrile 2 mmol of Se was produced and evaporation of solvent from the filtrate left a yellow solid which according to ^1H NMR and elemental analysis was a 1:1 mixture of *5* and the hydrazinium salt of *3*. The theoretical stoichiometries of the two reactions are given as eqns. 6 and 7, respectively.

$$4(R_2NCSe)_2Se+10N_2H_4+4O_2 \xrightarrow{DMF} 8R_2NCSeNHNH_2+4Se+2N_2+8H_2O \quad (6)$$

$$4(R_2NCSe)_2Se+8N_2H_4+2O_2 \xrightarrow{CH_3CN} 8R_2NCSeNHNH_2+4Se+4H_2O \quad (7)$$

This solvent dependence may be explained in terms of the structure of *4* which is intermediary between a coordination compound of selenium(II) and a covalent triselenide.[6] Thus, in the better solvating medium, DMF, *4* behaves like a selenium(II) species and oxidizes hydrazine while in the poorer solvating acetonitrile the covalent character of *4* predominates and it undergoes nucleophilic substitution in analogy with *1*.

EXPERIMENTAL

^1H NMR spectra were obtained with a Varian T-60A instrument ($CDCl_3$ and CCl_4; $SiMe_4$ as internal standard). IR spectra were obtained with a Perkin-Elmer 157P infrared spectrophotometer and UV spectra on a Unicam SP 1800 instrument. GLC-MS were obtained with a VG Micromass 7070 instrument operated at 70 eV with an ion source temperature at 220 °C in combination with a 5 % OV 101 column.

4-Monosubstituted selenosemicarbazides were prepared according to known methods.[5]

Preparation of 4,4-diethyl selenosemicarbazide. Monoselenide *1* (1 mmol) was dissolved in acetonitrile (100 ml) hydrazine hydrate (3 mmol) added and the mixture left with stirring for 24 h. The mixture was filtered through a silicone treated filter or a layer of a porous material ($MgSO_4$) to remove elemental selenium. Toluene (25 ml) was added and the solution concentrated *in vacuo* at 20 °C to *ca.* 10 ml. The solution was transferred to a centrifuge tube, cooled in acetone–dry-ice and the precipitated crystals isolated by centrifugation and dried over conc. sulfuric acid in a desiccator. *4,4-Diethylselenosemicarbazide,* 76 % yield, m.p. 85–88 °C. Found: C, 30.96; H, 6.84; N, 21.87. Calc. for $C_5H_{13}N_3Se$: C, 30.77; H, 6.67; N, 21.54.

Preparation and properties of 5-diethylamino-1,2,3,4-selenatriazole. Path a. Bis(*N,N*-diethylselenocarbamoyl)selenide (*1*) (1 mmol) was suspended in methanol (20 ml), ammonium azide (2 mmol) was added and the mixture left for 24 h at room temperature with stirring and protected against light. Water (20 ml) was added and the precipitated triselenide removed by filtration. The solution was saturated with sodium chloride, transferred to a separating funnel and extracted with ether (2×10 ml). The combined ether extracts were filtered through a silicone treated filter to remove elemental selenium dried over $MgSO_4$ at 0 °C followed by evaporation of the solvent *in vacuo* at 0 °C and protected against light. 85 % yield, oil. MS: 206.0122 ($M^{\cdot+}$, 0.62 %, calc. for $C_5H_{10}N_4Se$: 206.0070), 178 ($M^{\cdot+}-N_2$, 1.9 %), 122 ($C_2H_5CHSe^{\cdot+}$, 2.4 %), 98 ($(C_2H_5)_2NCN^{\cdot+}$, 100 %), 83 ($C_2H_5N(=CH_2)CN^+$, 80 %), 70 (C_2H_5NHCN, 11 %), 69 (9.2 %), 55 (54 %), 42 (3.8 %), 41 (4.6 %). NMR ($CDCl_3$), δ 1.40 (CH_3), 3.68 (CH_2) IR: Lack of azide band in the heterocumulene region (*ca.* 1800–2400 cm^{-1}). UV (CCl_4): λ 278 nm, ε 5875±2 %. The decomposition of the selenatriazole in CCl_4 at room temperature to diethylcyanamide was monitored by NMR and a half-life of 8 days was calculated. Diethylcyanamide was identified by comparison with authentic

material. The selenatriazole may be submitted to column chromatography (silica gel) with ether as eluent.

Preparation of 5-methyl(phenyl)amino-1,2,3,4-selenatriazole. Bis(methyl(phenyl)selenocarbamoyl)selenide (0.25 mmol) was dissolved in methanol–dioxane (1:1), ammonium azide (1.66 mmol) was added and the mixture left for 24 h at 0 °C. The solvent was removed *in vacuo* with protection against light. The remains were extracted with ether, followed by drying over $MgSO_4$ at 0 °C, and the solvent removed *in vacuo* at 0 °C to give an oil. NMR (CCl_4): Bands are observed at δ 3.34 and 3.66 (ratio *ca.* 3:1) and around δ 7.0–7.5 (phenyl). The band at δ 3.66 gradually disappears at room temperature and is tentatively assigned to 5-methyl(phenyl)amino-1,2,3,4-selenatriazole. The cyanamide was identified by comparison with authentic material.

Aza-transfer reaction. Path b. The mono or disubstituted selenosemicarbazide (0.1 mmol) was suspended in methanol (10 ml) cooled to 0 °C and protected against light. Benzenediazonium tetrafluoroborate (0.11 mmol) was added gradually as a solid or added slowly as a methanol suspension. The reaction mixture was stirred for 30 min, water (20 ml) was added, the solution was saturated with sodium chloride, transferred to a separating funnel and extracted with ether (10×2 ml). The combined ether extracts were dried over $MgSO_4$ at 0 °C and filtered through a silicone treated filter to remove elemental selenium. The solvent was removed *in vacuo* at 0 °C.

Diazotized sulfanilic acid prepared according to literature [4] may be used instead of benzenediazonium tetrafluoroborate. It is used in 10 % excess as a suspension in methanol.

5-Diethylamino-1,2,3,4-selenatriazole was prepared in this manner with benzenediazonium tetrafluoroborate in 70 % yield.

On attempted preparation of 5-cyclohexylamino-1,2,3,4-selenatriazole, an oil was obtained which exhibited a strong IR absorption ($CHCl_3$) at 2200 cm^{-1} indicating formation of cyclohexylisoselenocyanate. A GLC–MS investigation demonstrated the presence of cyclohexylisoselenocyanate (and absence of cyclohexylcyanamide) and of diphenylselenide while MS directly on the oil showed the presence of diphenyldiselenide as well. Cyclohexylisoselenocyanate is assumed to be formed from 5-cyclohexylamino-1,2,3,4-selenatriazole but we have not been able to demonstrate its presence in the oil obtained (IR and ^1H NMR).

A similar result was obtained on attempted preparation of 5-benzylamino-1,2,3,4-selenatriazole. The isolated oil was shown to contain benzylisoselenocyanate identical (TLC and ^1H NMR) with an authentic sample.

Acknowledgement. We thank Dr. J. Øgaard Madsen for recording the GLC-mass spectra.

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Photocyclisations of Unsaturated [2$_4$]Paracyclophanes

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The photocyclisation of [2$_4$]paracyclophanes with one or two unsaturated bridges, compounds *7* and *3*, gives [2$_3$](3,6)phenanthrenodiparacyclophane, *8*, and [2$_2$](2,13)pentahelicenoparacyclophane, *4*, respectively. In contrast, [2$_4$]paracyclophanetriene, *1*, does not give the heptahelicene derivative *2* on irradiation. The introduction of iodosubstituents in [2$_4$]paracyclophanetetraene allows for successful photocyclisations under nitrogen.

The photocyclisation of stilbenes under oxidative conditions to give phenanthrenes is one of the most studied and widely used organic photoreactions.[1–3] It has been applied to the synthesis of condensed aromatic systems and has proved especially useful for the synthesis of helicenes [4] and bridged annulenes.[5] In this paper we report an investigation of the photocyclisation of [2$_4$]paracyclophanes with one, two or three bridging double bonds as well as that of some iodosubstituted [2$_4$]paracyclophanetetraenes.

As previsouly reported, [2$_4$]paracyclophanetetraene, *13*, slowly decomposes on irradiation in the presence of air and iodine.[6] Mass spectra of the product mixture showed the presence of aromatic aldehydes, probably formed by photooxidation of the double bonds. Other cyclophanes, closely related to *13*, also decomposed on irradiation [7] and an attempt to use tetracyanoethylene as a hydrogen acceptor, as has been proposed,[8] was also unsuccessful. There might be several reasons for the failure of these photocyclisation. One is that the products might be photolabile and decompose as soon as they are formed, and this is clearly a risk for the more strained products when the irradiation is performed in the presence of air. However, all unsuccessful photocyclisations showed a slow disappearance of the starting materials rather than fast formation of decomposition products and, in at least one case, we were indeed able to prepare the desired product, bi-2,13-pentahelicenylene, by a similar photocyclisation.[9]

Unfavourable electronic effects could be another reason for the unsuccessful photocyclisation. [2$_4$]Paracyclophanetetraene, *13*, as well as (2,5)-furan, (2,5)-thiophene or (4,4′)-biphenyl analogues all contain a perimeter of conjugated π-electrons. The delocalisation of the π-electrons around the ring, which affects the UV and NMR spectra of these compounds and also their electrochemical behaviour on reduction to the anion radicals [10] and dianions,[11] could also effect the photocyclisation. Interruption of the conjugated π-electron system around the perimeter by hydrogenation of some of the double bonds in [2$_4$]paracyclophanetetraene, *13*, would make it possible to test this hypothesis.

During this work it also became clear that steric requirements for the photocyclisations must be considered. It is generally accepted that the photochemical formation of a dihydrophenanthrene from a *cis*-stilbene is a conrotatory process which leads to *trans* orientation of the two inner hydrogens in the product.[12] The proper conformation of the reactant is that of *cis*-stilbene with a twofold axis of symmetry perpendicular to the double bond (Scheme 1). The reaction is reversible and considered to occur in the singlet state.[1,12] The barrier of activation for the cyclisation of excited *cis*-stilbene [1] and *cis*-1,2-(2-naphtyl)ethylene [13] has been determined. In the latter case, the barriers are different for the three

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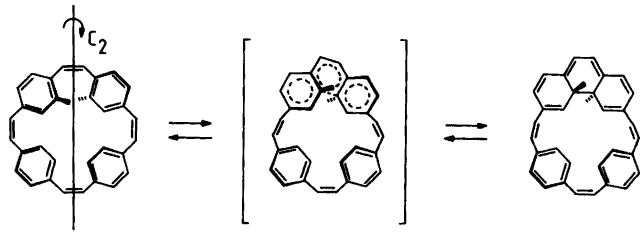
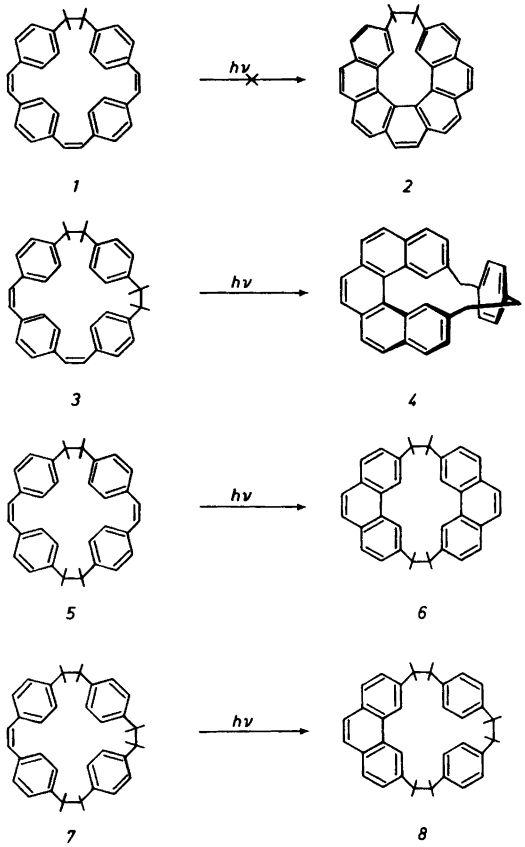
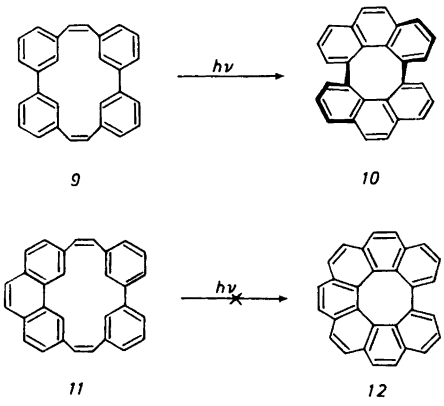


Scheme 1.

possible photocyclisations, and this can be rationalized as being due to steric effects.[14] Similarly, steric effects could be responsible for the failure of the photocyclisation of *13*. Successive saturation of the double bonds in *13* should result in more flexible cyclophanes and possibly decrease the contribution of steric effects to the barriers to cyclisation. Recently, we have been able to selectively reduce the double bonds in *13* by electrochemical reduction at constant



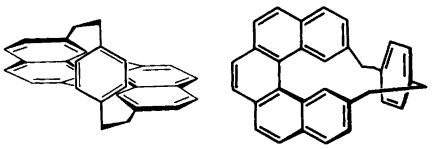


potential.[15] Thus, we have prepared [2$_4$]paracyclophanes with three double bonds, *1*, two double bonds, isomers *3* and *5*, and one double bond, *7*. The cyclophane *5* has also been prepared by a twofold Witting reaction from 4,4′-bibenzyldicarbaldehyde and the bistriphenylphosphonium salt from 4,4′-bis(bromomethyl)bibenzyl.[19]

RESULTS AND DISCUSSION

Photocyclisation of [2$_4$]*parabyclophanes.* Irradiation of [2$_4$]paracyclophanetriene, *1*, led to slow decomposition of the starting material. Apparently, cleavage of the cyclic π-electron system at only one site is not a sufficient condition for a successful photocyclisation in this series of cyclophanes.

[2$_4$]Paracyclophanediene, isomer *3* with the longer conjugated π-system, was converted nicely to a pentahelicene derivative, *4*, upon irradiation in cyclohexane at 254 nm. The structure of the product, [2$_2$](2,13)pentahelicenoparacyclophane, was determined from its UV, ^{1}H NMR and mass spectra. The UV spectrum is almost identical



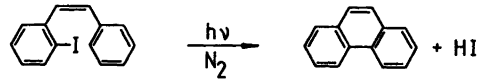
[2.2](2,13)Pentahelicenoparacyclophane *4*

with that of [5]helicene.[16] The chemical shifts for the protons in the pentahelicene part of the molecule are similar to those of the protons in [5]helicene, except for the inner protons which show an upfield shift of the signal by 1.2 ppm.[17] The shielding effect of the benzene ring (which must be essentially perpendicular to the pentahelicene part of *4*) is estimated to 0.5 ppm from the Bovey-Johnson equations.[18] Inspection of molecular models (CPK) of *4* reveals that the benzene ring must force the outer end of the pentahelicene apart, thereby increasing the angle and the distance between the inner hydrogens which results in decreased mutual deshielding. The protons in the bridges give rise to an ABCD spin system, and the shifts and coupling constants are consistent with a rather rigid structure.

The photocyclisation of the other isomer of [2_4]paracyclophanediene, *5*, gave [2_2](3,6)-phenanthrenophane, *6*, under the same conditions as above,the product being identical with that from hydrogenation of the corresponding diene *15*.[19]

Similarly, [2_4]paracyclophanemonoene, *7*, gives [2_3](3,6)phenanthrenodiparacyclophane, *8*, on irradiation. The ^{1}H NMR spectrum of the product shows a singlet and an AMX pattern for the phenanthrene protons, an AA'BB' pattern for the benzene protons and an AA'BB' pattern and a singlet for the protons on the bridges.

It has been suggested that the unsuccessful photocyclisation of *15* to *16* [20] and *11* to *12* [21] are due to the unfavourable *syn* conformations of the dienes. However, the closely related photocyclisation of *9* to *10* occurs readily.[19,22,23] The cyclophane *9* probably has a ground state conformation of D_2-symmetry,[19] and further approach of the benzene rings towards each other in the two *cis*-stilbene units to form the new carbon–carbon bonds should be facile.

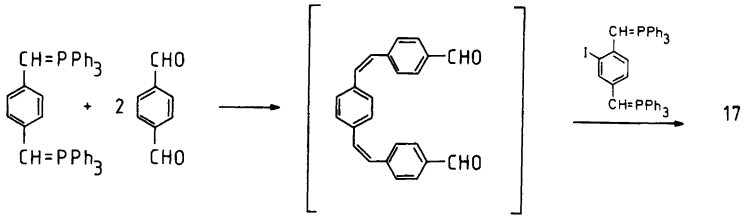


Scheme 2.

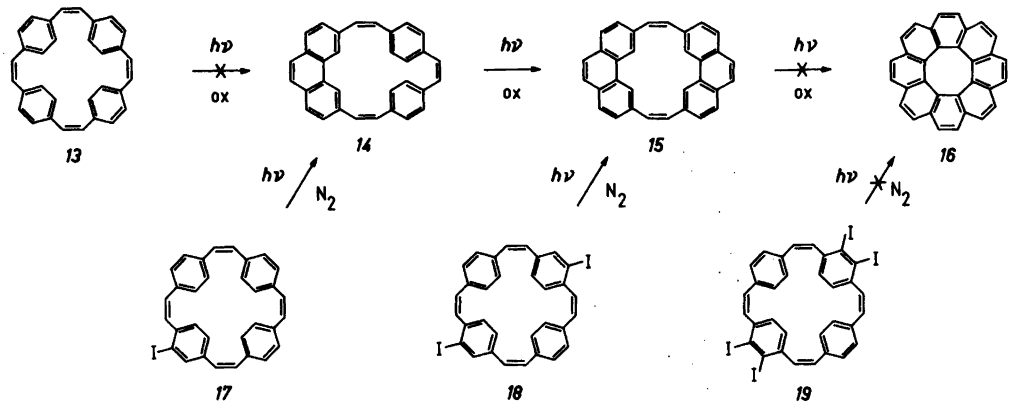
Photocyclisation of iodo-[2_4]*paracyclophanetetraenes.* As exemplified above, the photocyclisation of cyclophanes with *cis*-stilbene units is governed by rather subtle steric factors. Often, prolonged reaction times are needed which, due to the presence of air, can lead to photooxidation of the reactants and products. It may thus be advantageous to run the photocyclisation under an inert atmosphere, and this can be achieved if the cyclophane contains an iodine substituent adjacent to the double bond (see also Scheme 2).[24]

Multiple Wittig reactions can be used for the convenient synthesis of iodo-[2_4]paracyclophanetetraenes. Previously, we have reported the synthesis and photocyclisation of a di-iodo[2_4]-paracyclophanetetraene, *18*, to give *15*, [2_2]-(3,6)phenanthrenophanediene.[20] Similarly, photocyclisation of di-iodo[2_4](4,4')biphenyl-paracyclophanetetraene gave bi-2,13-pentahelicenyylene.[9] We have found that it is possible to prepare iodo[2_4]paracyclophanetetraene, *17*, by a fourfold Wittig reaction in a one pot reaction sequence from two mol of benzene-1,4-dicarbaldehyde and one mol each of the bistriphenylphosphonium salts from 1,4-bis(bromomethyl)benzene and 1,4-bis(bromomethyl)-2-iodobenzene (Scheme 3).

On irradiation, the monoiodocyclophane *17* gives *15* in the presence of air. If nitrogen is



Scheme 3.

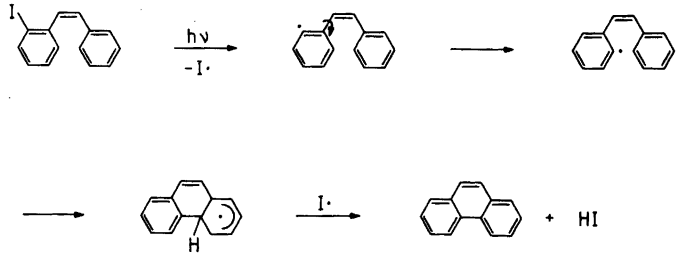


Scheme 4.

bubbled through the solution during the irradiation, the main product is [2_3](3,6)phenanthrenodiparacyclophanetriene, *14*. Hydrogenation of the product gives the saturated cyclophane *8*, described above.

We have also made an attempt to prepare [8]circulene, *16*, from a tetraiodo[2_4]paracyclophanetetraene, *19* (Scheme 4). The substitution pattern is set to ensure the proper location of the two iodines in the hypothetical intermediate (a di-iodo[2_2](3,6)phenanthrenophanediene) which might close to give [8]circulene. Unfortunately, this substitution pattern also favours the formation of benzyne derivatives.[24] The tetraiodo[2_4]-paracyclophanetetraene *19* was prepared from benzene-1,4-dicarbaldehyde and the bistriphenylphosphonium salt from 1,4-bis(bromomethyl)-2,3-di-iodobenzene by the usual Wittig reaction procedure. The bisphosphonium salt was prepared from 1,4-dimethylbenzene by a rather lengthy sequence of standard reactions. Irradiation of the cyclophane *19* under various conditions did not result in any detectable amount of [8]circulene, no product with the mass of 400 being observed by mass spectrometry.

Conclusion. Steric effects are of lesser importance in the photocyclisations of iodo[2_4]paracyclophanes than in photocyclisations of unsubstituted [2]paracyclophanes, which points to different mechanisms for the reactions. It is well-known that aryl iodides readily lose an iodine atom on irradiation,[24] and this is probably the first step in the photocyclisation of the iodocyclophanes discussed here. The hypothetical reaction sequence is shown in Scheme 5. For the photocyclisation of unsubstituted [2_4]paracyclophanes with bridging double bonds it is necessary to consider steric effects in the first excited singlet state and not only the ground state conformations. If the photocyclisation is too slow, or if the products are photo-oxidised, it should be advantageous to use a properly substituted iodo-stilbene-derivative.



Scheme 5.

EXPERIMENTAL

Melting points are uncorrected. UV spectra were recorded on a Beckman DK 2A, IR spectra on a Beckman IR 9, MS on an AEI MS 902 and NMR on a Bruker WH 270 instrument.

The [2$_4$]paracyclophanes *1*, *3*, *5* and *7* were prepared by selective electrochemical reduction of [2$_4$]paracyclophanetetraene, *13*, as previously described.[15]

Photocyclisations were performed using a Rayonet reactor (RPR-100) with low-pressure mercury lamps at 254 nm. Air was not excluded and catalytic amounts of iodine added in most reactions. The photoreactions were carried out in the standard water-coated quartz vessel in cyclohexane (spectroscopic grade) or benzene (A.R.) solution. The reactions were monitored by TLC and stopped when no starting material could be detected, usually after a few hours. The products were recrystallised from dichloromethane–methanol or sometimes purified by chromatography on silica gel.

[2$_4$]Paracyclophanediene, isomer *3*, (50 mg in 75 ml cyclohexane) gave after irradiation for 3.5 h at 254 nm *[2$_2$](2,13)pentahelicenoparacyclophane, 4* (29 mg, 60 %, m.p. 285 °C). UV [ethanol] (ε): 235 (81 500), 263 (sh), 271 (31 400), 293 (sh), 295 (31 600) nm. MS [IP 45 eV; *m/e* (% rel. int.)]: 408 (100, M^+), 303 (11), 302 (14), 301 (21), 276 (18). Abs. mass, obs. 408.187, calc. for $C_{32}H_{24}$ 408.188. ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.86 (2 H, d, H_4), 7.81 and 7.65 (4 H, dd, $H_{5,6}$, J 8.5 Hz), 7.60 (2 H, s, H_7), 7.38 (2 H, dd, H_3, J 8.5 and 1.8 Hz), 7.26 (2 H, d, H_1), helicene protons, 6.91 and 6.77 (4 H, AA'BB'-pattern, J 8.5 Hz) parasubstituted ring protons, 3.12 (2 H, dt), 2.93 (4 H, m), and 2.18 (2 H, dt), J:s 13.5, 13.5, 13.5, 4.5, 4.5, and 4.5 Hz, methylene protons.

[2$_4$]Paracyclophanediene, isomer *5* (50 mg in 75 ml cyclohexane) gave after irradiation for 5 h at 254 nm *[2.2](3,6)phenanthrenophanne, 6* (30 mg, 58 %, m.p. 335–340 °C). UV [cyclohexane (ε)]: 247 nm (105 000). MS [IP 68 eV; *m/e* (% rel. int.)]: 408 (100, m^+), 217 (41), 204.5 (5), 204 (63), 203 (13), 202 (25), 191 (32). Abs. mass, obs. 408.18±0.01, calc. for $C_{32}H_{24}$ 408.19. ^{1}H NMR (270 MHz, $CDCl_2$): δ 9.17 (4 H, broad s, H_4), 7.64 (4 H, d, H_1), 7.48 (4 H, s, H_9), 7.37 (4 H, dd, H_2, J 1.7 and 8.3 Hz) phenanthrene protons, 3.62 (8 H, s) methylene protons.

[2$_4$]Paracyclophanemonoene, *7* (50 mg in 75 ml cyclohexane) gave after irradiation for 3.5 h at 254 nm *[2.2.2](3,6)phenanthrenodiparracyclophane, 8*, MS [IP 60 eV; *m/e* (% rel. int.)]: 412 (100, M^+), 307 (11), 217 (13), 207 (20), 206 (16), 205 (28), 204 (23), 203 (12), 202 (13), 191 (25). Abs. mass, obs. 412.203, calc. for $C_{32}H_{28}$ 412.219. ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.80 (2 H, d, H_1) 7.63 (2 H, s, H_9), 7.53 (2 H, broad s, H_4), 7.48 (2 H, dd, H_2, J 1.5 and 8 Hz), phenanthrene protons, 6.82 and 6.60 (8 H, AA'BB'-pattern, J 8 Hz) parasubstituted ring protons, 3.04 and 2.95 (8 H, m), 2.94 (4 H, s) methylene protons.

[Iodo]2$_4$Paracyclophanetetraene, *17* (40 mg in 75 ml benzene) gave after irradiation, under nitrogen without addition of iodine, for 2 h at 254 nm *[2.2.2](3,6)phenanthrenodiparacyclophanediene, 14*,, after separation by preparative TLC in CCl_4 (13 mg, 41 %, m.p. 209–213 °C). MS [IP 60 eV; *m/e* (% rel. int.)]: 406 (100, M^+), 405 (10), 203 (12), and 202 (11). Abs. mass, obs. 406.171, calc. for $C_{32}H_{22}$ 406.172.

^{1}H NMR (270 MHz, $CDCl_3$: δ 8.16 (2 H, broad s, H_A), 7.87 (2 H, d, H_C), 7.69 (2 H, s, H_D), 7.47 (2 H, dd, H_B, J_{AB} 1 Hz, J_{BC} 8 Hz), 7.01 (4 H, d, H_E or $H_{E'}$), 6.86 (2 H, s, H_F), 6.75 (2 H, d, H_G or $H_{G'}$), 6.71 (4 H, d, H_E or $H_{E'}$l, $J_{EE'}$ 8 Hz) and 6.56 (2 H, d, H_G or $H_{G'}$, $J_{GG'}$ 12 Hz).

Some [2.2](3,6)phenanthrenophanediene, *15* (5 mg, 16 %, identified by comparison with authentic sample [20]) was also isolated.

The *Wittig reaction* was carried out as previously described.

Iodo[2$_4$]Paracyclophanetetraene, *17*, was prepared from 1,4-benzene dicarbaldehyde (10 mmol), the bisphosphonium salt from 1,4-bis-(bromomethyl)benzene (5 mmol), and the bisphosphonium salt from 2-iodo-1,4-bis(bromomethyl)benzene (5 mmol) in DMF at −40 °C. The dialdehyde and the unsubstituted bisphosphonium salt were mixed in DMF and lithium ethoxide in ethanol added slowly. When no further colour developed on addition of base, the iodo-substituted bisphosphonium salt was added, followed by more base. Work-up and chromatographic separation gave cyclophane *17* (180 mg, 7 %) together with small amounts of the unsubstituted and di-iodo [2$_4$]paracyclophanetetraenes. MS [IP 70 eV; *m/e* (rel. int.)]: 534 (100 %, M^+), 407 (12), 406 (16), 203 (12), 201 (15), 200 (16). Mol. wt., obs. 534.075±0.015, calc. for $C_{32}H_{23}I$ 534.086. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.75 (1 H, s, *ortho* to iodine), 7.34–7.13 (14 H, m, aromatic protons), 6.51–6.38 (8 H, m, olefinic protons).

Tetraiodo[2$_4$]paracyclophanetetraene, *19*. Benzene 1,4-dicarbaldehyde (3 mmol) and the bisphosphonium salt from 2,3-diiodo-1,4-bis-(bromomethyl)benzene (3 mmol) were reacted under the standard conditions to give the cyclophane *19* (90 mg, 6 %). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.25 (4 H, s), 7.12 (8 H, s) aromatic protons, 6.48 (4 H, d) and 6.38 (4 H, d, J 12 Hz)

olefinic protons. MS [IP 70 eV; *m/e* (rel. int.)]: 912 (M^+, 3 %), 785 (25), 658 (18), 531 (10), 406 (39), 405 (47), 404 (100), 403 (25), 402 (28), 401 (22), 400 (28), 399 (17), 398 (31), 387 (31), 374 (31), 203 (33), 202 (50), 201 (33), 200 (39), and 199 (33). Abs. mass, obs. 911.776; calc. for $C_{32}H_{20}I_4$ 911.775.

The bistriphenylphosphonium salt from 2,3-diiodo-1,4-bis(bromomethyl)benzene was prepared from *p*-xylene as follows. A mixture of isomers (216 g) of dinitro-*p*-xylenes, obtained by nitration of *p*-xylene, was partially hydrogenated in toluene–ethanol 1:1 with palladium on charcoal as catalyst. The formation of a yellow compound, 2-amino-3-nitro-*p*-xylene, was followed by TLC. The product was purified by column chromatography with chloroform as eluent. The first yellow fractions gave *2-amino-3-nitro*-p-*xylene* (36 g, 20 %, m.p. 69–71 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ 6.85 and 6.32 (2 H, dd, J 7.3 Hz), 5.14 (2 H, s), 2.36 (3 H, s) and 2.11 (3 H, s). 2-Amino-3-nitro-*p*-xylene (36 g) was further hydrogenated under the same conditions as above to give *2,3-diamino*-p-xylene, which was recrystallised from iso-butanol (15 g, 51 %). IR (KBr): 3370 (s), 3260 (s), 3100–2600 (s, broad band), 2570 (s), 1670 (s), 1625(s), 1475 (s), 1310 (s), 1100 (s), 1025 (s), 792 (s) cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_2$): δ 6.78 (2 H, s), 3.34 (4 H, s), and 2.25 (6 H, s). MS [IP 70 eV; *m/e* (rel. int.)]: 136 (M^+, 100 %), 135 (30), 121 (9), 119 (12), 118 (17), 108 (11) and 104 (11).

2,3-Diamino-*p*-xylene (1.36 g) and sodium nitrite (2 g) were mixed with sulfuric acid (conc., 25 and 20 ml, respectively) in two separate flasks. The former solution was slowly added to the latter while the temperature was kept below 0 °C. Phosphoric acid (25 ml) was then added to the mixture and the temperature kept below 10 °C. The reaction mixture was poured onto crushed ice (300 ml) and potassium iodide (8 g). The mixture was stirred overnight, extracted with chloroform (3×150 ml) and treated with sodiumbisulphite. The solvent was distilled off and the residue, a light yellow oil, was chromatographed with tetrachloromethane as eluant. The first fractions gave *2,3-diiodo*-p-*xylene* (1.4 g, 40 %, m.p. 20 °C). ^{1}H NMR ($CDCl_3$): δ 7.1 (2 H, s) and 2.5 (6 H, s). MS [IP 70 eV; *m/e* (rel. int.)]: 358 (M^+, 100 %), 231 (33), 127 (14), 104 (67), 103 (33). 2,3-Diiodo-*p*-xylene (3.58 g) was refluxed in tetrachloromethane with NBS (3.9 g) and dibenzoyl peroxide as initiator for 24 h. The hot solution was filtered. From the cooled filtrate, a white crystalline precipitate was collected and purified by column chromatography with tetrachloromethane as eluent. The major fractions yielded *2,3-diiodo-1,4-bis(bromomethyl)benzene* (1.3 g, 225 %, m.p. 135–137 °C). IR: 2925 (m), 1460 (s), 1210 (s), 860 (s), and 650 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.46 (2 H, s), and 4.75 (4 H, s). MS [IP 70 eV; *m/e* (rel. int.)]: 516 (M^+, 22 %), 515 (17), 437 (80), 435 (80), 356 (57), 310 (14), 308 (14), 254 (12), 229 (14), 127 (30), 102 (100). 2,3-Diiodo-1,4-bis(bromomethyl)benzene (10 mmol) and triphenylphosphine (20 mmol) were heated in dry DMF overnight. After cooling, the solution was added to dry diethyl ether (500 ml). The precipitated phosphonium salt was collected, washed with dry ether and dried in vacuum at 110 °C before use.

Hydrogenations were carried out at room temperature and atmospheric pressure. The cyclophanes *15* and *14*, respectively, were dissolved in benzene (P.A.) and palladium on charcoal added and the mixtures were stirred under hydrogen. After 24 h the catalyst was filtered off, the solvent evaporated, and cyclophanes *6* and *8*, respectively, were collected and found to be identical with the products from the photocyclisations of cyclophanes *5* and *7*, respectively.

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The Quantitative Comparison of Experimental to Theoretical Electrode Mechanism Data Using Normalized Working Curves

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The theoretical working curve is a plot of dimensionless observables on one axis and dimensionless variables on the other and is used for the comparison of experimental with theoretical electrode measurement data during electrode mechanism studies. This comparison is highly subjective and gives no quantitative measure of the correspondence of experimental and theoretical data. A method is described to remedy this situation. Theoretical and experimental values of variables (V) necessary for the observable (O) to be equal to fixed values are related by eqn. (i),

$$\ln (V_{exp}y) = m \ln (V_{theory}) + c \qquad \text{(i)}$$

where m and c are the slope and intercept and y is a constant. Providing that the experimental and theoretical data correspond to the same process and y is properly chosen, m will be unity. The analysis provides a quantitative measure of the fit of experimental data to the working curve. Normalized theoretical data for double potential step chronoamperometry (DPSC) and derivative cyclic voltammetry (DCV) analysis of several electrode mechanisms are presented. The method is demonstrated using experimental data for the dimerization of 9-diazofluorene anion radical in acetonitrile.

The most common way to relate experimental electrode measurements to the theoretical values for a particular electrode mechanism is to use a theoretical working curve. The theoretical working curve is a plot of some dimensionless observable such as the peak current ratio in cyclic voltammetry on one axis and the variables, in dimensionless form, on the other axis. The quantities that must be taken into account on the variable axis are evident from the units of the kinetic constants of the rate law for the process. For example, if the theoretical data correspond to a first order reaction of the primary intermediate (B) according to reactions (1) and (2), the unit of k_2 is s^{-1} or for the second order reaction (3), the units of k_3 are $M^{-1}\ s^{-1}$.

$$A \pm e^- \rightleftarrows B \qquad (1)$$

$$B \xrightarrow{k_2} C \qquad (2)$$

$$2B \xrightarrow{k_3} D \qquad (3)$$

In the first case, it is necessary that the other variable, such as the reciprocal of a ($=Fv/RT$), v is the voltage sweep rate, in cyclic voltammetry be expressed in s so that the units cancel. For the second order reaction (3) a dimensionless set of variables is obtained by the product of k_3, the electrochemical variable in s and the substrate concentration (C_A) in M.

Kinetic constants are estimated by finding the theoretical value of the variable corresponding to the experimental value of the observable. An undesirable feature of this procedure is that there is usually some question as to how well the experimental data fit the theoretical working curve. When data are presented in figures the deviations of the experimental data from the theoretical data are only evident when they are quite large. It is most often impossible for the reader of a paper making use of working curves

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to evaluate the reliability of the conclusions based on the data. It is the purpose of this paper to present a quantitative method for the comparison of experimental and theoretical electrochemical data related to electrode mechanisms. The method is similar to the procedure used for normalized potential sweep voltammetry.[1-4]

RESULTS AND DISCUSSION

The method is illustrated by the three dimensional plot in the figure. The observable (O) is represented as the z axis, the theoretical variables (V_{theory}) on the x axis and the experimental variables ($V_{exp}y$) on the y axis. Providing that the experimental and the theoretical data correspond to the same process and the constant y is properly chosen, the projection of the curve onto the x–y plane will define a straight line of unit slope and zero intercept. The value of y can be determined from linear regression relationship (4) by adjusting the scale so that $c=0$.

$$\ln(V_{exp}y) = m \ln(V_{theory}) + c \quad (4)$$

This can be done as a mechanism test by assuming the data at some arbitrary point correspond to that mechanism. That point is conveniently a value of 0.500 of the normalized observable such as the ratio of the derivative peaks during DCV (R'_I) or the normalized current ratio ($R_I=(1-2^{-1/2})i_b/i_f$) durring DPSC. In order to carry out the analysis both theoretical and experimental data must be obtained at the same values of the observable. Preferably, the data represent an as wide as possible range of O.

The deviations of m and c from unity and zero are numerical measures of the fidelity of the fit of the experimental to the theoretical data. The most effective way to test for linearity in the data is to divide the data into two equal segments of V_{theory} and then carry out three correlations; one on each data segment (m_1 and m_2) and one encompassing all of the data (m_T). The required data fit is $m_1=m_2=m_T=$unity. Any deviations from this relationship must be accounted for before assigning a mechanism to an electrode process.

Electrode mechanisms. Four different mechanisms will be considered and are listed below.

I. First order EC.

$$B \xrightarrow{k_I} \text{products} \quad (5)$$

II. First order ECE (or ECE_h).

$$B \xrightarrow{k_{II}} C \text{ (rate determining)} \quad (6)$$

$$C \pm e^- \rightarrow D \text{ (fast)} \quad (7)$$

$$C + B \rightarrow D + A \text{ (fast)} \quad (8)$$

III. Second order ion radical-substrate.

$$B + A \xrightarrow{k_{III}} B-A \text{ (rate determining)} \quad (9)$$

$$B-A \pm e^- \rightarrow \text{dimer (fast)} \quad (10)$$

IV. Second order dimerization.

$$2B \xrightarrow{k_{VI}} \text{dimer} \quad (11)$$

The working curve variables (V_{theory}) for the mechanisms are summarized both for DPSC and DCV in Table 1 along with the V_{exp} and the significance of y.

Theoretical data for all mechanisms are tabulated in Tables 2 and 3 for DPSC and DCV,

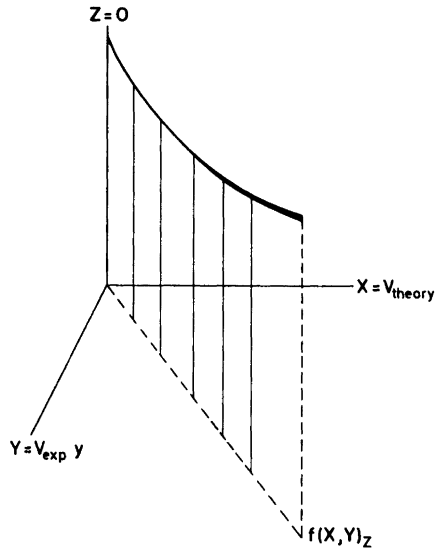

Fig. 1. Representation of the normalized working curve.

Table 1. Working curve parameters for electrode mechanisms.

Mechanism	Technique	V_{theory}	V_{exp}	y
I	DPSC	$k_I\tau$	τ	k_I
I	DCV	k_I/a	a^{-1}	k_I
II	DPSC	$k_{II}\tau$	τ	k_{II}
II	DCV	k_{II}/a	a^{-1}	k_{II}
III	DPSC	$k_{III}C_a\tau$	$C_A\tau$	k_{III}
III	DCV	$k_{III}C_A/a$	C_A/a	k_{III}
IV	DPSC	$k_{IV}C_A\tau$	$C_A\tau$	k_{IV}
IV	DCV	$k_{IV}C_A/a$	C_A/a	k_{IV}

respectively. In the case of DCV, a working curve is necessary for each value of $E_{sw}-E_{rev}$ which is the difference in switching and reversible potentials. The values given are for $E_{sw}-E_{rev}$ equal to 300 mV for mechanisms II–IV and 200 mV for mechanism I. Very nearly linear relationships have been reported for DCV mechanism analysis for all mechanisms but the first order EC so that the full working curve is not generally necessary.[5] The theoretical data used to construct

Table 2. Double potential step chronoamperometric theoretical data for normalized analysis.

R_I	$k\tau$(I)[a]	$k\tau$(II)[a]	$kC_A\tau$(III)[a]	$kC_A\tau$(IV)[a]
0.90	0.058	0.035	0.095	0.083
0.85	0.093	0.057	0.14	0.13
0.80	0.12	0.079	0.20	0.20
0.75	0.16	0.11	0.26	0.26
0.70	0.21	0.13	0.33	0.35
0.65	0.25	0.16	0.40	0.43
0.60	0.30	0.20	0.49	0.54
0.55	0.35	0.23	0.58	0.66
0.50	0.405	0.272	0.678	0.830
0.45	0.47	0.32	0.80	1.03
0.40	0.53	0.37	0.94	1.26
0.35	0.61	0.43	1.10	1.58
0.30	0.71	0.50	1.30	2.07
0.25	0.81	0.58	1.53	2.67
0.20	0.95	0.70	1.85	3.69
0.15	1.12	0.84	2.22	5.55
0.10	1.37	1.05	2.88	9.16
0.05	1.83	1.45	4.04	21.6

[a] The Roman numerals refer to the number of the mechanism in the text.

Table 3. Derivative cyclic voltammetric theoretical data for normalized analysis.

R'_I	k/a(I)[a]	k/a(II)[b]	kC_A/a(III)[b]	kC_A/a(IV)[b]
0.85	0.025	0.0152		
0.80	0.034	0.022		0.020
0.75	0.044	0.030	0.045	0.027
0.70	0.056	0.038	0.060	0.037
0.65	0.069	0.052	0.077	0.049
0.60	0.085	0.066	0.097	0.065
0.55	0.103	0.085	0.122	0.088
0.50	0.124	0.111	0.157	0.120
0.45	0.153	0.148	0.20	0.168
0.40	0.193	0.21	0.27	0.25
0.35	0.262	0.30	0.39	0.38
0.30	0.382	0.48	0.59	0.62
0.25	0.664	0.85	0.99	1.12

[a] For $E_{switch}-E_{rev}$ equal to 200 mV. [b] For $E_{switch}-E_{rev}$ equal to 300 mV.

Table 2 are those reported by Childs [6] and those for Table 3 were obtained by Ahlberg in connection with the analysis given in Ref. 5. For both DPSC and DCV relationship (12) was used to interpolate between the available theoretical values of the observable.

$$\ln O = m \ln V_{theory} + c \qquad (12)$$

This linear relationship holds over a very large range of the theoretical data for DCV[5] and over shorter intervals for the DPSC theoretical data. In no case is error introduced in the theoretical values due to inapplicability of (12).

The use of the normalized working curves is illustrated using DCV and experimental data obtained for the dimerization of 9-diazofluorene anion radical [7] in acetonitrile. The data are correlated with theoretical data for mechanism IV and the data are gathered in Table 4. Where possible, four different substrate concentrations were used. Some indication as to how well the experimental data correspond to the theoretical data can be obtained by noting the degree of deviations in y. The standard deviations at each R'_I value are no greater than ±10 % of y. Results from linear regression analysis of the data are summarized in Table 5. Some deviations from unity are observed in the slopes m. However, the

Table 4. Derivative cyclic voltammetry working curve data for the dimerization of 9-diazofluorene anion radical.

R'_I	$k_{IV}C_A/a$	C_A/mM	v/V s^{-1}	10^7 (C_A/a)	$10^{-5}y$/M^{-1}s^{-1}	
0.70	0.037	0.25	32.9	1.92	1.93	
0.65	0.049	0.25	27.2	2.32	2.11	
0.60	0.065	0.25	21.6	2.92	2.23	
0.55	0.088	0.25	16.2	3.90	2.26	2.13(0.18)
0.55	0.088	0.50	28.7	4.40	2.00	
0.50	0.120	0.25	11.9	5.30	2.26	2.34(0.09)
0.50	0.120	0.50	24.5	5.15	2.33	
0.50	0.120	0.75	38.4	4.93	2.43	
0.45	0.168	0.25	8.62	7.32	2.30	2.39(0.09)
0.45	0.168	0.50	18.1	6.67	2.41	
0.45	0.168	0.75	27.8	6.81	2.47	
0.45	0.168	1.00	33.2	7.60	2.21	
0.40	0.25	0.25	6.05	10.4	2.40	2.49(0.10)
0.40	0.25	0.50	12.5	10.1	2.48	
0.40	0.25	0.75	20.0	9.46	2.64	
0.40	0.25	1.00	24.7	10.2	2.45	
0.35	0.38	0.25	4.00	15.8	2.41	2.46(0.10)
0.35	0.38	0.50	8.26	15.3	2.48	
0.35	0.38	0.75	12.9	14.7	2.59	
0.35	0.38	1.00	15.7	16.1	2.36	
0.30	0.62	0.25	2.08	30.3	2.05	2.29(0.22)
0.30	0.62	0.50	4.91	25.7	2.41	
0.30	0.62	0.75	7.70	24.6	2.52	
0.30	0.62	1.00	8.82	28.6	2.17	
0.25	1.12	0.50	2.57	49.1	2.28	2.25(0.09)
0.25	1.12	0.75	4.00	47.3	2.37	
0.25	1.12	1.00	4.92	51.3	2.18	
					2.32(0.17)	

Table 5. Correlation parameters for the dimerization reaction.

Slope	R'_I	m [a]	r [b]	(s/Y_{mean})100/% [c]
m_1	0.70–0.45	0.89(3.4 %)	0.994	2.3
m_2	0.40–0.25	1.07(2.6 %)	0.996	8.1
m_T	0.70–0.25	0.97(1.6 %)	0.997	5.3

[a] From eqn. (4) and the data in Table 4. The error was calculated according to Ref. 12. [b] Linear regression correlation coefficient. [c] The standard deviation about the regression divided by the mean value of Y expressed in percent.

only significant deviation occurs in m_1. There are two likely reasons for the deviation. The first is that the rate of charge transfer may be interfering at the higher sweep rates necessary for high R'_I and secondly the data for the highest R'_I values are all from the lowest substrate concentrations which were necessary to limit the rate of the reaction. The R'_I data for the lowest C_A are expected to be in greater error than that for the higher concentrations.

Since there are plausible reasons for the deviations of m from the theoretical values, it can

be concluded that the experimental data for the dimerization of 9-diazofluorene anion radical correspond reasonably well to the theoretical data for the second order dimerization mechanism (IV). The slope m_T calculated for mechanism III is 1.19 with a standard deviation of 0.016 and is therefore significantly different from unity.

It has recently been emphasized that theoretical working curves may not be unique for a particular electrode mechanism.[8] Other drawbacks to the use of the theoretical working curve were also pointed out and an alternative method, using reaction orders without calculations, has been presented.[9] If the theoretical working curves are to be used in electrode mechanism determination it is surely highly desirable that some indication be given of how well the experimental and theoretical data correspond. The method presented here provides a quantitative comparison of experimental to theoretical data for electrode mechanisms.

EXPERIMENTAL

The instrumentation, electrodes, cells and data handling procedures were those described earlier.[10] Reagent grade acetonitrile containing Bu_4NBF_4 (0.1 M) was passed through a column containing neutral alumina before use. 9-Diazofluorene was that used in earlier work.[7] Derivative cyclic voltammetry experiments were carried out as previously described.[11]

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A New Sensitive, Thiol Tolerant Method for the Determination of Inorganic Pyrophosphatase

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In this work a new sensitive, thiol tolerant method for the determination of inorganic pyrophosphatase (EC 3.6.1.1) was developed. It is based on the formation of phosphomolybdate–triethylamine complex, which in acid–acetone mixture forms a white, homogenous precipitate, the turbidity of which can be accurately measured spectrophotometrically at 355 nm. This method can be easily applied to all the P_i-producing enzymes whose substrates are stable enough in our acidic experimental conditions.

We have recently shown that inorganic pyrophosphatase (EC 3.6.1.1, hereafter referred to as PPase) of *Streptococcus faecalis* is regulated at the activity level *via* the ratio of reduced glutathione to oxidized glutathione.[1,2] The enzyme exists in two interconvertible forms which differ in activity. The highly active form of enzyme is labile, and is stabilized by thiol compounds *in vitro*.[3] Our purpose is to study the kinetics of these two enzyme forms. We have recently introduced a new method for the determination of inorganic orthophosphate which is suitable for most purposes.[4] However, the sensitivity of that method is somewhat too low when the kinetics of inorganic pyrophosphatase is studied at low pyrophosphate concentrations. Furthermore, the various modifications of the Fiske-SubbaRow method generally used for studies on PPase kinetics [5–7] are highly sensitive for thiol compounds. The kinetics of the highly active form of *S. faecalis* PPase has to be measured in the presence of thiol compounds, and so a method insensitive to thiols had to be developed.

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MATERIALS AND METHODS

Chemicals. Commercially available chemicals of analytical grade were used without any purification. ATAM solution (acetone–triethylamine–acid–molybdate) was prepared daily by mixing 4 vol of 10 mM $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ with 2 vol of 2.5 N H_2SO_4, 1 vol of acetone and 0.075 vol of triethylamine.

Assay of inorganic pyrophosphatase. The reaction mixture was as follows: 150 μl of the appropriate dilution (about 1.9–0.2 mUnits; U=μmol product formed/min, dilution with 0.05 M Tris/HCl pH 8.0) of the homogenous enzyme preparation purified according to Lahti and Niemi,[8] 25 μl of 9 mM $MgCl_2$, 25 μl of 9 mM Na_4PP_i and 25 μl of 0.05 M Tris/HCl pH 8.0. The reaction took place at 25 °C, and it was started by the addition of PP_i. After the appropriate incubation time the reaction was stopped by adding 25 μl of 1.2 M trichloroacetic acid. ATAM solution (0.9 ml) was pipetted to the reaction mixture. After vigorous mixing with a vortex mixer 0.1 ml of 1 M citric acid was added to the tube, and the white, homogenous turbidity of the phosphomolybdate–triethylamine complex was measured spectrophotometrically at 355 nm. A reaction mixture, where trichloroacetic acid was added before PP_i, was used as a blank. Furthermore, for the determination of P_i standard for PPase assay 25 μl of Tris/HCl pH 8.0 was substituted by 25 μl of appropriate P_i solutions (9–180 nmol P_i, dissolved in 0.05 M Tris/HCl pH 8.0), and trichloroacetic acid was again added before PP_i to prevent the enzymatic reaction.

RESULTS

Based on the observation that inorganic pyrophosphate and orthophosphate can be separated by selective precipitation of P_i as a phosphomolybdate–triethylamine complex [9] we decided to search for conditions where the turbidity of the precipitate could be accurately determined. The precipitate formed as described by Cartier and Thuillier [9] is flocculent, and cannot be measured as such. In addition, if triethylamine is added to the acetone–acid–molybdate (AAM) mixture described by us [4] no precipitation occurs. However, when the ratio of the concentrations of the components in the AAM is changed to that of ATAM presented in Materials and Methods the phosphomolybdate–triethylamine precipitate becomes homogenous enough to be measured spectrophotometrically.

The turbidity was developed in a couple of minutes as the contents of the test tube were thoroughly mixed after the addition of ATAM solution. After that the turbidity decreased by about 10 % in 30 min but the relative decrease was independent of the initial amount of the turbidity. The blank value did not change significantly in 30 min (Fig. 1). To overcome the interference of slow decrease in turbidity the samples were measured at fixed times after the addition of ATAM solution.

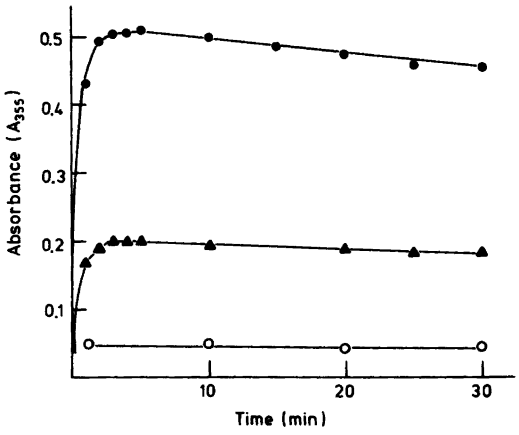

Fig. 1. Stability of the turbidity of the phosphomolybdate–triethylamine complex. Enzymatic hydrolysis of inorganic pyrophosphate catalyzed by inorganic pyrophosphatase was arrested after the reaction time of 0 min (○), 5 min (▲), and 15 min (●), and the turbidity of the white precipitate was measured at intervals spectrophotometrically at 355 nm. Distilled water was used as a blank.

High salt (1 M NaCl) and sucrose (200 mg/ml) concentrations exerted no measurable effects on the turbidity formed or on the blank value. Furthermore, a strong reductant, ascorbic acid, at 1 mM concentration had no effect on the formation or stability of the precipitate. Thiol compounds, such as L-cysteine and 2-mercaptoethanol, increased the blank value when present in high concentrations. This interference could be easily overcome by making the standard in the presence of the thiol compounds. When the concentration of thiol compounds was less than 10 mM, no corrective measures were required.

The standard curve for the routine PPase assay is presented in Fig. 2. The lower limit of the assay is about 1 nmol P_i. In the routine PPase assay the amount of P_i produced was a linear function of the amount of the enzyme up to about 20 nmol of P_i liberated (corresponding to about A_{355}=0.7). The precision of the PPase assay was determined by using aliquots of the same enzyme preparation in ten different reaction mixtures. The arithmetic mean of the measured activities was 3.5 nmol of P_i formed in 15 min (0.130 A_{355}/15 min), and the range of the values was 0.32 nmol (0.125–0.137 A_{355}) or 9.2 % of the mean value.

DISCUSSION

The aim of this work was to develop a method for the determination of inorganic pyrophosphatase that is sensitive enough for kinetic studies and is resistant to thiol compounds. The latter

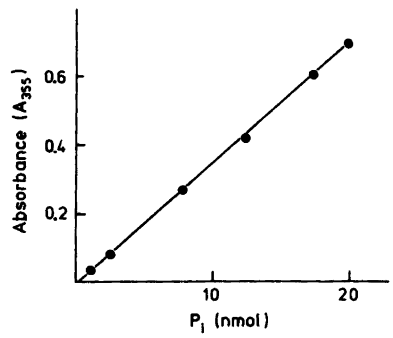

Fig. 2. Standard curve for the assay of inorganic pyrophosphatase. For further details see Materials and Methods.

demand is especially important when the kinetics of the highly active form of *S. faecalis* inorganic pyrophosphatase is studied.[3] The radiochemical method presented by Heinonen [10] fulfills both of these demands excellently, but the price of the labelled substrate and the inconvenience of working with radioactive solutions encouraged us to look for an alternative. Our new method, which is a modified combination of the methods described by Cartier and Thuillier [9] and Heinonen and Lahti,[4] is tolerant to thiol compounds and it is about fifty times as sensitive as that described by us recently.[4] This method is easily applicable to all the P_i producing enzymes whose substrates are so stable that they do not hydrolyze significantly between the additions of trichloroacetic acid and citric acid (see Materials and Methods). When citric acid has been added the acid catalyzed release of P_i causes no problems because all extra molybdate is complexed with citrate. This method requires no expensive materials and special instruments, and hence can be used in any laboratory.

While this manuscript was in preparation we learned a method described by Eibl and Lands [11] where phosphomolybdate precipitate was formed by Triton X-100. However, this method was not checked for possible interferences, and evidently it has not been much used.

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Characterization of Papaya Peptidase A as an Enzyme of Extreme Basicity

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Papaya peptidase A, a papain-like enzyme, has formerly been shown to contain a larger excess of basic amino acids and to have a higher isoelectric point than any of the other enzymes in the papaya latex. Determinations of the free electrophoretic mobility as a function of pH now establishes the isoelectric point of papaya peptidase A as 11.7 and that of succinylated papaya peptidase A as 3.8. Although the specific activity of the enzyme appears only slightly affected by the succinylation, the accompanying change in the charge/mobility ratio seems to indicate a relatively large conformational change upon succinylation.

Papaya peptidase A [1] is a proteolytic enzyme of the latex of *Carica papaya* very similar to papain (EC 3.4.22.2). Among the few differences revealed by comparison of the two enzymes, a prominent one is the high content of lysine in papaya peptidase A compared with that of papain. Whereas papain is known to possess 10 lysine residues [2] a number of 22–23 has been reported for papaya peptidase A.[1,3] Due to its extreme basicity the isoelectric point of papaya peptidase A has never been exactly determined. Schack [1] estimated an isoelectric point of "near 11" using free electrophoresis, I=0.1 M, whereas Brocklehurst *et al.*[4] claimed "above 11" for the protein prepared from spray dried latex and a value of 10.9 for other preparations of the enzyme. In the latter cases, however, neither the method nor the ionic strength were stated.

We here report electrophoretic mobility determinations at constant current for papaya peptidase A in the pH-range 9.8–12.4 using glycine/NaOH buffers, I=0.14 M. This reveals an isoelectric point of 11.7 for papaya peptidase A, to our knowledge the highest value reported for any protein.

Furthermore, it is demonstrated that succinylation of papaya peptidase A is associated with a loss of at least 20 free amino groups and a concomitant decrease in isoelectric point of approximately 8 pH units without any decrease in specific activity towards *N*-benzoyl-L-arginine ethyl ester (BAEE) at pH 6.0.

EXPERIMENTAL

A lyophilized extract of commercial dried papaya latex (Sigma, crude type I, lot 127C-0334) was prepared according to Schack.[1] From this material papaya peptidase A was prepared by means of a batchwise procedure: 5 g lyophilized extract dissolved in 100 ml 0.4 M NaCl was equilibrated with 50 ml CM-Sepharose CL-6B slurry (Pharmacia, Sweden). The suspension was transferred to a sintered glass filter and washed with 500 ml 0.4 M NaCl added in several portions. Papaya peptidase A was subsequently eluted by washing with 2×50 ml 1.2 M NaCl, the fractions of which were pooled, dialyzed and lyophilized according to Schack.[1] The preparations thus obtained appeared homogeneous by the criteria of disc-electrophoresis and ion-exchange chromatography. Upon activation with dithiothreitol the preparation exhibited a thiol content of typically 0.35 mol of SH per mol of protein as determined by titration with 2,2′-dipyridyl disulfide.[5]

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Succinylation of the unactivated enzyme was performed according to the method of Sluyterman and De Graaf [6] for the succinylation of papain. The modified enzyme was stored lyophilized.

Cytochrome *c* type VI from horse heart was the product of Sigma. Free electrophoretic mobilities were determined by zone electrophoresis using the constant current method of Waldmann-Meyer.[7] Whatman 3 MM paper was used as the carrier and the following buffers were employed: pH 3.7–5.0 sodium acetate–acetic acid, pH 6.0–7.0 sodium barbiturate–acetic acid, pH 9.8–12.4 glycine–NaOH. The electrophoretic experiments were generally performed using 3 % (w/v) solutions of lyophilized protein in the respective buffers. In the case of papaya peptidase A control experiments were run employing the mercury derivative of fully active papaya peptidase A which was prepared by covalent chromatography using Thiopropyl-Sepharose 4B (Pharmacia, Sweden) and a method similar to that of Stuchbury *et al.*[8] for the preparation of papain. The pH dependence shown by the electrophoretic mobilities obtained for the mercury derivative of the fully active enzyme was indistinguishable from that obtained for only partly active preparations of the enzyme.

Free amino groups were determined by a modification of the trinitrobenzenesulfonic acid (TNBS) method of Snyder and Sobocinski [9] using $NaHCO_3$–Na_2CO_3 buffer, pH 9.3. Glycine was employed as reference. *O*-succinylation of hydroxyamino acids (serine and threonine) was determined by means of the alkaline hydroxylamine reaction of Hestrin.[10] Succinic monomethylester (a generous gift from the Department of Organic Chemistry, this University) was used as standard. Esterase activity towards *N*-benzoyl-L-arginine ethyl ester (BAEE, Sigma) at pH 6.0 was determined using a pH-stat (pH-meter 28/autoburette ABU 11/titrator TTT 11, Radiometer, Copenhagen). The concentrations of enzyme and substrate were typically 1 μM and 5 mM, respectively. Assay mixtures contained 10^{-4} M dithiothreitol. Specific activity was expressed as initial velocity divided by the substrate concentration and by the protein concentration. Protein concentrations were determined using a value of 18.3 for $A^{1\%}_{1\,cm}$ at 280 nm and M_r 24000 for papaya peptidase A [3] and M_r 26000 for succinyl-papaya peptidase A.

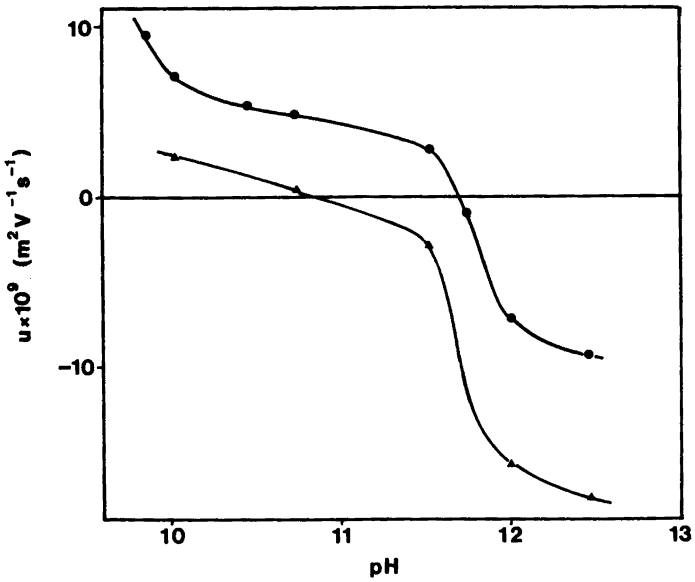

Fig. 1. Electrophoretic mobilities at 25 °C, glycine–NaOH buffers, I=0.14 M *versus* pH for (●) papaya peptidase A and (▲) cytochrome *c*.

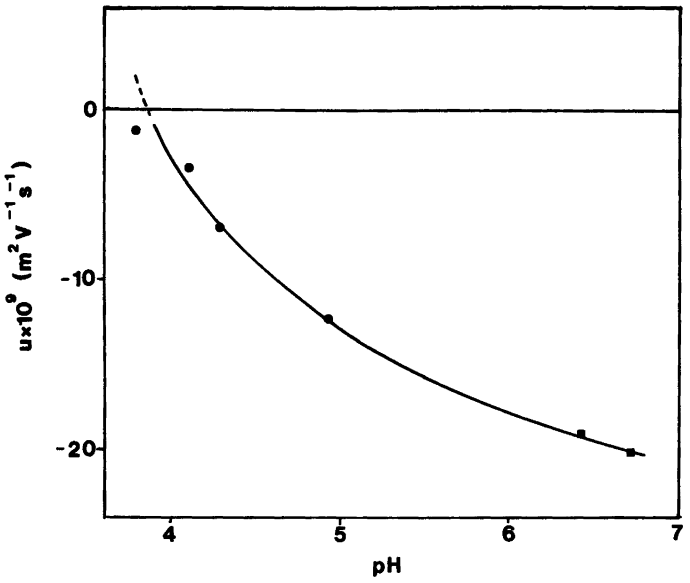

Fig. 2. Electrophoretic mobilities at 25 °C, I=0.1 M *versus* pH for succinyl-papaya peptidase A in (●) sodium acetate–acetic acid buffers and (■) sodium barbiturate–acetic acid buffers.

RESULTS AND DISCUSSION

The exact electrophoretic mobility of papaya peptidase A was determined at eight different pH values in the range 9.8–12.4. For comparison, cytochrome c was included in five of the runs as an example of a well-known basic protein. In Fig. 1, a plot of the electrophoretic mobility at 25 °C, I=0.14 M, against pH is shown for papaya peptidase A and cytochrome c. This reveals isoelectric points of 11.7 and 10.8, respectively. The corresponding value for cytochrome *c* given in the literature [11] is 10.65 determined by free electrophoresis, I=0.2 M. Considering the different ionic strength used here the agreement is very satisfactory.

Since the pI value of 11.7 apparently establishes papaya peptidase A as an enzyme of a hitherto unknown basicity, it was of interest to examine how the protein would respond to the introduction of a massive negative charge, *e.g.* to succinylation. The pH-dependence of the electrophoretic mobility for succinyl-papaya peptidase A is shown in Fig. 2. Compared to the unmodified enzyme, the solubility of succinyl-papaya peptidase A at low pH values is decreased by at least a factor of ten, thus causing difficulties in the determination of electrophoretic mobilities below pH 4.5. Consequently, the pI of succinyl-papaya peptidase A had to be obtained by extrapolation yielding a value of approximately

Table 1. Comparison of papaya peptidase A and succinyl-papaya peptidase A.[a]

	Papaya peptidase A	Succinyl-papaya peptidase A
NH_2 groups/molecule [b]	24.8±1.5	2.5±1.0
O-succinylated groups/molecule [c]	—	3.3±0.2
Specific activity/$M^{-1}s^{-1}$ [d]	310±30	415±90 [e]

[a] Assuming a value of 18.3 for $A^{1\%}_{1cm}$ at 280 nm, M_r 24000 for papaya peptidase *A* [3] and M_r 26000 for succinyl-papaya peptidase A. [b] TNBS-assay.[9] [c] Alkaline hydroxylamine reaction.[10] [d] Towards BAEE at pH 6.0. [e] Based on 4 determinations.

3.8, *i.e.* as a result of succinylation the pI of papaya peptidase A drops nearly 8 pH units.

In Table 1, papaya peptidase A and succinyl-papaya peptidase A are compared with respect to the content of free amino groups, the number of *O*-succinylated hydroxyamino acids and specific activity. It is seen that 3 hydroxyl groups and about 20 amino groups are succinylated under the conditions employed. Furthermore, when taking the *N*-terminal amino group into account, the estimated number of 24.8±1.5 amino groups per molecule of papaya peptidase A provides an independent check of the number of lysine residues listed in the amino acid analyses of Schack [1] and Robinson.[3] These investigators reported 22 and 23 lysine residues, respectively, per molecule of papaya peptidase A in excellent agreement with the result obtained by the TNBS-assay used here. The fact that conversion of 3 neutral hydroxyl groups and 20 positive amino groups into negative groups causes a change in pI as large as 8 pH units seems reasonable, especially when compared with papain, for which Sluyterman and De Graaf [6] found a corresponding change in pI of 5 pH units upon succinylation of 4 neutral hydroxyl groups and 8 positive amino groups.

Still, in accordance with the case of papain,[12] Table 1 shows that the specific activity towards BAEE at pH 6.0 is only slightly affected by succinylation, probably indicating a largely unchanged configuration of the active center residues upon modification. However, it seems unlikely that the protein as a whole should remain unaffected by this rather drastic modification, which around neutral pH constitutes a change of about 43 charges. This presumption was qualitatively confirmed by comparing the charge/mobility ratio as obtained experimentally with that predicted by the theories of Debye, Hückel, Henry and Gorin.[13] Electrophoresis of papaya peptidase A under conditions identical with those of the pH 6.71 run of Fig. 2 (*i.e.* sodium barbiturate–acetic acid buffer, I=0.1 M) yielded a mobility of 14.5×10^{-9} m^2 V^{-1} s^{-1}. For f/f_o=1.14, $\bar{v}$=0.727 cm^3/g and M_r 24 000[1,3] this mobility gives a charge/mobility ratio of 0.74×10^9 V s m^{-2}, whence papaya peptidase A possesses an average net charge of +10.73 at pH 6.71, I=0.1 M.

Since succinylation decreases the charge by 43 units (Table 1) and the mobility of the succinylated enzyme at pH 6.71 is -20.2×10^9 m^2 V^{-1} s^{-1} (*cf.* Fig. 2), the z/u ratio for the modified protein is 1.60×10^9 V s m^{-2}. In view of the fact that the z/u equation [13] has been clearly validated by experimental data for serum albumin,[14,15] it appears reasonable to ascribe the above difference in z/u ratios to a significant conformational change produced by succinylation and to different degrees of electrolyte binding.

In the absence of binding data, a tentative calculation using $z/u=1.6\times10^9$ V s m^{-2}, $\bar{v}$=0.727 cm^3/g and M_r 26 000 yields a frictional coefficient, f/f_o, of 1.35 for the succcinylated enzyme corresponding to an axial ratio of about 6.5 as compared to the f/f_o value of 1.14 for the unmodified enzyme, which corresponds to an axial ratio of about 3.

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Introduction of Carbon Substituents into Pyrimidines by Grignard Reagents

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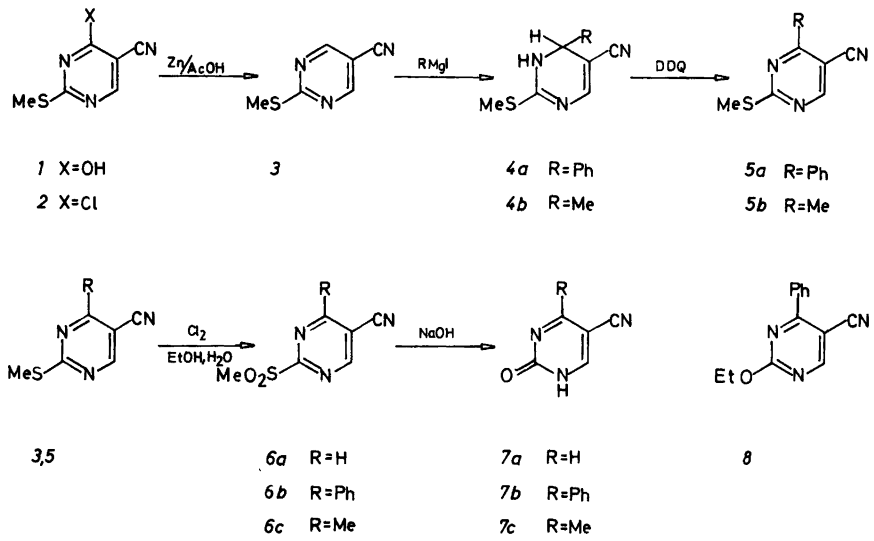
Alkyl- and arylmagnesium halides selectively form 1:1-adducts with the heterocyclic ring in substituted 5-cyanopyrimidines. Dehydrogenation gives the corresponding alkyl or aryl substituted pyrimidine.

Grignard reagents react with halopyrimidines under the influence of nickel-complex catalysis; the nature of the nucleophile is decisive for the preferred reaction path which is either cross-coupling or 1:1 adduct formation.[1] Carbon–carbon bond formation through replacement of thioether groups also seems to require nickel-complex catalysis.[2] We herein report our findings for the reactions of Grignard reagents with 5-cyano-2-methylthiopyrimidine *3* in the absence of a catalytic agent. In the reaction of *3*, addition to the 3,4-azomethine bond of the pyrimidine nucleus [3,4] or the usual addition of Grignard reagents to the nitrile group,[5] can be envisaged. Perhaps surprisingly, in view of the failure of the halopyrimidines to react with Grignard reagents at significant rates in the absence of nickel catalysis, the cyanopyrimidine *3* rapidly formed nuclear adducts as discussed below.

For the synthesis of the 5-cyanide *3*, 5-cyano-2-methylthiopyrimidin-4-one *1* was the starting material; *1* was available by the condensation between *S*-methylisothiouronium iodide and



1 X=OH
2 X=Cl
3
4a R=Ph
4b R=Me
5a R=Ph
5b R=Me
3,5
6a R=H
6b R=Ph
6c R=Me
7a R=H
7b R=Ph
7c R=Me
8

Scheme 1.

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ethyl 2-cyano-3-ethoxyacrylate.[6] *1* was converted to the chloride *2* using phosphorus oxychloride [7] and *2* subjected to hydrogenolysis using zinc in acetic acid; the previously reported synthesis from *2* in auqeous ethanol without acid catalysis, gives much inferior yield of *3*.[8]

In the Grignard reaction, methyl- and phenylmagnesium iodide were used in ether solution. In both cases the adduct *4* was the exclusive product. The reaction is rapid and goes under mild conditions as would be expected for the selectivity observed in the reaction. Dehydrogenation to the aromatic pyrimidine *5* was effected using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).

The net result of the above reaction is the introduction of a carbon substituent into the pyrimidine ring. This can be used with advantage in the synthesis of a variety of derivatives, such an example being illustrated by the preparation of the substituted pyrimidinones *7*. The methylthio substituent in the cyanide *3* could be hydrolyzed under alkaline conditions, but it was difficult to effect complete selectivity without attack on the cyano group. Therefore the sulfide substituent in *3* and *5* was oxidized to a sulfonyl group (*6*) using chlorine in aqueous ethanol. The phenyl derivative *5a*, however, furnished the 2-ethoxy derivative *8* under these conditions presumably because the exothermic reaction of *5a* resulted in solvolysis. In aqueous dioxane, however, the sulfone *6b* was smoothly formed from *5b*. Aqueous alkaline hydrolysis of *6* yielded the 5-cyanolactams *7*.

EXPERIMENTAL

The MS data are given as MS 70 eV; *m/z* (% rel.int.).

5-Cyano-2-methylthiopyrimidine [8] 3. 4-Chloro-5-cyano-2-methylthiopyrimidine [7] (7.3 g, 39 mmol) was dissolved in ethanol (65 ml) and water (12 ml) and zinc dust (12.4 g) added. Acetic acid (0.5 ml×5, 44 mmol) was added at intervals (0, 30, 60, 90 and 120 min) to the vigorously stirred mixture and the stirring continued at room temperature for 2.5 h when TLC (silica gel and chloroform) showed the reaction to be complete. The reaction mixture was then filtered, the solid washed with warm ethanol and the combined washings and filtrate evaporated to dryness at reduced pressure. The residue was extracted with chloroform and the chloroform solution filtered through an alumina column (neutral). Evaporation of the chloroform eluate left the title compound which was recrystallized from ethanol, yield 4.0 g (67 %), m.p. 79–80 °C. [5] ^{1}H NMR ($CDCl_3$): δ 2.62 (SMe), 8.76 (H-4, H-6).

5-Cyano-2-methylthio-4-phenyl-3,4-dihydropyrimidine 4a. A solution of 5-cyano-2-methylthiopyrimidine (2.3 g, 15.2 mmol) in dry ether (50 ml) was added dropwise with stirring at 0 °C to an ethereal solution of phenylmagnesium iodide, which had been prepared from iodobenzene (6.21 g, 31 mmol) and Mg (0.75 g, 31 mmol) in dry ether (100 ml). After the addition had been completed the mixture was stirred for 10 min at room temperature, the mixture poured into 20 % aqueous NH_4Cl, the ether layer separated, the aqueous layer extracted with ether, the combined ether solutions washed with water, the dried ($MgSO_4$) solution evaporated and the residue chromatographed on neutral alumina (Woelm-activity II) using $CHCl_3$: hexane 7:3 for elution; yield 2.1 g (60 %) of an oily material which was analyzed as such. Anal. $C_{12}H_{11}N_3S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.36 (S–Me), 4.87 (H-4), 6.20 (NH), 6.98 (H-6), 7.23 (Ph). MS: 229 (M, 31), 228 (19), 214 (23), 152 (100).

5-Cyano-4-methyl-2-methylthio-3,4-dihydropyrimidine 4b was prepared as above using methylmagnesium iodide. The product was purified by chromatography on basic alumina (activity II) using $CHCl_3$–EtOAc 7:3 after initial elution with $CHCl_3$–hexane 7:3 to remove some starting material; yield 1.8 g (54 %) of an oily material. ^{1}H NMR ($CDCl_3$) δ 1.38 (4-Me, *J* 6.5 Hz), 2.40 (SMe), 4.30 (H-4, *J* 6.5 Hz), 6.90 (H-6), 6.5–7 (NH).

5-Cyano-2-methylthio-4-phenylpyrimidine 5a. 5-Cyano-2-methylthio-4-phenyl-3,4-dihydropyrimidine (2.5 g, 10.9 mmol) and DDQ (2.7 g, 12.0 mmol) were stirred together in benzene (50 ml) for 10 min. The solution was then concentrated to one third of its volume, the precipitate removed and extracted with boiling benzene, the benzene solutions combined, most of the solvent distilled off and the concentrated solution passed through a short (4 cm) alumina column. The title compound was eluted with chloroform; yield 1.65 g (67 %), m.p. 141–143 °C (EtOH). Anal. $C_{12}H_9N_3S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.58 (S–Me), 7.3–7.6 and 7.8–8.2 (Ph), 8.65 (H-6). IR (KBr): 2200 cm^{-1} (CN). MS: 227 (M, 100), 226 (31), 181 (28), 180 (54), 154 (11), 127 (13), 77 (11).

5-Cyano-4-methyl-2-methylthiopyrimidine 5b was prepared as above from 5-cyano-4-methyl-2-methylthio-3,4-dihydropyrimidine. The reaction mixture was left at room temperature overnight

before isolation *via* chromatography; yield 83 %, m.p. 52–53 °C (EtOH). Anal. $C_7H_7N_3S$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.58 (Me), 2.66 (Me), 8.61 (H-6).

5-Cyano-2-methylsulfonylpyrimidine 6a was prepared from *3* by chlorine oxidation in aqueous ethanol as described for *6c*.

5-Cyano-2-methylsulfonyl-4-phenylpyrimidine 6b. Chlorine was bubbled through a stirred solution of 5-cyano-2-methylthio-4-phenylpyrimidine (0.70 g, 3.0 mmol) in dioxane (55 ml) and water (15 ml) at 0 °C for 15 min. On concentrating the solution at reduced pressure the product crystallized; yield 0.65 g (81 %), m.p. 155–157 °C. Anal. $C_{12}H_9N_3O_2S$: C, H. ^{1}H NMR ($CDCl_3$): δ 3.40 (Me), 7.4–7.7 and 8.0–8.3 (Ph), 9.10 (H-6). IR (KBr): 2200 cm^{-1} (CN). MS: 260 (14), 259 (M, 72), 196 (31), 181 (34), 180 (60), 128 (100), 77 (40).

5-Cyano-4-methyl-2-methylsulfonylpyrimidine 6c. Chlorine gas was bubbled through a stirred solution of 5-cyano-4-methyl-2-methylthiopyrimidine (0.72 g, 4.3 mmol) in ethanol (25 ml) and water (10 ml) at 10 °C for 10 min and left at this temperature for 4 h before the precipitate was collected and recrystallized from ethanol; yield 0.45 g (52 %), m.p. 115 °C. Anal. $C_7H_7N_3O_2S$: C, H. ^{1}H NMR (DMSO-d_6): δ 2.80 (4-Me), 3.43 ($MeSO_2$), 9.45 (H-6).

5-Cyanopyrimidin-2-one [9] 7a was prepared from 5-cyano-2-methylthiopyrimidine as described for *7b* below allowing the hydrolysis to proceed overnight; m.p. 260–262 °C after sublimation (190 °C/0.5 mmHg). ^{1}H NMR (TFA): δ 9.02 (H-4, H-6).

5-Cyano-4-phenylpyrimidin-2-one 7b. 2 M NaOH was added dropwise with stirring to a solution of 5-cyano-2-methylsulfonyl-4-phenylpyrimidine (0.20 g, 0.77 mmol) in dioxane (2 ml) and water (2 ml) at room temperature. The mixture was stirred for 15 min after the addition was completed and then acidified with HCl when the product crystallized; yield 0.11 g (72 %), m.p. 214 °C. Anal. $C_{11}H_7N_3O$: C, H. ^{1}H NMR (DMSO-d_6): δ 7.3–7.8 (Ph), 8.78 (H-6). IR (KBr): 2210 cm^{-1} (CN). MS: 197 (M, 87), 196 (100), 171 (10), 170 (5), 169 (14), 155 (6), 142 (7), 77 (19).

5-Cyano-4-methylpyrimidin-2-one 7c was prepared from 5-cyano-4-methyl-2-methylsulfonylpyrimidine as *7b* above by allowing the hydrolysis to proceed for 1 h. After acidification the product was obtained in 56 % yield, m.p. 258–260 °C (sublimed). Anal. $C_6H_5N_3O$: C, H. ^{1}H NMR (TFA): δ 3.01 (Me), 9.15 (H-6).

5-Cyano-2-ethyloxy-4-phenylpyrimidine 8 was obtained in a mixture with the lactam *7b* when chlorine gas was bubbled into a solution of 5-cyano-2-methylthio-4-phenylpyrimidine (1.0 g, 4.4 mmol) in a solution of ethanol (35 ml) and water (10 ml) at −10 °C for 10 min. Fractional sublimation from the precipitate gave the title compound in 60 % yield (0.60 g), m.p. 76–77 °C. Anal. $C_{13}H_{11}N_3O$: C, H. ^{1}H NMR ($CDCl_3$): δ 1.45 and 4.54 (Et), 7.3–7.6 and 7.8–8.1 (Ph), 8.70 (H-6). MS: 225 (M, 42), 224 (31), 197 (33), 196 (66), 181 (100), 180 (16), 104 (18).

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Ring–Chain Tautomerism of Pseudooxynicotine and Some Other Iminium Compounds

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The ring–chain tautomerism in aqueous solution of the nicotine metabolite pseudooxynicotine (*1*) has been studied. Of the four possible forms of *1*, only the chain form *1a* and the iminium form *1c* could be observed by NMR spectroscopy. The chain form *1a* was strongly preponderant at high or low pH. The proportion of *1c* reached a maximum level of 52–53 % in a neutral solution of *1*. These results differ strongly from those published by other workers. Some analogous compounds (*e.g.* *5* and *6*) have also been investigated.

Pseudooxynicotine (*1*) was first synthesized in 1892 [1] and was later found to be a bacterial metabolite of nicotine (*2*).[2] The compound is capable of displaying ring-chain tautomerism [3,4] and, according to classical theory, aqueous solutions of *1* may contain four forms in equilibrium, *1a*–*1d* (Fig. 1). The elemental composition and IR spectrum of the crystalline dihydrochloride indicate that this salt contains the dication derived from the chain form *1a*.[5] Extraction of an alkaline aqueous solution of *1* with chloroform followed by distillation yields the unstable enamine *1d*, also known as *N*-methylmyosmine.[5,6]

A large number of aromatic heterocyclic cations have been studied with respect to their formation of pseudobases by covalent addition of a hydroxyl ion, but much less information is available about the corresponding reactions in the aliphatic series, *e.g.* of iminium ions.[4,7] A structural investigation of *1*, mainly performed by means of ^{1}H NMR spectroscopy, was recently undertaken by Maeda *et al.*[8] and, to the best of our knowledge, their report constituted the first

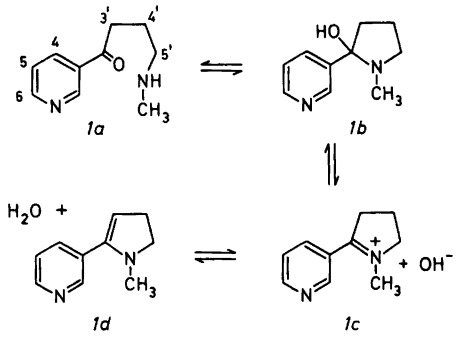

Fig. 1. Conceivable forms (*1a*–*1d*) of pseudooxynicotine in aqueous solution. The numbering of the carbons in nicotine has been kept throughout and is shown for *1a*.

study of the ring-chain tautomerism of a γ-(alkylamino) ketone in aqueous solution as a function of pH. Maeda *et al.* concluded that *1a* was present at pH 1–4, *1c* at pH 2–9.5, and *1d* at pH 4–11. We have now carried out a similar study of *1* and we obtained ^{1}H NMR spectra which were similar to those of Maeda *et al.* However, our interpretations of the spectra and the conclusions reported below differ strongly from those of Maeda *et al.*[8] Knowledge of the structures of nicotine metabolites in aqueous solution is necessary for the detailed mapping of the reaction pathways involved in the metabolism of nicotine. We have previously described a structural study of nicotine $\Delta^{1'(5')}$-iminium ion,[9] which is an isomer of *1c*, while others [10] have studied the 5'-oxo analogue of *1b*.*

* The nicotine numbering, as in *1a*, is used throughout.

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STUDIES OF PSEUDOOXYNICOTINE

^{1}H NMR spectra of neutral aqueous solutions of pseudooxynicotine (*1*) showed that two forms of *1* are present in approximately equal amounts and the structures *1a* and *1c* could be ascribed to these forms. Thus, the signals at δ 2.82 (48 %) and 3.71 (52 %) were ascribed to the *N*–CH_3 of *1a* and *1c*, respectively. The signals ascribed to the *N*-methylpyrrolinium moiety of *1c* agree well with those obtained from 3,4-dihydro-1-methyl-5-phenyl-2*H*-pyrrolium perchlorate in trifluoroacetic acid solution.[11] The assignments are further supported by the effects of pH on the NMR spectra. Changes in pH did not affect the positions of the signals ascribed to the protons of the five-membered ring of *1c*; on the other hand, the corresponding signals ascribed to *1a* varied with pH in the manner expected for an amine (see below). The ^{13}C NMR spectrum of a neutral solution of *1* in H_2O is in accord with the ^{1}H NMR spectrum; the chemical shifts obtained for *1a* and *1c* are given in Table 1. In the downfield region two weak signals were observed at 201.7 and 184.7 ppm, respectively. The former has practically the same shift as the signal from the carbonyl carbon of *3* and is therefore assigned to C-2′ of *1a*; the 184.7 ppm resonance is assigned to C-2′ of *1c*.

With the assignment of the NMR signals from *1a* and *1c* at hand, it was readily seen that either acidification or alkalization of the neutral solution leads to an increased content of *1a* and a corresponding decrease in *1c* (Fig. 2). Only these two forms could be observed by NMR measurements on aqueous solutions of *1*; we observed no signals which could be assigned to the carbinola-

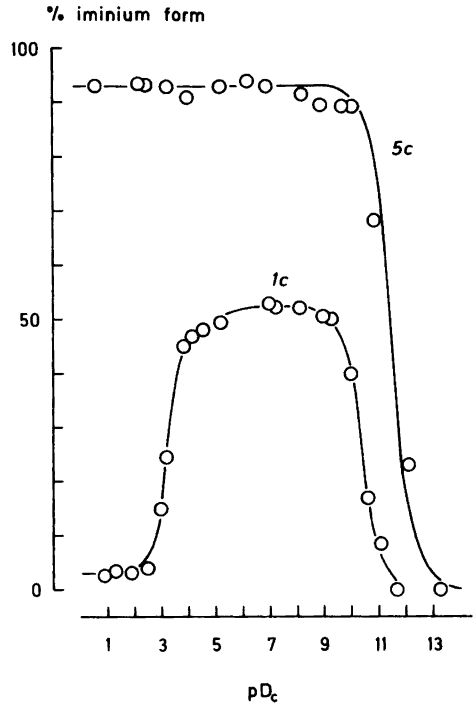


Fig. 2. Equilibrium contents of iminium forms *1c* and *5c* as a function of pD_c. For both compounds, the remaining part up to 100 %, as seen by ^{1}H NMR spectroscopy, is the amino ketone form, *i.e. 1a* and *5a*, respectively.

Table 1. ^{13}C NMR chemical shifts obtained for the pseudooxynicotine forms *1a*, *1c* and *1d*, for nicotine (*2*) and for the reference compound *3*.

Carbon No.	*1a* [a] pH 1	*1a* [a] pH 7	*1a* [a] pH 13	*1c* [a] pH 7	*1d* [b]	*2* [c] $pD_c>11$	*3* [d] $pD_c\approx13$
2	145.2	149.2	148.3	149.1	149.6*	149.0*	149.2
3	135.5	132.7	136.2*	124.6	148.6*	138.8*	133.0
4	142.4	137.6	136.9*	138.7	134.7*	137.3*	137.2
5	128.8	125.4	125.2	125.4	124.2*	125.5	125.3
6	146.5	153.7	151.1	154.3	152.0*	149.3*	153.7
2′	197.6	201.7	n.o.	184.7	130.4*	69.6	201.2
3′	36.8	36.4	39.9*	40.8	105.5	34.9	n.o.
4′	20.3	20.5	22.1	19.1	29.7	22.9	21.8
5′	49.1	49.4	51.9	64.7	57.0	57.4	58.7
N-CH_3	33.9	34.0	34.4*	39.9	40.5	40.4	45.1

[a] in H_2O; [b] in $(CD_3)_2SO$ with dioxane (67.40 ppm) as internal reference; [c] in D_2O ($pD_c>11$); shifts are relative to external TMS; [d] in D_2O ($pD_c\approx13$)+$(CD_3)_2SO$(≈1:2); *: tentative assignment; n.o.: not observed.

mine *1b* or the enamine *1d*. There was no spectral change near pD_c 4 in the ^1H and ^{13}C NMR spectra which Maeda *et al.*[8] claimed to occur in this pH region and which should be associated with a change from *1a* to *1d*. There is a gradual change in the NMR spectra between $pD_c \approx 2$ and 5 which is most pronounced between pD_c 3 and 4 but this must be attributed to the protonation of the pyridine ring; the protonation effect on the ^{13}C NMR spectrum of *1a* is evident from the values given in Table 1 (*cf.* the shifts for *1a* at pH 1 and 7).

Also at pH values higher than 7, the only forms of *1* observed by NMR spectroscopy were *1a* and *1c* (Fig. 2). The recording of the ^1H and, in particular, of the ^{13}C NMR spectra was, however, complicated by the instability of *1* in alkaline solutions. In the ^1H spectra, the signal ascribed to *N*-CH_3 in *1a* was at $\delta \approx 2.82$ up to $pD_c \approx 8.7$ (pD_c=pH meter reading+0.40 [12]). A further increase in pD_c led to upfield shifts; δ 2.18 being reached between pD_c 12 and 13. The magnitudes of pertinent chemical shifts indicate that the only form of *1* observed in strongly alkaline solution is *1a* rather than *1d* as claimed [8] by Maeda *et al.* Thus, in D_2O at pD_c 13 the two H-4′ hydrogens show a triplet at δ 1.89 but in distilled *1d*, dissolved in DMSO-d_6, they give a signal at δ 2.55. This difference in chemical shift seems too large to be due to a solvent effect on a C–H hydrogen but can be accounted for by assuming that the H-4′ hydrogens are allylic, as in *1d*, in DMSO-d_6 but not in D_2O solution, as in *1a*. Similar but smaller differences were also observed for the *N*-CH_3 and H-5′ hydrogens ($\Delta\delta$=0.27 and 0.45 ppm, respectively). The ^{13}C NMR spectra of *1a* in strongly alkaline aqueous solutions differed clearly from that of *1d* in DMSO-d_6 (Table 1). No signal from the carbonyl C-2′ in *1a* was detected under the actual recording conditions but this was considered less significant since *3*, which is more stable, behaved similarly and gave only a weak signal for C-2′ at pD_c 13.

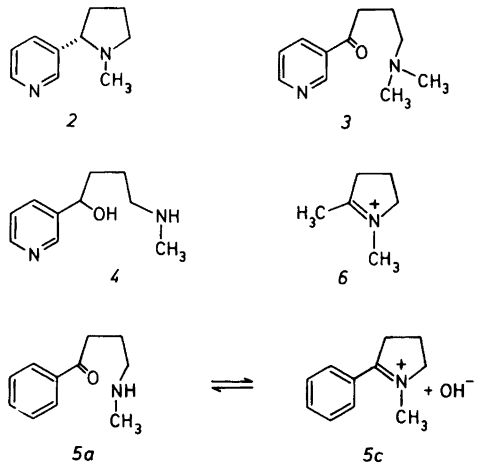
As seen in Table 1, the NMR signals obtained from the aromatic carbons of *1a* at pH 7 are close to those of *3* at $pD_c \approx 13$. This is reasonable since the pyridine ring in *1a* is unprotonated in neutral solution. However, a strongly alkaline solution of *1* gives a partly altered set of signals and this observation raises the question of whether the reactions *1a* ⇄ *1b* are rapid on the NMR time scale. Under the assumption that they are rapid, and if the equilibrium mol fraction of *1b* is large enough, one may expect alterations in the observed chemical shifts. To settle this matter we recorded the UV spectra of *1*, nicotine and 3-acetylpyridine in alkaline aqueous solutions (Fig. 3). The 230 nm band, which is common to *1* and 3-acetylpyridine only, seems to undergo a slight decrease in intensity with increasing pH but, since the two compounds behave similarly, this decrease could not be due to the formation of carbinolamine. If nicotine is accepted as a UV model compound for the carbinolamine *1b*, these spectra clearly show that the major form of pseudooxynicotine at pH 12 is the amino ketone *1a*. Similarly, the 3-indolyl analogue of *1c* gives an amino ketone on treatment with aqueous sodium hydroxide and extraction with ether.[13]

The enamine structure *1d* has been assigned to a tobacco alkaloid [14,16] and a metabolite [15] of nicotine or its *N*′-oxide. Our results show that if a single structure is to be assigned to this alkaloid (metabolite), the iminium form *1c* should be preferred as this form predominates at physiological pH.

Two chemical reactions with *1* in aqueous solution were carried out. When sodium borohydride was added to a weakly alkaline solution (pH 9.0) of *1* in water, nicotine was formed in large excess over the alcohol *4* (^1H NMR). At this pH, *1a* and *1c* are present in comparable amounts but *1c* is evidently reduced much faster than *1a*. When the reduction was started at pH 11.7, nicotine and *4* were formed in approximately equal amounts, demonstrating that the concentration of *1c* was much lower at this pH. These findings also show that the reaction *1a* → *1c* is fast enough to provide more *1c* as the reduction proceeds. Benzoylation of *1* with benzoyl chloride in strongly alkaline solution gave the *N*-benzoyl derivative of *1a* in an 87 % yield.

STUDIES OF ANALOGOUS COMPOUNDS

Some compounds analogous to *1* have also been studied with respect to ring-chain tautomerism. The crystalline perchlorates of *5c* [11] and *6* [21] were investigated by ^1H NMR spectroscopy as described above. The iminium ion *6* was the only form observed by ^1H NMR spectroscopy up to $pD_c \approx 12$. Compound *5*, like its pyridyl analogue



1, existed mainly as its amino ketone form (*5a*) and iminium form (*5c*); the percentage of *5c* is given as a function of pD_c in Fig. 2.

An investigation of neutral aqueous solutions of *5* and five analogues *p*- or *m*-substituted in the benzene ring shows that the equilibrium content of the iminium form increases when the substituent is electron-donating and decreases when it is electron-attracting. Moreover, some preliminary measurements indicate that the contents of iminium ion in acidic solutions are the same as in neutral solutions. Pseudooxynicotine (*1*) behaves differently and shows no constant level of iminium ion (*1c*) in the acidic region (Fig. 2). Two plateau levels can, however, be discerned, one at 2–3 % and the other at 52–53 %. These should correspond to the species in which the pyridine ring is protonated and unprotonated, respectively, and the mole fractions of these species are then in agreement with the substituent effects discussed above for *5* and its analogues.

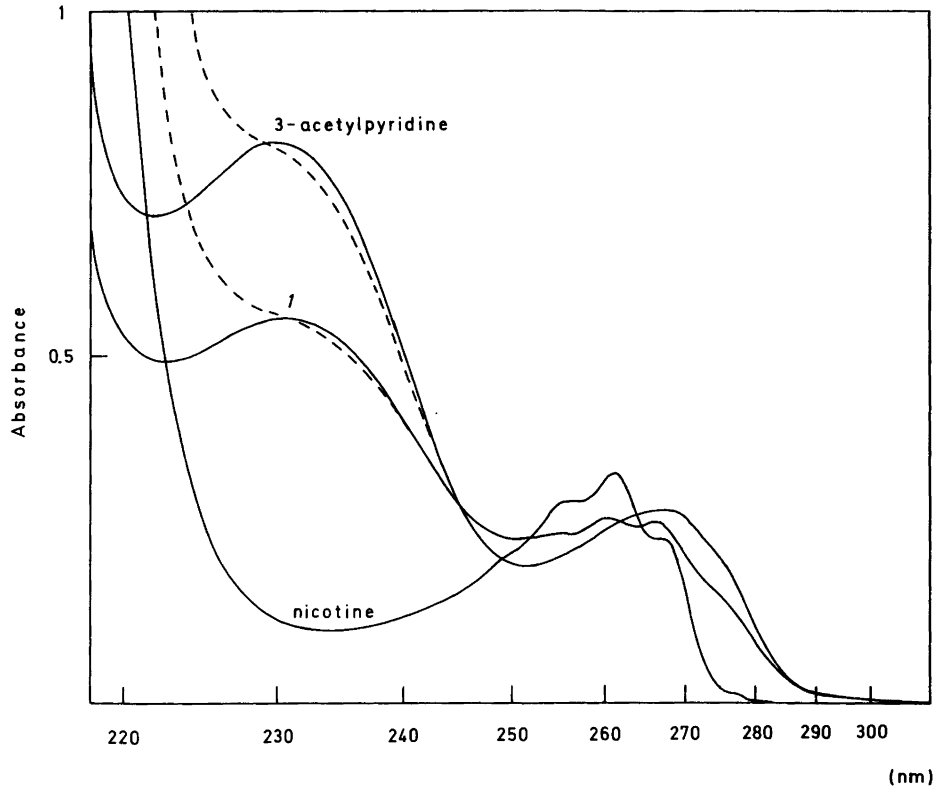


Fig. 3. UV spectra of nicotine (97 μM), pseudooxynicotine (*1*, 80 μM) and 3-acetylpyridine (83 μM) in H_2O at pH 11.9–12.0 (unbroken curves) and 12.9 (broken curves).

EXPERIMENTAL

^1H NMR spectra. In order to obtain simple spectra, isotope exchange of the H-3′ hydrogens was effected by treating the dihydrochloride twice with refluxing 20 % DCl in D_2O overnight. Solutions of *1* (0.04 M) were prepared by dissolving the resulting salt in D_2O and adjusting the pD_c with NaOD or DCl in D_2O. The ratios *1a*:*1c* are averages of the three ratios obtained from integration of the N-CH_3, H-4′ and H-4 signals. Spectra were recorded at 23–25 °C on a Varian XL-100 or JEOL FX-100 instrument using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal reference. ^1H NMR shifts measured in DMSO-d_6 or $CDCl_3$ are given relative to internal TMS.

^{13}C NMR spectra were recorded at 25 MHz using the same instruments. When the spectra of *1* were taken in strongly alkaline solutions (pH >10.5), the salt content was minimized by dissolving distilled *1d* in D_2O and then adding $NaOD/D_2O$. For the samples run in H_2O, a coaxial capillary containing D_2O was used for the locking and chemical shifts were calibrated from internal dioxane (67.40 ppm). The references used for the samples in D_2O and $(CD_3)_2SO$ were the same as those for the ^1H spectra. Single frequency off-resonance (SFOR) spectra were obtained by irradiating at 400 Hz upfield from TMS in the proton spectrum. Assignments of the signals were based on multiplicities obtained in SFOR spectra, chemical shift considerations,[17] results after exchanges of the H-3′ hydrogens in D_2O, comparison between *1* and *3*, and comparison of spectra run at pH 1 and pH 7.

Measurements of pD_c or pH were performed on a PHM 62 standard pH meter (Radiometer, Copenhagen, accuracy 0.01 unit); UV spectra were recorded on a Beckman DK-2 spectrometer. Melting points are corrected. Nicotine and 3-acetylpyridine were purchased from Merck and distilled before use.

Pseudooxynicotine dihydrochloride (*1a* dihydrochloride) was prepared from ethyl nicotinate and *N*-methylpyrrolidone as previously described,[18] but using sodium hydride in toluene (reflux, 3 h) as base instead of sodium ethoxide. After two recrystallizations from ethanol–light petroleum (1:1), the compound melted at 200–202 °C (sealed tube); lit.[5] m.p. 196–198 °C; IR (KBr): 1692 cm^{-1}, broad ammonium bands around 2400 cm^{-1}.

4-Dimethylamino-1-(3′-pyridinyl)-1-butanone (*3*) was prepared from ethyl nicotinate and ethyl 4-(dimethylamino)butanoate according to method B described for the diethylamino analogue.[19] ^1H NMR ($CDCl_3$): δ 9.2–7.1 (4 H), 2.95 (t, 2 H), 2.5–1.5 (10 H, including an N-CH_3 singlet at 2.11); ^{13}C NMR: See Table 1.

3,4-Dihydro-1-methyl-5-phenyl-2H-pyrrolium (5c) perchlorate and *3,4-dihydro-1,5-dimethyl-2H-pyrrolium (6) perchlorate* were prepared by reactions between the corresponding cyclic imines [20] and methyl iodide and subsequent ion exchange with silver perchlorate. The former iminium salt [11] showed m.p. 116–117 °C and the latter 241–243 °C; lit.[21] m.p. 239–241 °C.

5c: ^1H NMR (D_2O, pD_c 6.9): δ 7.7–7.6 (aromatic hydrogens), 4.36 (t, H-5′), 3.60 (s, N-CH_3), 3.24 (t, H-3′), 2.56–2.20 (m, H-4′).

5a: ^1H NMR (D_2O, pD_c 13.3): δ 8.0–7.4 (aromatic hydrogens), 2.58 (t, H-5′), 2.26 (s, N-CH_3), 1.84 (t, H-4′).

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Antagonists of Substance P from Emphasis on Position 11

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Ten analogs of substance P (SP) were designed and synthesized. The agonist and antagonist activities against SP were assayed on the isolated guinea pig ileum. The prime designs were changes of the important Met^{11} to include Leu^{11}, Thr^{11}, D-Leu^{11} and D-Ala^{11}. Step-wise designs of changing D-Arg^1 and D-Pro^2 to the corresponding L-configurations resulted in decreasing antagonist activity. Changing Leu^{11} to D-Leu^{11} and D-Ala^{11} reduced antagonist activity. [D-Arg_1,D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP is the most potent antagonist of this group of analogs, and required a 100-fold increase in the concentration of SP to give 50 % of the maximal response caused by SP.

The design and synthesis of effective antagonists of substrance P (SP), H-Arg,Pro,Lys,Pro,Gln,-Gln,Phe,Phe,Gly,Leu,Met,NH_2, evolved,[1–3] and ultimately two analogs were achieved which were found to be useful in physiological systems to study the actions of SP. These two analogs were [D-Pro^2,D-Phe^7,D-Trp^9]-SP and [D-Pro^2,D-$Trp^{7,9}$]-SP.[4] [D-Pro^2,D-$Trp^{7,9}$]-SP was the most potent of these two analogs, which at a concentration of 10^{-4} required a 22-fold increase in SP to allow the same twitch response of the guinea pig ileum as was obtained in the absence of the analog. Mizrahi *et al.*[5] investigated [D-Pro^4,D-$Trp^{7,9}$]-SP-(4–11) and [D-Pro^2,D$Trp^{7,9,10}$]-SP-(4–11) and reported that these analogs inhibited a hypotensive effect of SP in rats and were specific for SP in all pharmacological systems which they studied.

Exemplifying the extensive biological studies which have been made upon [D-Pro^2,D-Phe^7,D-Trp^9]-SP and [D-Pro^2,D-$Trp^{7,9}$]-SP are the following citations. Rosell *et al.*[6] studied the inhibition of antidromic and substance P-induced vasodilatation. Caranikas *et al.*[7] pharmacologically studied SP-antagonists. Bjorkroth *et al.*[8] pharmacologically characterized four SP-antagonists. Rosell and Folkers [9] critiqued SP-antagonists as a new type of pharmacological tool.

Holmdahl *et al.*[10] reported that [D-Pro^2,D-$Trp^{7,9}$]-SP inhibited inflammatory responses in the rabbit eye. Hokfelt *et al.*[11] described immunohistochemical evidence for a neurotoxic action of [D-Pro^2,D-$Trp^{7,9}$]-SP. Piercey *et al.*[12] described sensory and motor functions of spinal cord substance P and intraspinal antagonism of spinal cord substance P receptors which cause sensory and motor deficits.

Although some but not all of our early SP-antagonists [3,4] such as [D-Pro^2,D-$Trp^{7,9}$]-SP showed weak and transient agonist activity,the overriding antagonist activity proved to be useful in the early physiological studies on the actions of SP. For example, [D-Pro^2,D-$Trp^{7,9}$]-SP showed an agonist activity of 0.0007 in comparison with the relative potency of 100 for SP.[4] Even such very low agonist activity was considered to impair perhaps the use of such an antagonist in the pharmacological analysis of SP effects in the spinal cord and sympathetic ganglia, *etc.* Consequently, the design and synthesis of new analogs of SP toward peptides having zero agonist activity and a potency of antagonism considerably greater than that of the early antagonists was sought. We describe herein improved antagonists of substance P, from continuing designs, syntheses and bioassays, which have yielded [D-Arg^1,D-Pro^2,D-$Trp^{7,9}$,Leu^{11}]-SP. The first three reports of biological studies on [D-Arg^1,D-Pro^2,D-$Trp^{7,9}$,Leu^{11}]-

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SP are as follows. Rosell *et al.*[13] described the pharmacological profile of this analog as a new and specific antagonist of substance P, and evidence for the existence of subpopulations of SP-receptors. Lundberg *et al.*[14] found that this new antagonist inhibits vagally induced inflammation and bronchial smooth muscle contraction in the guinea pig. Yanagisawa *et al.*[15] showed that [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-SP blocks slow reflex responses in the spinal cord.

METHODS

The acquisition of protected amino acids, the synthesis of the peptides, the cleavage of the peptides from the resin, were conducted as described for the previous analogs and antagonists of substance P.[3]

The modified purification of the crude peptides, as carried out for the peptides described herein, was conducted as follows. Samples of about 200 mg of the crude peptides were applied to a column of Sephadex G-25 (100×2.5 cm) which had been equilibrated with 12 % acetic acid, and then the chromatography was carried out with the same solvent. Fractions of 10 ml were collected. The peptides were detected by spotting samples of the individual fractions on silica gel plates and conducting the chromatography with n-BuOH–HOAc–H_2O=4:1:2. The fractions containing the desired peptides were pooled and lyophilized, and generally yielded 75–100 mg of material. This lyophilized material was purified over a column of silica gel (1×60 cm) which had been equilibrated with the solvent mixture, n-BuOH–HOAc–H_2O=4:1:2. The chromatography was carried out with this same solvent system, and fractions of 4 ml were collected. The desired peptides, in general, were found in fractions 30–40. Those fractions which contained the pure or nearly pure peptide were collected and lyophilized. If the desired peptide were not sufficiently pure, it was again purified over silica gel using the same solvent system. The yields of the peptides were 20–50 %. Purity was more important than the yield, and ranged from 90 to 99 %, which was acceptable for the first sample for the assay and its error. The time for achievement of purity was balanced with the time of synthesis and assay and the achievement of higher potency of antagonism which was the important goal. The peptidic impurities were not a deterrent toward the goal. High performance liquid chromatography was conducted on a Water Liquid Chromatograph equipped with a Waters 660 solvent programmer. The samples were chromatographed on a μ-Bondapak C_{18} column (10μ), 3.9×300 mm. For elution of the peptides, a linear gradient from 20–100 % of a solvent system was used during 25 min. The solvent system consisted of 70 % of CH_3CN and 30 % 0.1M K H_2PO_4 buffer, pH 3. The flow rate was 2.0 ml/min and 10 μl of a 0.1 % solution of the peptide was injected. The eluted peptide was detected by its UV absorbance at 206 nm.

The amino acid analyses and the optical rotations were conducted as described.[4] The amino acid analytical data are as follows.

III. Glu 1.91(2); Pro 2.03(2); Leu 0.97(1); Phe 0.95(1); Met 0.95(1); Lys 0.95(1); Arg 0.95(1); NH_3 3.27(3); Trp +.

IV. Glu 2.00(2); Pro 2.04(2); Leu 2.07(2); Phe 0.98(1); Lys 0.96(1); Arg 0.96(1); Trp +; NH_3 +.

V. Glu 1.03(2); Pro 2.04(2); Leu 1.01(1); Phe 0.98(1); Thr 0.94(1); Lys 1.13(1); Arg 0.97(1); Trp +; NH_3 +.

VI. Glu 2.03(2); Pro 2.07(2); Leu 1.95(2); Phe 0.98(1); Lys 0.99(1); Arg 0.99(1); Trp +; NH_3 +.

VII. Glu 2.00(2); Pro 2.01(2); Leu 0.95(1); Phe 0.99(1); Ala 1.06(1); Lys 0.99(1); Arg 1.00(1); Trp +; NH_3 +.

VIII. Glu 1.96(2); Pro 2.12(2); Leu 2.01(2); Phe 0.95(1); Lys 1.00(1); Arg 0.96(1); Trp +; NH_3 +.

IX. Glu 1.98(2); Pro 2.35(2); Leu 1.97(2); Phe 0.96(1); Lys 0.97(1); Arg 0.93(1); Trp +; NH_3 +.

X. Glu 1.99(2); Pro 2.03(2); Leu 0.99(1); Val 0.96(1); Phe 1.02(1); Lys 1.01(1); Arg 1.01(1); Trp +; NH_3 +.

XI. Glu 2.04(2); Pro 3.03(2); Leu 2.00(2); Phe 0.87(1); Lys 1.01(1); Arg 1.06(1); Trp +; NH_3 +.

XII. Glu 2.05(2); Pro 1.98(2); Leu 0.94(1); Phe 0.93(1); Ala 1.11(1); Arg 0.98(1); Trp +; NH_3 +.

Table 1. Chemical data of the analogs of substance P Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2.

	Analog	R_F in solvent system [a] I	II	III	IV	V	Retention time in HPLC (min)	Purity (HPLC) *ca.* (%)	$[\alpha]_D$
III.	[D-Arg^1,D-Pro^2,D-Trp^{7,9}]-SP	0.89	0.07	0.60	0.45	0.18	15.6	97	−24 [c]
IV.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP	0.82	0	0.54	0.28	0.20	15.0	96	−50.4 [b]
V.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},Thr^{11}]-SP	0.93	0.01	0.52	0.28	0.22	14.5	96	−38.6 [b]
VI.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},D-Leu^{11}]-SP	0.92	0	0.57	0.23	0.16	13.2	94	−20.8 [b]
VII.	[D-Arg^1,D-Pro^2,D-Trp^{7,9},D-Ala^{11}]-SP	0.85	0.04	0.57	0.25	0.23	12.0	98	−20.4 [b]
VIII.	[D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP	0.82	0	0.57	0.35	0.18	16.5	95	−38.7 [b]
IX.	[D-Trp^{7,9},Leu^{11}]-SP	0.89	0	0.56	0.29	0.27	15.0	97	−36.4 [b]
X.	[D-Trp^{7,9},Val^{11}]-SP	0.89	0	0.59	0.27	0.27	14.5	95	−64.8 [b]
XI.	[D-Trp^{7,9},D-Leu^{11}]-SP	0.97	0	0.58	0.24	0.13	12.8	90	—
XII.	[D-Trp^{7,9},D-Ala^{11}]-SP	0.84	0.02	0.52	0.25	0.27	12.0	98	−10.4 [b]

[a] I: EtOAc:Py:HOAc:H_2O(5:5:1:3); II: n-BuOH:EtOAc:HOAc:H_2O(2:2:1:1); III: n-BuOH:Py:HOAc:H_2O-(30:30:6:24); IV: n-BuOH:Py:HOAc:H_2O(50:33:1:40); V; n-BuOH:HOAc:H_2O(4:1:2). [b] C 0.5 %, 12 % HOAc. [c] C 1 %, MeOH.

Table 2. Assay data from guinea pig ileum system.

	Analog	Agonist activity relative to SP at 100	Antagonist activity.[a] Analog conc. 10^{-4}
III.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9]-SP	0.002	16
IV.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP	0.0001	100
V.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,Thr^{11}]-SP	0.0002	5
VI.	[D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,D-Leu^{11}]-SP	0.0001	9
VII.	[D-Arg^1,D-Pro^2, D-Trp^7,D-Trp^9,D-Ala^{11}]-SP	0.0001	6
VIII.	[D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP	0.0001	70
IX.	[D-Trp^7,D-Trp^9,Leu^{11}]-SP	0.0001	34
X.	[D-Trp^7,D-Trp^9,Val^{11}]-SP	0.0001	31
XI.	[D-Trp^7,D-Trp^9,D-Leu^{11}]-SP	0.0001	14
XII.	[D-Trp^7,D-Trp^9,D-Ala^{11}]-SP	0.0001	2

[a] Fold-increase in SP conc. to give 50 % of max. response.

RESULTS AND DISCUSSION

Structure I represents substance P (SP). The antagonist activity of an analog of SP is expressed as the fold-increase in the concentration of SP in the presence of the antagonist to give 50 % of the maximal response caused by SP alone. The chemical data are in Table 1. The assay data are in Table 2. The biological activity of the SP-analogs was tested using the terminal portion of the guinea pig ileum in a 5-ml organ bath. The tests were carried out as described by Yamaguchi *et al.*[16]

[D-Pro^2,D-Trp^7,D-Trp^9]-SP (II) was found to require a 22-fold increase in SP at a concentration of 10^{-4} to give 50 % of maximal response by SP alone (Table 3).[4]

Arg^1 of II was changed to D-Arg^1 to give III, which reqquired a 16-fold increase, and showed for the pair, II and III, a reduction in activity on changing configuration from L to D for position 1.

It has been known that [desMet^{11}]-SP is substantially less active than SP. For example, on the basis of a relative activity of 1 for SP, [desMet^{11}]-SP had a relative activity of 0.0003, but when

Table 3. Fold increases.

					Fold increase in conc.
I.	Arg^1	$Pro^2Lys^3Pro^4Gln^5Gln^6$	Phe^7Phe^8	$Gly^9Leu^{10}Met^{11}$	
II.	[	Pro^2	D-Trp^7	D-Trp^9]-SP	22
III.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9]-SP	16
IV.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 Leu^{11}]-SP	100
V.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 Thr^{11}]-SP	5
VI.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 D-Leu^{11}]-SP	9
VII.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 D-Ala^{11}]-SP	6

Met^{11} was changed to Leu^{11}, the relative activity was 0.3 according to Yanaihara *et al.*[17] Weak agonist activity was improved 100-fold by the presence of the "non-functional" Leu^{11} in place of deletion of Met^{11}. In other words, the presence of a steric or carbon structure in position 11 improved agonist activity over that of no substituent in position 11.

The exchange of Met^{11} in III for Leu^{11} to give IV increased antagonist activity 6 times, since a 100-fold increase in the concentration of SP was required for a 50 % response in the presence of [D-Arg^1,D-Pro^2,D-Trp^7,D-Trp^9,Leu^{11}]-SP.

The change of Leu^{11} in IV to Thr^{11} in V reduced antagonist activity from 100- to 5-fold, showing some specificity in the requirement of the nature of the amino acid in position 11.

Changing Leu^{11} in analog IV to D-Leu^{11} for analog VI reduced activity from 100- to 9-fold, and showed the apparent essentiality of an L-configuration in position 11 in contrast to the corresponding D-configuration.

Substituting D-Ala^{11} as in analog VII for D-Leu^{11} as in analog VI resulted in a negligible difference between these two D-amino acids in position 11 (Table 4)

When D-Arg^1 of the analog IV was changed to Arg^1 of the natural configuration in analog VIII, the increase in concentration was decreased from 100 to 70.

When D-Pro^2 of analog VIII was changed to Pro^2 of the natural configuration to give analog IV, the concentration was decreased from 70 to 34.

When Leu^{11} of analog IX was changed to the closely related Val^{11} to give analog X, there was negligible change in the concentration, *i.e.*, 34 to 31.

When Leu^{11} of analog IV was changed to give the two analogs, D-Leu^{11} and D-Ala^{11} for analogs XI and XII, there were significant decreases in the concentration which were from 34 to 14 and to 2, respectively.

The ten analogs were tested for agonist activity in comparison with SP on the guinea pig ileum. The data are in Table 2 and show that these analogs had little or no agonist activity. It is understood that a most effective antagonist will likely have zero agonist activity.

Table 4. Fold increases.

					Fold increase in conc.
IV.	[D-Arg^1D-Pro^2		D-Trp^7	D-Trp^9 Leu^{11}]-SP	100
VIII.	[	D-Pro^2	D-Trp^7	D-Trp^9 Leu^{11}]-SP	70
IX.	[		D-Trp^7	D-Trp^9 Leu^{11}]-SP	34
X.	[		D-Trp^7	D-Trp^9 Val^{11}]-SP	31
XI.	[		D-Trp^7	D-Trp^9 D-Leu^{11}]-SP	14
XII.	[		D-Trp^7	D-Trp^9 D-Ala^{11}]-SP	2

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Synthesis of Disaccharides Related to the *O*-Specific Polysaccharide of *Salmonella typhimurium*

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Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (*13*) has been glycosylated with 3,6-dideoxy-2,4-di-*O*-*p*-nitrobenzoyl-α-D-*xylo*-hexopyranosyl bromide (*4*) and its enantiomer (*5*) using mercury cyanide as catalyst and toluene and nitromethane as solvent. The anomeric ratio has been determined by ^{1}H NMR spectroscopy and is reversed going from the D to the L compound. Glycosylation of *13* with 2-*O*-benzyl-3,6-dideoxy-4-*O*-*p*-nitrobenzoyl-α-D-*xylo*-hexopyranosyl bromide (*9*) under similar reaction conditions gives exclusively the α-linked disaccharide while glycosylation using 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl chloride (*12*) gives a 1:3 mixture of β- and α-linked disaccharides. Glycosylation of *13* with *12*, catalyzed by tetrabutylammonium bromide at elevated temperature, yields exclusively the α-linked disaccharide. The conformation of two of the deprotected disaccharides has been determined using hard sphere calculations and high field NMR data.

The disaccharide 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranose constitutes an important part of the determinant of the *O*-specific polysaccharide of *Salmonella typhimurium* and other species of the *Salmonella* serogroup B. The synthesis of glycosides of this disaccharide, and of larger oligosaccharide parts of the *O*-specific chain, has been previously published by Garegg *et al.*[1–5] As part of a programme to synthesize larger oligosaccharides containing α-linked 3,6-dideoxy-α-D- (or L)-hexopyranoses we have developed a novel route to crystalline derivatives of 3,6-dideoxy-α-D-*xylo*-hexopyranosyl halides, which have been subjected to reaction with methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside under varying conditions in order to optimize the glycosylation reaction. The conformation in aqueous solution of the disaccharides, possessing the β-D and α-L glycosidic configurations, has been deduced from NMR data and hard sphere calculations (HSEA).[6,7] Conformational data on the disaccharide with the α-D glycosidic configuration will be discussed in a forthcoming publication [8] together with other oligosaccharides related to the *O*-specific polysaccharide of a *Salmonella* strain.

RESULTS

Reduction of 3,6-dideoxy-D-*xylo*-hexono-1,4-lactone (*1*) (or the L form), obtained from D-(respectively-L)-galactono-1,4-lactone,[9] with diisoamyl borane in tetrahydrofuran afforded 3,6-dideoxy-D-*xylo*-hexose (*2*) in an overall yield from galactone-1,4-lactone of 62 %. Low temperature acylation with *p*-nitrobenzoyl chloride in pyridine, with 4-(dimethylamino)-pyridine as a catalyst, gave a 91 % yield of *3* as a mixture containing 80 % of the β-pyranose form. Treatment of the mixture with hydrogen bromide in acetic acid and dichloromethane gave, after chromatographic separation, the crystalline 3,6-dideoxy-2,4-di-*O*-*p*-nitrobenzoyl-α-D-*xylo*-hexopyranosyl bromide, (*4*) in 31 % yield. Analogous reactions were carried out within the L-series giving the crystalline bromide (*5*). When the crude bromide was reacted with methanol and silver carbonate in dichloromethane, crystalline

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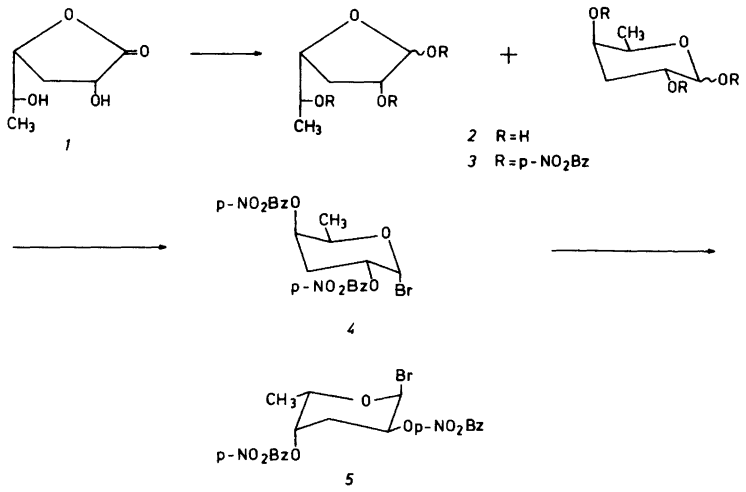


methyl 3,6-dideoxy-2,4-di-*O*-*p*-nitrobenzoyl-β-D-*xylo*-hexopyranoside (*6*) was obtained in a 55 % overall yield from *3*. Selective deacylation of *6* with potassium carbonate in methanol gave a 47 % yield of *7*, which was benzylated according to Klemer [10] to give *8* (88 % yield), and converted on treatment with hydrogen bromide in a dichloromethane, into the unstable bromide (*9*). Some decomposition was observed during the last step of this reaction.

Complete deacylation of *6* with sodium methoxide in methanol gave a 95 % yield of methyl 3,6-dideoxy-β-D-*xylo*-hexopyranoside (*10*), benzylation of which afforded the dibenzyl derivative (*11*) in 84 % yield. The glycoside (*11*) was reacted with hydrogen chloride in ether to give the chloride (*12*) in 95 % yield. The glycosyl halides (*4*), (*5*), (*9*) and (*12*) was individually reacted with excess of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (*13*), prepared according to Garegg *et al.*,[11] in order to compare the selectivity in the oligosaccharide synthesis. The results and conditions are presented in Table 1.

It is obvious that the highest selectivity in the preparation of 3,6-dideoxy-α-D-*xylo*-hexopyranosyl derivatives is achieved with tetrabutylammonium bromide as a catalyst and DMF–toluene (1:20) as the solvent. The reactions of halides (*4*) and (*5*) with *13* are not stereoselective and cannot be recommended for the synthesis of larger oligosaccharides. The glycosyl chloride (*12*), reacted under conditions A (Table 1), produces a high yield of disaccharide (70 %) with an acceptable stereoselectivity.

The disaccharides (*14*), (*15*), (*16*) and (*17*) were separated, purified and deacylated, followed by removal of the benzylidene groups

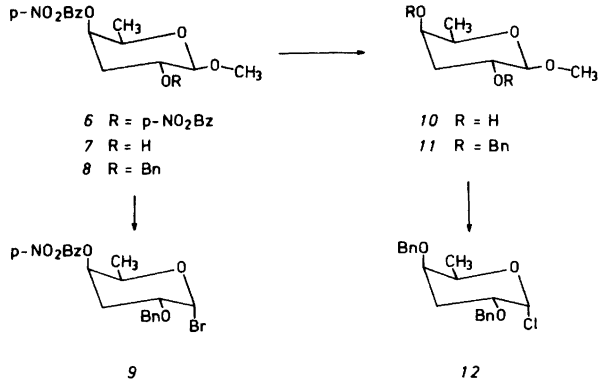


Table 1. Reactions and yields.

Compound	Reaction conditions [a]	Time/h	Temp./°C	Anomeric ratio (α/β) measured by NMR [b]	Anomeric ratio (α/β) of isolated products	Total yield of isolated disaccharides/%
4+13	A	40	20	0.56/0.44	0.77/0.23 [c]	43
5+13	A	96	−15	0.42/0.58	0.52/0.48 [d]	71
9+13	A	14	0	0.90/0.10 [e]	0.97/0.03	39
12+13	A	13	0	0.71/0.29	0.71/0.29	70
12+13	B	24	−50	decomposition	—	—
12+13	C	96	50	0.94/0.06	0.95/0.05	40

[a] A: $Hg(CN)_2$, toluene–nitromethane (1:1). B: AgTfl, CH_3CN. C: $(Bu)_4NBr$, DMF–toluene (1:20). [b] H6.1 and –OMe signals were integrated on the crude reaction mixtures. [c] Difficulties encountered in distinguishing the β-anomer from excess of aglycone. Minor by-products as reported.[1] [d] Difficulties in making β-anomer free from excess of aglycone. No by-products observed. [e] Several by-products obscured the interpretation of the ^1H NMR spectrum.

using aqueous acetic acid. Finally, the *O*-benzyl groups were removed by hydrogenolysis with palladium on charcoal and the free disaccharides (*18*), (*19*), (*20*) and (*21*) were isolated in yields of 57, 36, 57 and 19 %, respectively.

CONFORMATIONAL ANALYSIS

The disaccharides (*19*), (*20*) and (*21*) were subjected to conformational analysis using a combination of HSEA calculations and NMR data as published previously.[6,7] The resulting minimum energy conformations are given in Table 2 together with the ^1H NMR chemical shift changes. In Fig. 1 are shown the CPK models of the disaccharides (*19*) and (*20*) in their minimum energy conformations.

The results of nuclear Overhauser enhancement (n.O.e.) experiments are presented in Table 3; it is seen that both these and the chemical shift changes are in good agreement with the calculated minimum energy conformations of (*19*) and (*20*). Thus in (*19*) the observed

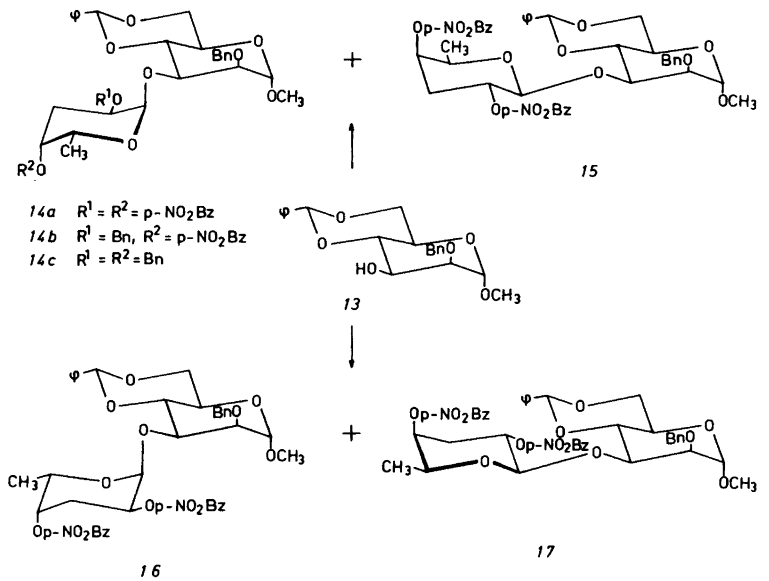


Table 2. Calculated minimum energy conformations [a] and observed ^{1}H NMR deshieldings [b] of methyl 3-*O*-(3,6-dideoxy-*xylo*-hexopyranosyl)-α-D-mannopyranosides.

Compound	ϕ_H°	ψ_H°	Interaction	Calculated Distance/Å	Deshielding ppm
19	54	14	H-3.2–O-5.1	2.52	0.18
20	45	30	H-5.1–O-3.2	2.49	
			H-5.1–O-4.2	2.60	0.30
			H-3.2–O-5.1	2.59	0.07
21	300	10	H-2.2–O-5.1	2.70	0.25
			H-3.2–O-5.1	2.57	0.13

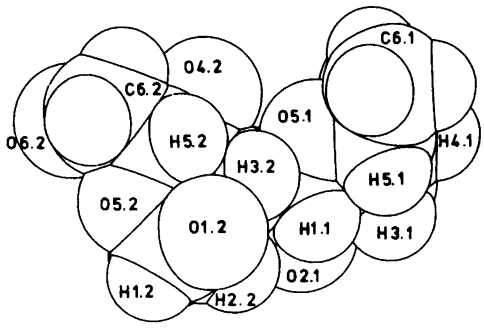
[a] The ϕ_H, ψ_H angles have been calculated in 2° steps and are considered significant within ±10°. [b] Comparison with model methyl glycosides.

relative n.O.e. from H-1.1 to H-2.2 and H-3.2 of 0.23 and 0.42, respectively, are in reasonable agreement with the calculated values of 0.27 and 0.38, respectively. Furthermore, H-3.2 is deshielded 0.18 ppm by *O*-5.1. In *20* both *O*-3.2 and *O*-4.2 are close to H-5.1 and H-5.1 is therefore deshielded 0.30 ppm. H-1.1 of *20* is relaxed by both H-2.2 and H-3.2 and the observed relative n.O.e.'s of 0.35 and 0.25, respectively, are in excellent agreement with the calculated values of 0.36 and 0.24, respectively. The expected deshielding of H-3.2 by the ring-oxygen in *19* and *20* is not considered significant,[12] this can best be explained by the reduced electronegativity of the ring-oxygen compared to that of a hydroxyl group.

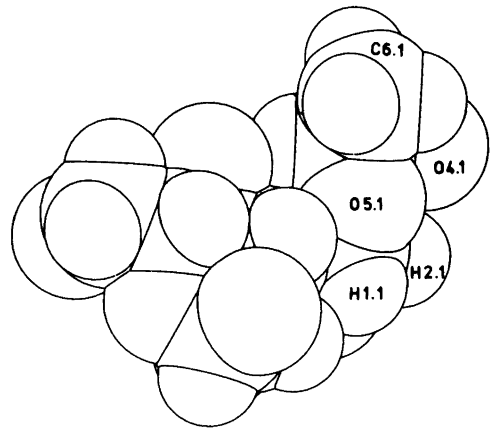
EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. NMR spectra were obtained on Bruker WH-90 and HX 270 instruments. The spectra were measured in $CDCl_3$ or in D_2O solution with acetone as the internal reference (2.12 ppm). ^{13}C NMR spectra were measured in D_2O with dioxane as internal reference (67.4 ppm). Microanalyses were performed by Novo microanalytical laboratory.

3,6-Dideoxy-D-xylo-*hexose* (*2*). 2-Methyl-2-butene (34.0 ml, 321 mmol) was added dropwise over a period of 45 min to a stirred solution of

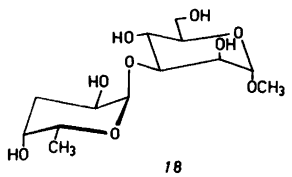


α-D-Man β-D-3,6-Dideoxy-*xylo*-hexose



α-D-Man α-L-3,6-Dideoxy-*xylo*-hexose

Fig. 1. CPK models of disaccharides (*19*) and (*20*) in the minimum energy conformation given in Table 1.



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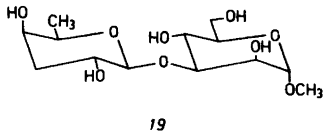

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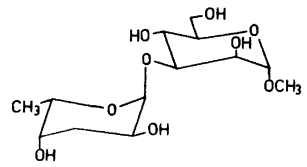

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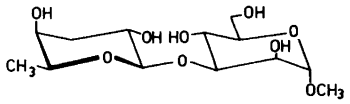

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borane dimethyl sulfide-complex (16.0 ml, 160 mmol BH_3) in freshly distilled THF (16 ml) at 5 °C in an atmosphere of argon. The mixture was stirred at 25 °C for 3 h and cooled to 5 °C. 3,6-Dideoxy-D-*xylo*-hexono-1,4-lactone [9] (4.09 g, 28.0 mmol) dissolved in THF (60 ml), was added dropwise at 5 °C over a period of 1 h. The mixture was stirred for 15 h at 20 °C. Water (20 ml) was added dropwise with stirring over a period of 45 min, whereupon the mixture was refluxed for 4 h, and concentrated *in vacuo*. The organic phase was extracted twice with water (50 ml). The combined aqueous phases were extracted twice with dichloromethane (100 and 50 ml) followed by diethyl ether (25 ml). Evaporation at 40 °C, 0.5 mm Hg gave 4.19 g (100 %) of glassy material (*2*). ^{13}C NMR: α-pyranose: 92.4 ppm (C-1); 63.9 (C-2); 33.0 (C-3); 66.8 (C-4); 69.2 (C-5); 16.4 (C-6), β-pyranose: 99.0 ppm (C-1); 67.2 (C-2); 37.9 (C-3); 69.2 (C-4); 74.9 (C-5) 16.7 (C-6), α-furanose: 95.9 ppm (C-1); 71.9 (C-2); 32.3 (C-3); 81.9 (C-4); 71.9 (C-5); 18.7 (C-6), β-furanose: 103.0 ppm (C-1); 76.2 (C-2); 34.5 (C-3); 83.0 (C-4); 70.4 (C-5); 19.1 (C-6).

3,6-Dideoxy-1,2,4-tri-O-p-*nitrobenzoyl*-β-D-xylo-*hexopyranose (3)*. The anomeric mixture of *2* (1.30 g, 8.8 mmol) was dissolved in pyridine (60 ml) and 4-dimethylamino-pyridine (100 mg) was added. The mixture was stirred and cooled to 0 °C. Finally, powdered *p*-nitrobenzoyl chloride (9.3 g, 50 mmol) was added in small portions over a period of 4 h at 0 °C. The mixture was stirred overnight at 20 °C. Water (20 ml) and dichloromethane (10 ml) were added and the stirring was continued for 2 h. The mixture was diluted with

Table 3. Observed [a] and calculated [b] nuclear Overhauser enhancements for disaccharides containing 3,6-dideoxy-*xylo*-hexopyranose.

Compound	Proton saturated	Relative and observed [a] NOE for protons			Calculated relative [b] NOE for protons		
19	H-1.1	H-5.1 0.36 (12.5 %)	H-2.2 0.23 (8.0 %)	H-3.2 0.42 (14.7 %)	H-5.1 0.35	H-2.2 0.27	H-3.2 0.38
20	H-1.1	H-2.1 0.41 (15.7 %)	H-2.2 0.35 (13.3 %)	H-3.2 0.25 (9.6 %)	H-2.1 0.41	H-2.2 0.36	H-3.2 0.24

[a] Performed in the difference mode. Accuracy considered to be of the order ±10 %. [b] Calculated for the minimum energy conformation as described in Ref. 6.

dichloromethane (75 ml) and extracted with water (250 ml). Successive washings with saturated aqueous sodium hydrogencarbonate, 2 M sulfuric acid, water, sodium hydrogencarbonate solution and water gave after drying and concentration *in vacuo* 4.76 g (91 %) of a mixture of *2* containing 80 % pyranose derivatives according to a proton NMR spectrum. ^{1}H NMR: *α*-pyranose: δ 6.73 (H-1); 5.62 (H-2); 2.56 (H-3a); 2.56 (H-3e); 5.46 (H-4); 4.42 (H-5); 1.30 (H-6). J_{12} 3.8 Hz, J_{45} 2.0, J_{56} 6.0. *β*-pyranose: δ 6.18 (H-1); 5.58 (H-2); 2.23 (H-3a); 2.77 (H-3e); 5.36 (H-4); 4.24 (H-5); 1.35 (H-6). J_{12} 8.1 Hz; J_{23a} 12.2; J_{23e} 5.6; J_{3a3e} 14.5; J_{3a4} 3.0; J_{3e4} 3.2; J_{45} 1.3; J_{56} 6.4.

3,6-Dideoxy-2,4 di-O-p-*nitrobenzoyl-α*-D-xylo-*hexopyranosyl bromide (4).* Crude *3* (4.5 g, 7.6 mmol) was dissolved in dichloromethane (15 ml) and cooled to 0 °C. Acetic acid saturated with hydrogen bromide (20 ml) was added and the mixture was stirred at 0 °C for 1 h. Filtration followed by dilution with dichloromethane (30 ml) and washing of the organic phase twice with ice water (50 ml), cold saturated sodium hydrogencarbonate (50 ml) and ice water, followed by drying (magnesium sulfate) and evaporation *in vacuo* gave a syrup which was purified on a silica gel column (400 g, ethyl acetate–pentane: 1:3). Concentration of the eluent at 30 °C and crystallization from diethyl ether (50 ml) gave 1.37 g bromide (*4*) (31 % from *2*), $[\alpha]_D^{20}$=+249° (c 0.2, $CHCl_3$), m.p.=91–95 °C, ^{1}H NMR: δ 6.83 (H-1); 5.28 (H-2); 2.59 (H-3a); 2.30 (H-3e); 5.39 (H-4); 4.44 (H-5); 1.31 (H-6). J_{12} 3.3 Hz; J_{23a} 11.6; J_{23e} 5,3; J_{3a3e} 12; J_{3a4} 3; J_{3e4} 3.2; J_{45} 1.3; J_{56} 6.8.

3,6 Dideoxy-2,4-di-O-p-*nitrobenzoyl-α*-L-xylo-*hexopyranosyl bromide (5).* This compound was prepared in the same way, starting from L-galactono-1,4-lactone, giving 30 % yield of bromide, *5*. $[\alpha]_D^{20}$=−255° (c 0.2, $CHCl_3$) m.p. 94–100 °C.

Methyl 3,6-dideoxy-2,4-di-O-p-*nitrobenzoyl-β*-D-xylo-*hexopyranoside (6).* The bromide *4* (prepared from *3* (4.50 g, 7.6 mmol)) was stirred with dry methanol (2.5 ml) in dry dichloromethane (15 ml) in the presence of silver carbonate (4.28 g, 14 mmol) for 16 h under a nitrogen atmosphere. The mixture was filtered through celite and evaporated *in vacuo* to give 3.3 g of syrup, which was dissolved in hot ethyl acetate (8 ml) and crystallized by slow addition of pentane (17 ml). Cooling to 0 °C and filtration afforded 1.43 g of *6*. Concentration of the mother liquor and chromatographic separation on silica gel (ethyl acetate–light petroleum 2:3) followed by crystallization gave another 0.49 g (total yield from *3*: 55 %). $[\alpha]_D^{20}$=+81° (c 0.3, $CHCl_3$), m.p. 177–178 °C, ^{1}H NMR: δ 4.59 (H-1); 5.23 (H-2); 2.02 (H-3a); 2.62 (H-3e); 5.26 (H-4); 3.98 (H-5); 1.33 (H-6). J_{12} 7.9; Hz; J_{23a} 10.8; J_{23e} 5.3; J_{3a3e} 14.1; J_{3a4} 3.0; J_{3e4} 3.3; J_{45} 1.3; J_{56} 6.5. Anal. $C_{21}H_{20}N_2O_{10}$: C, H, N.

Methyl 3,6-dideoxy-4-O-p-*nitrobenzoyl-β*-D-xylo-*hexopyranoside (7).* The methyl glycoside (*6*) (0.61 g, 1.3 mmol) was dissolved in hot methanol (50 ml) and cooled to 0 °C with stirring. Anhydrous potassium carbonate (60 mg, 0.43 mmol) was added and the stirring was continued for 75 min at 0 °C. The reaction was stopped by addition of concentrated hydrochloric acid (60 μl) followed by evaporation *in vacuo*. The residue was dissolved in dichloromethane (50 ml) and washed twice with water (10 ml). After drying (magnesium sulfate), filtration and evaporation *in vacuo* the product was purified by column chromatography (ethyl acetate–light petroleum: 7:5) giving 195 mg of *7* (47 %). $[\alpha]_D^{23}$ −65° (c 0.6, $CHCl_3$) m.p.: 129.5–131.0 °C ^{1}H NMR: δ 4.21 (H-1); 3.76 (H-2); 1.80 (H-3a); 2.41 (H-3e); 5.19 (H-4); 3.87 (H-5); 1.27 (H-6). J_{12} 7.5 Hz; J_{23a} 11.3; J_{23e} 5.6; J_{3a3e} 14.3; J_{3a4} 3.0; J_{3e4} 3.0; J_{45} 1.3; J_{56} 6.3. Anal. $C_{14}H_{17}NO_7$: C, H.

Methyl 2-O-*benzyl-3,6-dideoxy-4*-O-p-*nitrobenzoyl-β*-D-xylo-*hexopyranoside (8).* The glycoside *7* (177 mg, 0.57 mmol) was dissolved in dry toluene (5 ml), benzyl bromide (0.23 ml, 1.9 mmol) and silver oxide (350 mg 1.5 mmol) was added in the dark. The mixture was stirred for 24 h at 20 °C. Benzyl bromide (0.115 ml, 0.95 mmol) and silver oxide (150 mg, 0.65 mmol) were added and stirring continued in the dark for 24 h at 20 °C. After filtration and evaporation the material was purified on a silica gel column (ethyl acetate–light petroleum 3:7) giving 200 mg of *8* (88 %). $[\alpha]_D^{20}$ +47° (c 0.1, $CHCl_3$)(reported [1] +50° ($CHCl_3$)) ^{1}H NMR: δ 4.35 (H-1); 3.55 (H-2); 1.82 (H-3a); 2.36 (H-3e); 5.17 (H-4); 3.83 (H-5); 1.25 (H6). J_{12} 7.5 Hz; J_{23a} 11.0; J_{23e} 5.0; J_{3a3e} 14.3; J_{3a4} 3.0; J_{3e4} 3.0; J_{45} 1.3; J_{56} 6.6.

2-O-*Benzyl-3,6-dideoxy-4*-O-p-*nitrobenzoyl-β*-D-xylo-*hexopyranosyl bromide (9).* The methyl glycoside (*8*) (170 mg, 0.423 mmol) was dissolved in dry dichloromethane (10 ml) and cooled to −20 °C with stirring. Dry hydrogen bromide (5.7 mmol) in dichloromethane (10 ml) was added and the stirring was continued for 1 h and 45 min at −20 °C. The mixture was evaporated *in vacuo* at 0 °C and the evaporation was repeated with chloroform (5 ml) to give 195 mg (~100 %) of a light yellow syrup, which was used immediately for condensation. ^{1}H NMR: δ 6.65 (H-1); 3.73 (H-2); 2.28 (H-3a); 2.23 (H-3e); 5.26 (H-4); 4.32 (H-5); 1.23 (H-6). J_{12} 3.0 Hz; J_{23a} 10.5; J_{23e} 6.0; J_{3a4} 3.0; J_{3e4} 3.0; J_{45} 1.5; J_{56} 6.2.

Methyl 3,6-dideoxy-β-D-xylo-*hexopyranoside*

(10). Methyl 3,6-dideoxy-2,4-di-*O*-*p*-nitrobenzoyl-β-D-*xylo*-hexopyranoside (*6*) (1.23 g 2.68 mmol) was suspended in methanol (5 ml) and sodium methoxide (0.1 M, 20 ml) was added. The mixture was stirred for 2.5 h at 20 °C, cooled to 0 °C and filtered. The filtrate was neutralized by stirring with ion exchange resin (Amberlite IRC 50) for 1 h. Concentration to 10 ml followed by addition of water (20 ml), filtration and evaporation gave 530 mg of a syrup which was purified by preparative TLC (methanol–ethyl acetate: 3:17) to give 413 mg (95 %) of *10* as a syrup. $[\alpha]_D^{23}$ −66° (c 0.9, MeOH), (Reported [13] −69 °) ^{1}H NMR: δ 4.17 (H-1); 3.54 (H-2); 1.61 (H-3a): 2.04 (H-3e); 3.68 (H-4); 3.70 (H-5); 1.10 (H-6). J_{12} 8.0 Hz; J_{23a} 12.4; J_{23e} 4.4; J_{3a3e} 14.2; J_{3a4} 3.2; J_{3e4} 3.2; J_{45} 1.2; J_{56} 6.2.

*Methyl 2,4-di-*O*-benzyl-3,6-dideoxy-β-*D*-xylo-hexopyranoside (11)*. The glycoside (*10*) (413 mg, 2.56 mmol) was dissolved n dry *N,N*-dimethylformamide (DMF) (8 ml) and added dropwise to a stirred suspension of sodium hydride (500 mg, 11.5 mmol) in DMF. The mixture was stirred for 30 min and benzyl bromide (1.22 ml, 10.3 mmol) was added dropwise. After 3 h of stirring at 20 °C TLC (ethyl acetate–light petroleum 3:17) showed the reaction to be complete. The mixture was left overnight and methanol (2 ml) was added by stirring, which was continued for 2 h.

The mixture was poured into water (50 ml) and extracted 3 times with ethyl acetate (30 ml). The extract was washed twice with water (30 ml) dried (magnesium sulfate),filtered and evaporated *in vacuo* to give 1.18 g. Separation on a silica gel column (ethyl acetate–light petroleum 3:17) gave 730 mg (84 %) of *11*. $[\alpha]_D^{20}$ −55° (c 0.3, $CHCl_3$). ^{1}H NMR: δ 4.26 (H-1); 3.54 (H-2); 1.46 (H-3a); 2.31 (H-3e); 3.34 (H-4); 3.59 (H-5); 1.25 (H-6). J_{12} 7.5 Hz; J_{23a} 10.5; J_{23e} 5.3; J_{3a3e} 14.2; J_{3a4} 2.9; J_{3e4} 3.4; J_{45} 1.4; J_{56} 6.0. Anal. $C_{21}H_{26}O_4$: C, H.

*2,4-Di-*O*-benzyl-3,6-dideoxy-α-*D*-xylo-hexopyranosyl chloride (12)*. The glycoside (*11*) (200 mg, 0.58 mmol) was dissolved by stirring in dry diethyl ether (15 ml) and the solution was saturated with dry hydrogen chloride. After stirring at 20 °C for 35 min the solution was again saturated with HCl and the stirring was continued for 1 h and 10 min. Toluene (3 ml) was added and the mixture was evaporated *in vacuo*. Evaporation was repeated with toluene (5 ml) giving 190 mg (95 %) of *12*. ^{1}H NMR: δ 6.23 (H-1); 4.00 (H-2); 1.95 (H-3a); 2.18 (H-3e); 3.46 (H-4); 4.13 (H-5); 1.22 (H-6). J_{12} 3.4 Hz; J_{23a} 11.2; J_{23e} 4.7; J_{3a3e} 12.5; J_{3a4} 2.3; J_{3e4} 1.2; J_{45} 1.7; J_{56} 6.5. The chloride was used immediately in the next step due to instability.

*Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(3,6-dideoxy-2,4-di-*O*-p-nitrobenzoyl-α-*D*-xylo-hexopyranosyl)-α-*D*-mannopyranoside (14a) and β-isomer (15)*. Methyl 2-*O*-benzyl-4,6-*O*-benzyliden-α-D-mannopyranoside (*13*) [11] (558 mg, 1.5 mmol) was dissolved in dry toluene (20 ml) and toluene (15 ml) was distilled off at atmospheric pressure. Dry nitromethane (20 ml), mercury cyanide (2.53 g, 10 mmol) and molecular sieves (4Å, 5g) were added and the mixture was stirred under a nitrogen atmosphere for 16 h at 20 °C. A solution of *4* (559 mg, 1.1 mmol) in toluene (12 ml) was dried over molecular sieves for 1 h and added dropwise during 6 h. The mixture was stirred for 40 h at 20 °C. It was filtered through celite and the filter was washed with dichloromethane (50 ml). The filtrate was washed with water (100 ml), saturated sodium hydrogencarbonate solution (100 ml) and water (100 ml). Drying (magnesium sulfate), filtration and evaporation gave 1.00 g of a glassy syrup. ^{1}H NMR (270 MHz) showed that the glycosylation was almost quantitative with the anomeric ratio of α/β=0.56/0.44. The disaccharides were separated on a silica gel column (ethyl acetate–toluene: 1:4) and the first fraction gave 283 mg (32 %) of α anomer (*14a*) which was crystallized from ether–pentane (1:2), yield 232 mg $[\alpha]_D^{23}$ +95° (c 0.1, $CHCl_3$) (reported [1] +90°) m.p. 104.5–109.0 °C (reported: 105–108 °C).[1] ^{1}H NMR: δ 5.40 (H-1.1); 5.37 (H-2.1); 2.40 (H-3.1a); 2.19 (H-3.1e); 5.17 (H-4.1); 3.39 (H-5.1); 1.09 (H-6.1); 4.83 (H-1.2); 3.82 (H-2.2); 4.28 (H-3.2); 4.05 (H-4.2); 3.72 (H-5.2); 4.14 (H-6.2); 3.77 (H-6.2′). $J_{12.1}$ 3.5 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 4.5; $J_{3a3e.1}$ 13.5; $J_{3a4.1}$ 2.0; $J_{3e4.1}$ 1.0; $J_{45.1}$ 2.0; $J_{56.1}$ 7.0; $J_{12.2}$ 2.0; $J_{23.2}$ 3.2; $J_{34.2}$ 10.0.

The second fraction was acetylated with acetic anhydride in pyridine in order to change the polarity of the aglycone. Purification by preparative TLC (toluen–ethyl acetate: 4:1) gave 100 mg (11 %) crystalline (*15*), which was recrystallized from ether–pentane, $[\alpha]_D^{22}$ +51° (c 0.2 $CHCl_3$), m.p. 108–113 °C. ^{1}H NMR: δ 4.93 (H-1.1); 5.29 (H-2.1); 1.97 (H-3.1a); 2.66 (H-3.1e); 5.24 (H-4.1); 3.87 (H-5.1); 1.21 (H-6.1); 4.61 (H-1.2); 3.66 (H-2.2); 4.28 (H-3.2); 4.22 (H-4.2); 3.87 (H-5.2); 4.26 (H-6.2); 3.87 (H-6.2′). $J_{12.1}$ 8.0 Hz; $J_{23a.1}$ 11.5; $J_{23e.1}$ 5.0; $J_{3a3e.1}$ 14.0; $J_{3a4.1}$ 3.5; $J_{3e4.1}$ 3.0; $J_{45.1}$ 1.5; $J_{56.1}$ 6.5; $J_{12.2}$ 1.5; $J_{23.2}$ 2.5; $J_{34.2}$ 10.0. Anal. $C_{41}H_{40}N_2O_{15}$: C, H.

*Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(3,6-dideoxy-2,4-di-*O*-p-nitrobenzoyl-α-*L*-xylo-hexopyranosyl)-α-*D*-mannopyranoside (16) and β-isomer (17)*. The glycoside *13* (612 mg, 1.65 mmol) and *5* (670 mg, 1.32 mmol) were reacted in the presence of mercury cyanide (1.66 g, 6.6 mmol) essentially as described above though keeping the temperature at −15 °C for 72 h and then at 20 °C for 24 h. The same work-up

procedure gave 1.07 g of a glassy syrup. ^{1}H NMR showed the glycosylation to be quantitative with a ratio α/β=0.42/0.58. Separation on a silica gel column (toluene–ethyl acetate: 8:13) gave 387 mg (37 %) of *16* and 358 mg (34 %) of *17*.

The disaccharide *16* was crystallized from ethyl acetate–light petroleum at −78 °C. $[\alpha]_D^{22}$ −149° (c 0.3, $CHCl_3$) m.p. 115−122 °C. ^{1}H NMR: δ 5.39 (H-1.1); 5.36 (H-2.1); 2.55 (H-3.1a); 2.26 (H-3.1e); 5.26 (H-4.1); 4.46 (H-5.1); 0.87 (H-6.1); 4.64 (H-1.2); 3.76 (H-2.2); 4.32 (H-3.2); 4.24 (H-4.2); 3.84 (H-5.2); 4.25 (H-6.2); 3.87 (H-6.2′). $J_{12.1}$ 4.0 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 3.5; $J_{3a3e.1}$ 14.5; $J_{3a4.1}$ 3.0; $J_{3e4.1}$ 3.5; $J_{45.1}$ 1.5; $J_{56.1}$ 8.0; $J_{12.2}$ 1.7; $J_{23.2}$ 3.0; $J_{34.2}$ 8.0; $J_{56.2}$ 5.5; $J_{66'.2}$ 11.5. Anal. $C_{41}H_{40}N_2O_{15}$: C, H.

Crystallization of *17* was performed under similar conditions. $[\alpha]_D^{23}$ −2.1° (c 0.1, $CHCl_3$) m.p. 158−165 °C. ^{1}H NMR: δ 5.06 (H-1.1); 5.31 (H-2.1); 2.00 (H-3.1a); 2.62 (H-3.1e); 5.29 (H-4.1); 4.00 (H-5.1); 1.41 (H-6.1); 4.65 (H-1.2); 4.04 (H-2.2); 4.27 (H-3.2); 4.08 (H-4.2); 3.78 (H-5.2); 4.19 (H-6.2); 3.81 (H-6.2′); $J_{12.1}$ 8.0 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 5.0; $J_{3a3e.1}$ 13.5; $J_{3a4.1}$ 3.0; $J_{3e4.1}$ 3.0; $J_{45.1}$ 1.2; $J_{56.1}$ 6.5; $J_{12.2}$ 1.5; $J_{23.2}$ 3.2; $J_{34.2}$ 10.0; $J_{45.2}$ 8.0. Anal. $C_{41}H_{40}N_2O_{15}$: C, H.

Methyl 2-O-benzyl-3-O-(2-O-benzyl-3,6-dideoxy-4-O-p-nitrobenzoyl-α-D-xylo-hexopyranosyl)-4,6-O-benzylidene-α-D-mannopyranoside (14b). The glycoside *13* (150 mg, 0.40 mmol) was dissolved in dry toluene (10 ml) and 8 ml was removed by distillation at atmospheric pressure. Mercury cyanide (500 mg, 2.0 mmol), dry nitromethane (5 ml) and molecular sieves (4Å, 2 g) were added and the mixture was stirred under nitrogen at 20 °C for 6 h followed by cooling to 0 °C. The bromide (*9*) (prepared from *8* 170 mg, 0.423 mmol) and dried in toluene (3 ml over molecular sieves for 1 h) was added dropwise during 45 min at 0 °C. Stirring was continued for 4 h at 0 °C and 10 h at 20 °C. The mixture was filtered through celite and the filter was washed 3 times with toluene (10 ml). The filtrate was washed twice with water (15 ml), dried (magnesium sulfate) and filtered. Evaporation and separation on a silica gel column (ethyl acetate−toluene 1:10) gave 116 mg (39 %) of *14b*. $[\alpha]_D^{20}$ +130° (c 0.6, $CHCl_3$)(reported 129° ($CHCl_3$)). ^{1}H NMR: δ 5.42 (H-1.1); 3.72 (H-2.1); 2.18 (H-3.1a); 2.03 (H-3.1e); 5.08 (H-4.1); 4.32 (H-5.1); 1.04 (H-6.1); 4.82 (H-1.2); 3.79 (H-2.2); 4.24 (H-3.2); 3.74 (H-4.2); 4.34 (H-5.2); 3.82 (H-6.2); 3.88 (H-6.2′). $J_{12.1}$ 3.6 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 4.0; $J_{3a3e.1}$ 14.4; $J_{3a4.1}$ 3.2; $J_{3e4.1}$ 3.2; $J_{45.1}$ 1.5; $J_{56.1}$ 6.0; $J_{12.2}$ 1.5; $J_{34.2}$ 5.2; $J_{45.2}$ 2.0; $J_{56.2}$ 0; $J_{56'.2}$ 0; $J_{66'.2}$ 9.2.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (14c). The glycoside *13* (220 mg, 0.59 mmol) was dissolved in toluene (5 ml) and toluene (4 ml) was distilled off. *N,N*-Dimethylformamide (0.4 ml), toluene (8 ml), molecular sieves (1 g, 4Å) and tetrabutylammonium bromide (200 mg, 0.65 mmol) were added and the mixture stirred for 6 h. The chloride (*12*) (190 mg, 0.55 mmol) dissolved in toluene (2 ml) was added and the mixture was stirred at 50 °C for 4 d. The mixture was filtered, diluted with toluene (10 ml), washed with saturated sodium hydrogencarbonate solution (10 ml), water (10 ml) and dried (magnesium sulfate). Filtration and evaporation gave 460 mg of a syrup. Separation on a silica gel column (toluene–ethyl acetate 7:1) gave 143 mg (38 %) of *19* as the main fraction. $[\alpha]_D^{22}$ +28° (c 2.5, $CHCl_3$). ^{1}H NMR: δ 5.38 (H-1.1); 3.74 (H-2.1); 1.85 (H-3.1a); 2.09 (H-3.1e); 3.30 (H-4.1); 3.67 (H-5.1); 1.12 (H-6.1); 4.73 (H-1.2); 3.74 (H-2.2); 4.33 (H-3.2); 4.24 (H-4.2); 3.79 (H-5.2); 4.03 (H-6.2); 3.83 (H-6.2′). $J_{12.1}$ 3.1 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 4.5; $J_{3a3e.1}$ 13.0; $J_{3a4.1}$ 2.2; $J_{3e4.1}$ 3.0; $J_{45.1}$ 1.0; $J_{56.1}$ 6.7; $J_{12.2}$ 1.1; $J_{23.2}$ 3.0; $J_{34.2}$ 9.0; $J_{45.2}$ 9.0.

A small fraction (7 mg, 2 %), which according to ^{1}H NMR was the β-linked disaccharide, was eluted after *14c*.

Methyl 2-O-benzyl-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (22). The aglycone (*13*) (223 mg, 0.60 mmol) was dissolved in toluene (6 ml) and 5 ml was distilled off. Mercury cyanide (200 mg, 0.8 mmol), nitromethane (5 ml), and molecular sieves (4Å, 1 g) were added and the mixture stirred for 6 h at 20 °C under nitrogen atmosphere. The chloride (*12*) (180 mg, 0.52 mmol), dissolved in toluene (3 ml) and dried for 2 h over molecular sieves (4Å, 0.3 g), was added dropwise with stirring at 0 °C. The mixture was stirred for 4 h at 0 °C and 9 h at 20 °C. The reaction mixture was filtered through celite, diluted with dichloromethane (40 ml), washed with sodium hydrogencarbonate solution, potassium iodide solution (30 ml), water (30 ml) and dried (magnesium sulfate). Filtration and evaporation gave 368 mg of syrup which was separated on a silica gel column (toluene–ethyl acetate 5:1) to give a disaccharide fraction of 245 mg (70 %, with an anomeric ratio β/α=2/5 seen from a ^{1}H NMR spectrum) and an aglyconic fraction of 80 mg. The disaccharides could not be separated chromatographically and the benzylidene group was therefore removed by stirring with 80 % acetic acid (10 ml) for 2 h at 60 °C. The mixture was evaporated and the resulting syrup was separated on a silica gel column (ethyl acetate–light petroleum: 7:5). The first fraction was evaporated giving 110 mg of *22* (36 % overall from *12* $[\alpha]_D^{25}$

+30° (c 1.1, $CHCl_3$). ^1H NMR: δ 4.96 (H-1.1); 3.83 (H-2.1); 1.82 (H-3.1a) 2.11 (H-3.1e); 3.33 (H-4.1); 3.85 (H-5.1); 1.06 (H-6.1); 4.67 (H-1.2); 3.76 (H-2.2); 3.85 (H-3.2); 3.97 (H-4.2); 3.60 (H-5.2); 3.80 (H-6.2); 3.75 (H-6.2'). $J_{12.1}$ 3.1 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 4.5; $J_{3a3e.1}$ 13.0; $J_{3a4.1}$ 2.3; $J_{3e4.1}$ 3.0; $J_{45.1}$ 1.0; $J_{56.1}$ 6.6; $J_{12.2}$ 1.2; $J_{23.2}$ 3.0; $J_{34.2}$ 9.5; $J_{45.2}$ 9.5; $J_{56.2}$ 3.8; $J_{56'.2}$ 5.0; $J_{66'.2}$ 11.5.

Evaporation of the second fraction gave 45 mg (15 % overall from *12*) of the β-isomer. $[\alpha]_D^{25}$ +5° (c 0.8, $CHCl_3$). ^1H NMR: δ 4.41 (H-1.1); 3.65 (H-2.1); 1.44 (H-3.1a); 2.34 (H-3.1e); 3.34 (H-4.1); 3.68 (H-5.1); 1.25 (H-6.1); 4.52 (H-1.2); 3.77 (H-2.2); 3.80 (H-3.2); 3.91 (H-4.2); 3.56 (H-5.2); 3.90 (H-6.2); 3.79 (H-6.2'). $J_{12.1}$ 7.5 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 4.7; $J_{3a3e.1}$ 13.9; $J_{3a4.1}$ 2.5; $J_{3e3.1}$ 3.2; $J_{45.1}$ 1.0; $J_{56.1}$ 6.5; $J_{12.2}$ 1.1; $J_{23.2}$ 3.0; $J_{34.2}$ 9.5; $J_{45.2}$ 9.5; $J_{56.2}$ 3.8; $J_{56'.2}$ 5.0; $J_{66'.2}$ 11.5.

*Methyl 3-O-(3,6-dideoxy-α-*D*-xylo-hexopyranosyl)-α-*D*-mannopyranoside (18).* The disaccharide (*14a*) (215 mg, 0.264 mmol) was stirred for 20 h at 20 °C with sodium methoxide in methanol (0.1 M, 10 ml). The mixture was neutralized with ion exchange resin (Amberlite) IRC 50) and filtered. After concentration *in vacuo* the residue was treated with charcoal in ethyl acetate for 1 h. Filtration through celite and evaporation left 130 mg of material. This was dissolved in 80 % acetic acid (5 ml) and left for 3.5 d. Water (10 ml) was added and the solution was concentrated at 40 °C and 10 mm Hg. The coevaporation with water was repeated twice to give 90 mg material, which was dissolved in methanol (40 ml) and hydrogenated at 110 atm. pressure of hydrogen over palladium on charcoal (50 mg, 5 %) for 20 h. Filtration through celite and evaporation left a residue which was separated on a silica gel plate (ethyl acetate–methanol 2:1) giving 50 mg (57 %) of *18*. $[\alpha]_D^{23}$ +117° (c 0.2, H_2O) [reported ^{1}+113° (H_2O)] ^1H NMR: δ 4.99 (H-1.1); 3.91 (H-2.1); 1.95 (H-3.1a); 1.87 (H-3.1e) 3.73 (H-4.1); 4.00 (H-5.1); 1.05 (H-6.1); 4.66 (H-1.2); 3.94 (H-2.2); 3.73 (H-3.2); 3.73 (H-4.2); 3.56 (H-5.2); 3.81 (H-6.2); 3.68 (H-6.2'). $J_{12.1}$ 3.9 Hz; $J_{23a.1}$ 12.5; $J_{23e.1}$ 5.6; $J_{3a3e.1}$ 14.1; $J_{3a4.1}$ 3.0; $J_{3e4.1}$ 3.4; $J_{45.1}$ 1.4; $J_{56.1}$ 6.4; $J_{12.2}$ 1.8; $J_{23.2}$ 2.8; $J_{34.2}$ 8.2; $J_{45.2}$ 8.3; $J_{56.2}$ 2.3; $J_{56'.2}$ 5.6; $J_{66'.2}$ 11.9. ^{13}C NMR: 101.4 ppm (C-1.1); 64.7 (C-2.1); 34.1 (C-3.1); 69.5 (C-4.1); 68.0 (C-5.1); 16.5 (C-6.1); 101.9 (C-1.2); 71.2 (C-2.2); 79.6 (C-3.2) 67.2 (C-4.2); 73.8 (C-5.2); 62.8 (C-6.2); 55.9 (OMe) $J_{ClHl.1}$ 170 Hz; $J_{ClHl.2}$ 171.

*Methyl 3-O-(3,6-dideoxy-β-*D*-xylo-hexopyranosyl)-α-*D*-mannopyranoside (19).* The disaccharide (*15*) was deprotected in essentially the same way to give *19* in 40 % yield. $[\alpha]_D^{22}$ −1.3° (c 0.3, H_2O), ^1H NMR: δ 4.39 (H-1.1); 3.67 (H-2.1); 1.63 (H-3.1a); 2.13 (H-3.1e); 3.74 (H-4.1); 3.76 (H-5.1); 1.11 (H-6.1); 4.73 (H-1.2); 4.03 (H-2.2); 3.84 (H-3.2); 3.70 (H-4.2); 3.57 (H-5.2); 3.84 (H-6.2); 3.70 (H-6.2'). $J_{12.1}$ 8.0 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 5.3; $J_{3a3e.1}$ 13.4; $J_{3a4.1}$ 2.9; $J_{3e4.1}$ 2.9; $J_{45.1}$ 0.8; $J_{56.1}$ 6.9; $J_{12.2}$ 1.9; $J_{23.2}$ 3.2; $J_{34.2}$ 9.5; $J_{45.2}$ 9.7; $J_{56.2}$ 2.1; $J_{56'.2}$ 5.6; $J_{66'.2}$ 12.0. ^{13}C NMR: 103.7 ppm (C-1.1); 66.1 (C-2.1); 37.6 (C-3.1); 73.2 (C-4.1); 68.9 (C-5.1); 16.4 (C-6.1); 101.4 (C-1.2); 68.6 (C-2.2); 79.3 (C-3.2); 66.1 (C-4.2); 75.2 (C-5.2); 61.7 (C-6.2); 55.9 (OMe). $J_{ClHl.1}$ 159 Hz; $J_{ClHl.2}$ 170.

*Methyl 3-O-(3,6-dideoxy-α-*L*-xylo-hexopyranosyl)-α-*D*-mannopyranoside (20).* The disaccharide (*16*) was deprotected essentially as described above to give *20* in 57 % yield. $[\alpha]_D^{23}$ −26.2° (c 0.5, H_2O). ^1H NMR: δ 4.86 (H-1.1); 3.94 (H-2.1); 1.97 (H-3.1a); 1.89 (H-3.1e); 3.77 (H-4.1); 4.14 (H-5.1); 1.04 (H-6.1); 4.72 (H-1.2); 4.02 (H-2.2); 3.73 (H-3.2); 3.69 (H-4.2); 3.56 (H-5.2); 3.83 (H-6.2); 3.70 (H-6.2'). $J_{12.1}$ 3.7 Hz; $J_{23a.1}$ 11.9; $J_{23e.1}$ 5.6; $J_{3a3e.1}$ 13.3; $J_{3a4.1}$ 3.2; $J_{3e4.1}$ 3.5; $J_{45.1}$ 1.5; $J_{56.1}$ 6.0; $J_{12.2}$ 2.1; $J_{23.2}$ 2.4; $J_{34.2}$ 9.1; $J_{45.2}$ 9.1; $J_{56.2}$ 2.2; $J_{56'.2}$ 5.6; $J_{66'.2}$ 11.9. ^{13}C NMR: 96.5 ppm (C-1.1); 64.3 (C-2.1); 34.1 (C-3.1); 69.6 (C-4.1); 67.8 (C-5.1); 16.5 (C-6.1); 101.8 (C-1.2); 68.0 (C-2.2); 77.2 (C-3.2); 66.4 (C-4.2); 73.8 (C-5.2); 62.2 (C-6.2); 56.0 (OMe) $J_{ClHl.1}$ 168 Hz; $J_{ClHl.2}$ 172.

*Methyl 3-O-(3,6-dideoxy-β-*L*-xylo-hexopyranosyl)-α-*D*-mannopyranoside (21).* Deprotection of *17* as described above gave *21* (8 mg) in 19 % yield. ^1H NMR: δ 4.51 (H-1.1); 3.64 (H-2.1); 1.63 (H-3.1a); 2.11 (H-3.1e); 3.71 (H-4.1); 3.71 (H-5.1); 1.10 (H-6.1); 4.67 (H-1.2); 4.08 (H-2.2); 3.79 (H-3.2); 3.72 (H-4.2); 3.56 (H-5.2); 3.80 (H-6.2); 3.68 (H-6.2'). $J_{12.1}$ 7.8 Hz; $J_{23a.1}$ 12.0; $J_{56.1}$ 5.8; $J_{12.2}$ 1.8; $J_{23.2}$ 3.0; $J_{34.2}$ 9.4; $J_{45.2}$ 9.3; $J_{56.2}$ 2.6; $J_{56'.2}$ 6.0; $J_{66'.2}$ 12.0.

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Methyl(cyanomethyl)phosphines. Synthesis and Nucleophilic Reactivity

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The preparation of Me_2PCH_2CN, $MeP(CH_2CN)_2$ and $P(CH_2CN)_3$ is described, and phosphorus lone-pair ionization potentials and second order rate constants for their reactions with benzyl bromide in acetonitrile at 35 °C are determined. The rate constant for Me_2PCH_2CN is approximately the same as that for Ph_3P, whereas that for $P(CH_2CN)_3$ is 2×10^4 times smaller. This low nucleophilic reactivity of cyanomethylphosphines is ascribed to polar effects from the cyano group(s) which contracts the phosphorus lone-pair.

(Cyanomethyl)phosphines, $R_{3-n}P(CH_2CN)_n$, are examples of *C*-functionalized tertiary phosphines which have recently attracted considerable interest, mainly because of their potential as chelating ligands.[1–3] Introduction of functional groups close to the phosphorus atom, however, changes the donor property of phosphorus for both steric and electronic reasons, and accordingly changes the coordinating ability (towards metal centers) and nucleophilicity (towards carbon centers). Obviously it would be valuable to have quantitative information on how different functional groups change the donor property in order to help designing new phosphines with specific properties. Functionalization at the α-carbon should give maximum effects, and many such substituted phosphines are now known, *e.g.* the trisubstituted phosphines $P(CH_2OH)_3$,[4] $P(CH_2NR_2)_3$,[5] $P(CH_2PMe_2)_3$,[6] $P(CH_2COOEt)_3$,[7] $P(CH_2COOH)_3$ [8] and $P(CH_2CN)_3$.[9] However, we are aware of only one quantitative study of the nucleophilic reactivity of α-substituted phosphines. The quaternization of some diphenylphosphinoacetic acid derivatives with ethyl iodide has been correlated with Taft σ^* values and phosphorus lone-pair ionization potentials.[10]

The present paper describes the preparation of two new α-substituted phosphines, Me_2PCH_2CN and $MeP(CH_2CN)_2$, a new route (eqn. (4)) to the known $P(CH_2CN)_3$, and quantitative results on their very different nucleophilic reactivities.

Synthesis. (Cyanomethyl)phosphines have hitherto been prepared by three methods [eqn. (1)–(3)]. The first method [eqn. (1)] [11]

$$R_2POEt + ClCH_2CN \rightarrow R_2P(O)CH_2CN \xrightarrow{Ph_2SiH_2} R_2PCH_2CN \quad (1)$$

$$Ph_{3-n}P(SiMe_3)_n + n\ ClCH_2CN \rightarrow Ph_{3-n}P(CH_2CN)_n + n\ Me_3SiCl \quad (2)$$

$$PCl_3 + 3\ Bu_3SnCH_2CN \rightarrow P(CH_2CN)_3 + 3\ Bu_3SnCl \quad (3)$$

allows only one cyanomethyl group to be introduced but is otherwise fairly general. This method was chosen to prepare Me_2PCH_2CN. The second route [eqn. (2)] [12] involves the preparation of silylphosphines which are very reactive and sometimes difficult to obtain, and the yield is low for $n=3$. The third method [eqn. (3)] [9] seems fairly general (Cl_2PCH_2CN [13] and $MeP(CH_2CN)_2$[14] have been similarly obtained) but its drawback is the low yield synthesis of Bu_3SnCH_2CN. To obtain $MeP(CH_2CN)_2$ and $P(CH_2CN)_3$ we therefore attempted a synthesis *via* the known [15] Reformatsky reagent $BrZnCH_2CN$ [eqn. (4)], in analogy with Podla-

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hová's successful syntheses of $Ph_{3-n}P(CH_2COOEt)_n$.[7] The reactions when run at low temperatures (−78 °C) gave $MeP(CH_2CN)_2$ and $P(CH_2CN)_3$ in fair yields. As it is a one pot synthesis from readily obtainable starting materials, this route is considered the most attractive one to prepare (cyanomethyl)phosphines with two or three cyanomethyl groups.

$$Me_{3-n}PCl_n + n\ BrZnCH_2CN \rightarrow Me_{3-n}P(CH_2CN)_n \quad (4)$$

Nucleophilic reactivity. $P(CH_2CN)_3$ reacts very slowly with alkyl halides. Several days, in refluxing acetonitrile with excess benzyl bromide, were necessary to prepare $PhCH_2P^+(CH_2CN)_3Br^-$, and refluxing with excess ethyl iodide in acetone did not give appreciable amounts of a phosphonium salt after 2 days. The above benzylphosphonium salt hydrolyzes easily at room temperature to give $PhCH_2P(O)(CH_2CN)_2$. This reflects the good leaving group ability of the cyanomethyl group which, more surprisingly, also manifests itself in the properties of $P(CH_2CN)_3$. Although fairly stable to water and dilute HCl, $P(CH_2CN)_3$ is rapidly hydrolyzed to HPO_3^{2-} and CH_3CN by aqueous NaOH at room temperature.[14] $MeP(CH_2CN)_2$ and Me_2PCH_2CN react more smoothly with alkyl halides, and only $PhCH_2(Me)P^+(CH_2CN)_2Br^-$ is somewhat prone to hydrolysis.

Quantitative results on the nucleophilic reactivity of Me_2PCH_2CN, $MeP(CH_2CN)_2$ and $P(CH_2CN)_3$ were obtained from following, by conductivity measurements, their reactions with benzyl bromide in acetonitrile at 35 °C. The second order rate constants for quaternization, including that of Ph_3P for comparison, are given in Table 1, together with the Taft σ^* values and the phosphorus lone-pair ionization potentials (IP). The rate data show that the cyanomethyl group decreases the nucleophilic reactivity of phosphorus very strongly. Me_2PCH_2CN reacts nearly as slowly as Ph_3P, and $P(CH_2CN)_3$ is approximately 20 000 times less reactive. The effects of multiple cyanomethyl substitution are additive as shown by a linear dependence of log k_2 with the Taft σ^* values (Fig. 1). The rate constants also correlate with the IP's (Fig. 2), although Ph_3P is anormalous. A correlation between gas phase (IP) and solution (log k_2) properties may fail for several reasons,[10] but the main cause of the deviation for Ph_3P is probably the different C−P−C bond angle (103° for Ph_3P,[16] about 98° for $P(CH_2CN)_3$[9] and Me_3P [17]). A larger C−P−C angle corresponds to a more extended lone-pair (one with more *p* character) with a lower IP, as observed. The correlation of

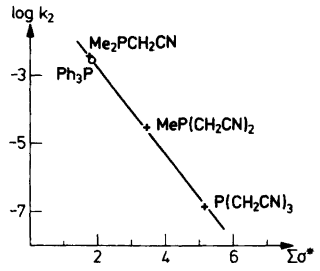

Fig. 1. Plot of rate constant *versus* Taft σ^* values for the reaction $R_3P+PhCH_2Br \rightarrow R_3PhCH_2P^+Br^-$ in acetonitrile at 35 °C.

Table 1. Rate constants for quaternization, Taft σ^* values and phosphorus lone-pair ionization potentials of $Me_{3-n}P(CH_2CN)_n$ and Ph_3P.

	k_2 [a] ($l\ mol^{-1}s^{-1}$)	$\Sigma\sigma^*$ [b]	IP [c] (eV)
$P(CH_2CN)_3$	1.3×10^{-7}	5.13	10.59
$MeP(CH_2CN)_2$	3.0×10^{-5}	3.42	9.83
Me_2PCH_2CN	3.9×10^{-3}	1.71	9.22
Ph_3P	3.0×10^{-3}	1.80	7.88

[a] Second order rate constant for quaternization with benzyl bromide in acetonitrile at 35 °C. [b] $\sigma^*=0.00$ for Me, 0.60 for Ph and 1.71 for CH_2CN.[10] [c] Vertical ionization potentials.

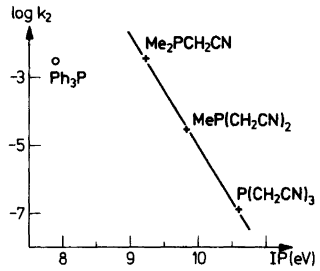

Fig. 2. Plot of rate constant *versus* phosphorus lone-pair ionization potential for the reaction $R_3P+PhCH_2Br \rightarrow R_3PhCH_2P^+Br^-$ in acetonitrile at 35 °C.

log k_2 with IP for Me_2PCH_2CN, $MeP(CH_2CN)_2$ and $P(CH_2CN)_3$ indicates that the cyano groups exert a field (or inductive) effect on the lone-pair which results in a contraction of it and thereby makes it less available for reactions.

This conclusion is corroborated by CNDO/2 calculations,[18] which show that the electron density in the lone pair region of $P(CH_2CN)_3$ is reduced relative to that of Me_3P. *Ab initio* calculations on Me_2PCH_2CN and low temperature X-ray diffraction studies on $P(CH_2CN)_3$ are in progress [19] with the aim to give more evidence on the electron distribution in these cyanomethylphosphines.

EXPERIMENTAL

Analyses were performed by the microanalytical department of this laboratory. ^1H and ^{31}P NMR spectra were recorded on a JEOL FX 90 Q Fourier NMR spectrometer. Chemical shifts (ppm) are given relative to internal TMS for ^1H spectra (δ_H) and external 85 % H_3PO_4 for ^{31}P spectra (δ_P), and are given as positive for low-field shifts. The He(I) photoelectron spectra were obtained on a Perkin-Elmer PS 18 spectrometer calibrated using the 2P3/2 peaks of Ar and Xe. Ionization potentials were reproducible within ±0.03 eV. The kinetic measurements were performed at 35.0±0.2° using the conductivity method of Henderson and Buckler.[20] A Radiometer CDM 2 Conductivity Meter and a cell with platinized platinum electrodes with a cell constant of 0.97 cm^{-1} was used. Acetonitrile (Merck "Uvasol") was purified through a column of neutral Al_2O_3 (Woelm N-Super I) and deoxygenated by bubbling Ar through it for 10 min. The solvent thus purified had a conductivity of less than 0.2 μohm^{-1} cm^{-1}. The solutions were prepared at 20 °C and corrected for concentration changes upon heating to 35 °C. The reactions were followed until a few percent of the phosphine had reacted. The rate constants were calculated from the linear approximation $kt=x/ab$.[20] Benzyl bromide and phosphine concentrations were varied to prove that the reactions were second order. Up to ten times molar excess of benzyl bromide was used for the more slowly reacting phosphines to ensure convenient rates. The calculated rate constants (Table 1) are the results of duplicate runs, which were reproducible within ±10 %. All manipulations with phosphines were carried out under N_2 or Ar.

P,P-*dimethylphosphinoylacetonitrile* (*$Me_2P(O)CH_2CN$*). Me_2POBu [21] (3.35 g, 25 mmol) was added in 0.5 ml portions to $ClCH_2CN$ (3.8 g, 50 mmol) at 75 °C with stirring. After 1 h at 75 °C BuCl and excess $ClCH_2CN$ were removed *in vacuo* and the residue recrystallized from ethyl acetate to give $Me_2P(O)CH_2CN$ (1.90 g, 65 %), m.p. 75–78 °C (hygroscopic). Anal. C_4H_8NOP: C, H, N. NMR ($CDCl_3$): δ_P 38.0; δ_H 1.76 (CH_3, d, $^2J_{PH}$ 13.2 Hz), 3.04 (CH_2, d, $^2J_{PH}$ 15.1 Hz).

P,P-*dimethylphosphinoacetonitrile* (*Me_2PCH_2CN*). A mixture of $Me_2P(O)CH_2CN$ (1.75 g, 15 mmol) and Ph_2SiH_2 (2.76 g, 15 mmol) was heated with stirring to 175 °C for 6 h. Vacuum distillation through a small Claisen head gave Me_2PCH_2CN (1.24 g, 82 %), b.p. 58–59 °C/12 mmHg. The purity was more than 97 % according to ^1H and ^{31}P NMR. NMR ($CDCl_3$): δ_p −50.1; δ_H 1.19 (CH_3, d, $^2J_{PH}$ 3.8 Hz), 2.40 (CH_2, d, $^2J_{PH}$ 5.6 Hz).

Benzyl(cyanomethyl)dimethylphosphonium bromide (*$PhCH_2Me_2P^+CH_2CN\ Br^-$*). A mixture of Me_2PCH_2CN (0.20 g, 2 mmol) and $PhCH_2Br$ (0.68 g, 4 mmol) in dry ether (10 ml) was kept at 20 °C for 6 days. The precipitate was washed with ether, dried and recrystallized from 2-propanol to give the product (0.33 g, 60 %), m.p. 120–121.5 °C. **Anal.** $C_{11}H_{15}BrNP$: C, H, N, Br. NMR (CD_3CN): δ_p 31.7.

P-*methylphosphinediyldiacetonitrile* (*$MeP(CH_2CN)_2$*). A solution of $BrCH_2CN$ (7.20 g, 60 mmol) in dry THF (30 ml) was added during 20 min to a stirred suspension of Zn (Riedel-de Haën "chem. rein. geraspelt", freshly etched with 0.2 M HCl followed by washing with water, acetone, ether and drying; 3.92 g, 60 mmol) in dry THF (10 ml). The temperature was kept at *ca.* 20 °C by occasional immersion in an ice-bath. The resulting green to brown solution was cooled to −78 °C and $MePCl_2$ (3.51 g, 30 mmol) in dry THF (10 ml) added with stirring during 20 min. The reaction mixture was allowed to reach room temperature slowly (over *ca.* 6 h), filtered through a glasswool plug and the precipitate washed with THF (2×10 ml). The solvent was removed at 10 mmHg and the residue dissolved in deoxygenated water (20 ml). The product was extracted with CH_2Cl_2 (2×20 ml) and the CH_2Cl_2 solution washed with water (20 ml) and dried (Na_2SO_4). Evaporation of the solvent and distillation *in vacuo* through a small Claisen head gave $MeP(CH_2CN)_2$ (1.13 g, 30 %), b.p. 99–102 °C/0.20 mmHg. The purity was higher than 97 % according to ^1H and ^{31}P NMR. NMR ($CDCl_3$): δ_p −38.2: δ_H 1.37 (CH_3, d, $^2J_{PH}$ 5.5 Hz), 2.63 (CH_2, AB part of ABX spectrum, Δ_{AB} 0.04 ppm, $^2J_{AB}$ 17.2, $^2J_{PH}$ 5.4 and 6.0 Hz).

P-*methylphosphonoyldiacetonitrile* (*$MeP(O)(CH_2CN)_2$*). $MeP(CH_2CN)_2$ (0.13 g, 1

mmol) in CH_2Cl_2 (5 ml) was oxidized by bubbling NO_2 through the solution until a green colour remained. Evaporation of the solvent and recrystallization of the residue from abs. ethanol gave the product (0.09 g, 60 %), m.p. 93–94 °C. Anal. $C_5H_7N_2OP$: C, H, N. NMR ($(CD_3)_2CO$): δ_P 31.1; δ_H 1.90 (CH_3, d, $^2J_{PH}$ 13.8 Hz), 3.49 (CH_2, d, $^2J_{PH}$ 14.5 Hz).

Benzyldi(cyanomethyl)methylphosphonium bromide ($PhCH_2MeP^+(CH_2CN)_2\ Br^-$). A mixture of $MeP(CH_2CN)_2$ (0.50 g, 4 mmol) and $PhCH_2Br$ (1.37 g, 8 mmol) in CH_3CN (5 ml) was refluxed for 20 h. The solvent was removed *in vacuo* and the residue washed with hexane to remove excess $PhCH_2Br$. Recrystallization from a small amount of CH_3CN gave the product (0.47 g, 40 %), m.p. 121.5–122.5 °C. Anal. $C_{12}H_{14}BrN_2P$: C, H, N, Br. NMR (CD_3CN): δ_P 33.2.

Phosphinetriyltriacetonitrile ($P(CH_2CN)_3$). This phosphine was prepared in the same way as $MeP(CH_2CN)_2$, from $BrCH_2CN$ (7.20 g, 60 mmol), Zn (3.92 g, 60 mmol) and PCl_3 (2.20 g, 16 mmol) in THF (50 ml). The reaction mixture, after reaching room temperature, was heated to reflux with active carbon and then filtered through a glasswool plug. The solvent was removed *in vacuo* and the brown residue was dissolved in hot, deoxygenated water (5 ml). Cooling to 0 °C gave a crystalline product which was recrystallized once from water, with addition of active carbon, to give $P(CH_2CN)_3$ (1.07 g, 45 %), m.p. 110–111.5 °C (lit.[9] 110.5–112.5 °C).

Benzyltris(cyanomethyl)phosphonium bromide ($PhCH_2P^+(CH_2CN)_3\ Br^-$). A mixture of $P(CH_2CN)_3$ (0.76 g, 5 mmol) and $PhCH_2Br$ (3.4 g, 20 mmol) in CH_3CN (7 ml) was heated to reflux for 4 days. The precipitate, after cooling, was filtered off and recrystallized from CH_3CN to give the product (1.02 g, 64 %), m.p. 199–201 °C. Anal. $C_{13}H_{13}BrN_3P$: C, H, N, Br. NMR (CD_3CN): δ_P 31.6; δ_H 4.69 (CH_2CN, d, $^2J_{PH}$ 15.5 Hz), 4.74 (CH_2Ph, d, $^2J_{PH}$ 14.9 Hz), 7.54 (Ph).

P-*benzylphosphonoyldiacetonitrile* ($PhCH_2P(O)(CH_2CN)_2$). $PhCH_2P^+(CH_2CN)_3\ Br^-$ (0.32 g, 1 mmol) and water (3 ml) were heated to give a clear solution. The product crystallized on cooling (0.19 g, 87 %), m.p. 163.5–165 °C. Anal. $C_{11}H_{11}N_2OP$: C, H, N. NMR (CD_3CN): δ_P 32.0; δ_H 3.18 (CH_2CN, d, $^2J_{PH}$ 13.4 Hz), 3.52 (CH_2Ph, d, $^2J_{PH}$ 14.8 Hz), 7.36 (Ph).

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Short Communications

Ring–Chain Tautomerism of Myosmine

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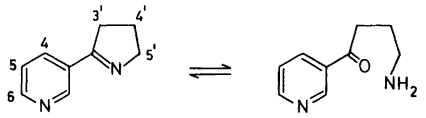
Myosmine (*1a*) is a minor tobacco alkaloid.[1] It is also formed by pyrolysis of nicotine [2] and has been detected in tobacco smoke.[3] On treatment with aqueous hydroxylamine or phenylhydrazine, it reacts as a carbonyl compound and it has therefore been assumed that aqueous solutions of myosmine contain equilibrium mixtures of *1a* and the amino ketone from *1b*, which has been called poikiline.[4–6]



1a *1b*

In connection with studies of the ring–chain tautomerism of nicotine metabolites,[7,8] we recorded ^1H and ^{13}C NMR spectra of aqueous solutions of myosmine and we here report the results. Signals from both *1a* and *1b* were seen in the spectra of acidic solutions and integrations of the signals ascribed to H-5'* gave the percentage of *1a* as a function of the acidity of the D_2O solution as shown in Fig. 1; similar values were obtained from the ^{13}C NMR spectra. There was no NMR evidence for the carbinolamine which reasonably should be an intermediate in the reactions *1a*⇄*1b*. Five ^{13}C NMR spectra of solutions of *1a* in H_2O demonstrate a marked, mixed origin, isotope effect on the equilibrium between *1a* and *1b* (Fig. 1). These latter spectra indicate that the content of *1b* in H_2O should be less than 1 % at physiological pH.

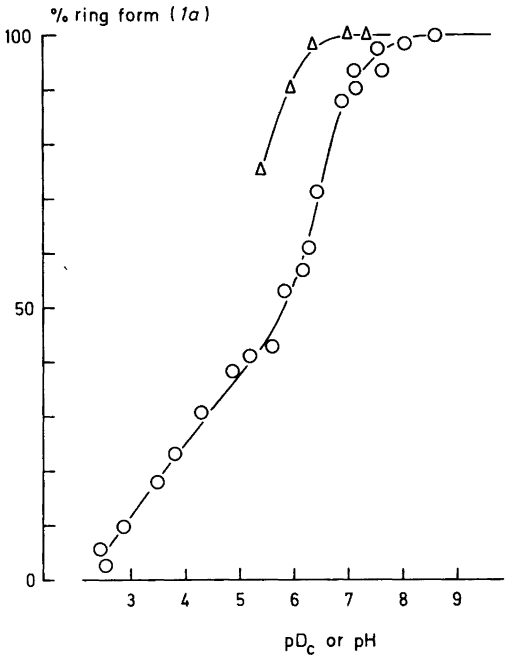


Fig. 1. Ring–chain tautomerism *1a*⇄*1b* as found by ^1H NMR spectroscopy of solutions of myosmine in D_2O (circles) and by ^{13}C NMR spectroscopy of solutions in H_2O (triangles).

As in a similar tautomeric system,[8] the nature of the aromatic ring has a decisive effect on the ring–chain tautomerism. Thus, the phenyl analogue [9] of myosmine is, as found by ^1H NMR, a *ca.* 15:85 mixture of the amino ketone and imino forms between pD_c 1.2 and 6.0 (pD_c=pH meter reading+0.40 [10]).

Experimental. The synthesis of myosmine [11] and the NMR investigation [8] were performed as described.

NMR spectra of 1a. ^1H NMR (D_2O, pD_c 8.6 to 13.9): δ 8.66 (dd, H-2), 8.46 (dd, H-6), 8.00 (m, H-4), 7.40 (ddd, H-5), 3.92 (m, H-5'), 2.92 (m, H-3'), 2.00 (quintet, H-4'). ^{13}C NMR (D_2O, pD_c

* The numbering of nicotine has been used throughout.

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10.2): 172.9 (C-2′), 151.0 (C-6)**, 148.1 (C-2)**, 136.4 (C-4), 129.2 (C-3), 125.0 (C-5), 61.3 (C-5′), 35.3 (C-3′), 22.2 ppm (C-4′). ^{1}H NMR (D_2O, pD_c 3.4): δ9.00 (broad s, H-2), 8.38 (m, H-4), 7.69 (ddd, H-5), 4.25 (m, H-5′), 3.63 (m, H-3′), 2.44 (quintet, H-4′). ^{13}C NMR (D_2O, pD_c 3.2): 155.2 (C-6)**, 150.1 (C-2)**, 139.8 (C-4), C-3 not observed, 126.1 (C-5), 55.4 (C-5′), C-3′ not observed, 20.5 ppm(C-4′).

NMR spectra of 1b. ^{1}H NMR (D_2O, pD_c 1.3): δ 9.28 (dd, H-2), 9.06 (m, H-4) 8.94 (dd, H-6), 8.18 (ddd, H-5), 3.38 (t, H-3′), 3.14 (t, H-5′), 2.12 (quintet, H-4′). ^{13}C NMR (D_2O, pD_c 0.9): 197.6 (C-2′), 146.6 (C-6),** 145.1 (C-2)**, 142.4 (C-4), 135.6 (C-3), 128.8 (C-5), 39.7 (C-5′), 36.8 (C-3′), 36.5 (t, monodeuterated C-3′), 21.5 ppm (C-4′).

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** Tentative assignment.

The Structure of the Stereoisomeric 3-Amino-2,4-diphenyl-2-butenenitriles

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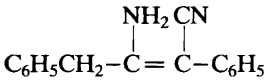
The β-amino- (*1*) or β-iminonitrile (*2*) formed by base-catalyzed dimerization of phenylacetonitrile [2] has been a subject for structure considerations several times in the literature. In older literature [1,2] the compound was always named β-iminonitrile although the structure was believed to be β-aminonitrile because hydrogenation gave a diamino compound.[2] Later,[3] on basis of UV investigations, the structure of a crystalline modification was determined as the amino compound and that of an oily form was believed to be the imino tautomer with the former predominating in solution.



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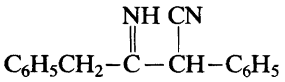


2

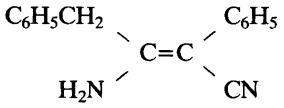
Since then the structures of the isomers have not been investigated specifically and the compounds are generally believed to be 3-amino-2,4-diphenyl-2-butenenitrile *1*.[4] The presence of two isomers with identical constitution formula has never been noticed.

The question regarding the two isomers came up because the dimer after purification by distillation [1] could be separated into two fractions by recrystallization, an oily and a crystalline fraction. The two fractions gave identical elemental analyses and hydrogenation products.[2]

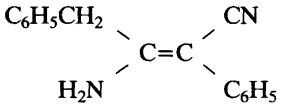
We have found that the dimer when analyzed on an HPLC Partisil 10 ODS column with acetonitrile–water as eluent gave two close but discrete peaks indicating the presence of two compounds in the ratio 2:1. Each isomer gave the same isomer distribution when analyzed on HPLC, indicating the isomerization to be very fast in that solvent. In chloroform, however, the isomerization was fairly slow so each isomer could be studied. The IR spectra of the two isomers recorded in $CHCl_3$ were almost identical. There were some small differences in band intensities. The most prominent differences were two absorptions at 1285 and 1270 cm^{-1} in the crystalline form missing in the oily modification. These dissimilarities can be ascribed to small differences in the carbon skeleton. Stretching vibrations at 3500 and 3400 cm^{-1} indicate that both isomers are amino compounds *1*. The C≡N stretch vibration at 2195 cm^{-1} is strong and indicates the nitrile is conjugated. No imino stretching vibration expected around 1660–1680 cm^{-1} was seen.

The ^1H NMR spectra of the two isomers also showed that only the amino form *1* was present in $CDCl_3$. The chemical shift value for the amino group was 4.81 ppm (2H). No CH signal from an imino form *2* was seen around 4.87 ppm, where the CH resonance for 3-oxo-2,4-diphenylbutanenitrile is found. The CH could, however, be covered by the amino signal but demasking with D_2O did not show any CH signal.

Since the IR and NMR spectroscopic evidence shows that both isomers are amino forms the two compounds must be *Z*/*E* isomers.



Z



E

The rotation is less hindered than the rotation around the normal CC double bond because the electron-donating and electron-attracting substituents attached to each end of the double bond is stabilizing the dipolar transition state for the rotation.

The significance of these resonance forms is stressed by the great diamagnetic shift for the ^{13}C NMR chemical shift for C-2 which is found at 81.1 ppm. The C-2 chemical shift for butenedinitrile is found at 120 ppm.[9]

The ^1H NMR spectra of the two isomers are almost identical except for the chemical shifts of

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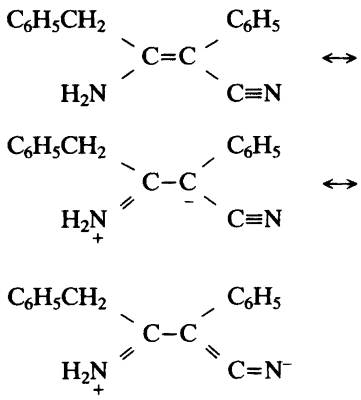

the methylene groups. For the crystalline isomer it was found at 3.65 ppm and for the oily isomer at 3.83 ppm. A solution of the crystalline isomer in $CDCl_3$ changes by standing at room temperature for 1–2 months into a 6:1 mixture of the two isomers with the oily isomer dominating. The oily isomer, on standing in solution is transformed into a mixture of the isomers in the same ratio giving an NMR identical with the NMR obtained for the crystalline isomer on standing in chloroform solution.

The oily isomer having the methylene group at lowest field must therefore be the *E*-form because of the greater deshielding effect of the cyano group compared to the phenyl group as seen for *e.g.* 2-methylbenzonitrile [5] and 2-methylbiphenyl [6] where the methyl chemical shifts are 2.51 and 2.18 ppm, respectively. For *cis* and *trans* 4-phenyl-2-butenenitrile the methylene group was found at 3.68 and 3.47, respectively,[7] also showing the deshielding effect of the cyano group. For *cis* and *trans* 2-benzyl-3-phenyl-2-butenenitrile [8] the methylene group was found at 3.70 and 3.43 ppm, respectively, demonstrating the shielding effect of the phenyl group.

In the ^{13}C NMR spectra the same trend is seen, the methylene carbon signal being shifted downfield from 37.1 ppm in the crystalline isomer to 40.3 ppm for the oily isomer. The phenyl group *cis* to the benzyl group causes more sterical pertubation than the cyano group [9] and is therefore shielding the methylene group.

From the spectroscopic evidence it can be seen that the compound formed by base catalyzed dimerization of phenylacetonitrile is 3-amino-2,4-diphenyl-2-butenenitrile. The isomers discussed in the literature for this compound are consequently not the amino–imino tautomers but the *Z*/*E* isomers with the oily *E* isomer dominating in chloroform solution.

Experimental. The experimental equipment was reported earlier.[10] Melting points are uncorrected, IR spectra were recorded on a Perkin Elmer model 298 grating spectrograph. 3-Amino-2,4-diphenyl-2-butenenitrile was prepared in accordance with the previously published method,[2] and purified by vacuum distillation. The two isomers were separated by recrystallization from ethanol.

3-Amino-2,4-diphenyl-2Z-butenenitrile. M.p. 103–108 °C. Anal. $C_{16}H_{14}N_2$: C, H, N. ^1H NMR ($CDCl_3$): δ 3.65 (2 H, s), 4.75 (2 H, broad), 7.05–7.45 (10 H, m). ^{13}C NMR ($CDCl_3$): δ 158.1, 135.8, 133.7, 129.4, 129.2, 128.9, 128.7, 127.3, 126.8, 120.3, 81.0, 37.1. IR ($CHCl_3$, cm^{-1}): 3500 (m), 3400 (m), 3000 (m), 2195 (s), 1625 (s), 1580 (s), 1495 (m), 1285 (m), 1270 (m).

3-Amino-2,4-diphenyl-2E-butenenitrile. B.p. 190–195 °C/0.5 mmHg. Anal. $C_{16}H_{14}N_2$: C, H, N. ^1H NMR ($CDCl_3$): δ 3.60 (0.3 H, s), 3.83 (1.7 H, s), 4.76 (2 H, broad), 7.05–7.45 (10 H, m). ^{13}C NMR ($CDCl_3$): δ 158.3, 157.0, 135.9, 133.3, 129.4, 129.2, 128.6, 128.8, 128.5, 127.3, 127.1, 126.9, 122.1, 120.3, 81.1, 40.3, 37.0. IR ($CHCl_3$, cm^{-1}): 3500 (m), 3400 (m), 3000 (m), 2195 (s), 1625 (s), 1580 (m), 1495 (m).

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In Vitro Immunization of Mouse Spleen Cells and the Production of Monoclonal Antibodies *

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The production of antibodies and lymphokines by immortalization of these functions in lymphoid cells can be achieved by somatic cell hybridization. The technology of immortalizing the ability of immune spleen cells to produce antibodies by fusing them with myeloma cells for the subsequent isolation of monoclonal antibodies is already well established. To further facilitate this technique and to introduce several advantages we have developed a new approach to the immunization procedure which allowes the cells to be immunized in culture.

This *in vitro* immunization technique utilizes T cell replacing factors (TCRF) to support the antigen specific immunization of non-immune spleen cells. TCRF can be generated by thymocytes from 10 day old mice [1,2] or in a mixed lymphocyte culture [3,4] and they apparently mimic the action of soluble factors produced by T helper cells during an *in vivo* immunization. A lymphokine preparation with the ability to support an antigen specific immunization of mouse spleen cells *in vitro* was produced by a mixed thymocyte culture. Thymocytes from 5–6 week old BALB/c and C57Bl/6 mice were co-cultivated for 48 h at a cell density of $4–5\times10^6$ cells/ml of supplemented Dulbeccos modification of Eagles medium (s-DMEM) (streptomycin 50 μg/ml, penicillin 50 IU/ml, L-glutamine 4 mM, ×100 non-essential amino acids 1 % (v/v)) containing 16 μM thymidine, 100 μM hypoxanthine, 50μM 2-mercaptoethanol and 2 % (v/v) Quadroma type 100 rabbit serum (Diagnostic Biochemistry Inc., CA., USA).[4] The medium was then collected by centrifugation at 800× g for 10 min, filtered (0.22 μm) and stored in 10 ml aliqouts at –70 °C for up to one year. Non-immunized BALB/c splenocytes (5×10^6 cells/ml) were then immunized in a 75 cm^2 tissue culture flask using s-DMEM containing 50 μM 2-mercaptoethanol and supplemented with 50 % (v/v) of supernatant from the mixed thymocyte culture together with filtered antigen of choice. After five days in culture at 37 °C, using an 8 % CO_2 and 92 % air gas phase, the cells were collected and used for cell hybridization.[4]

This *in vitro* immunization technique, briefly described above, has been used to specifically produce antigen-activated spleen cells subsequently used as parental cells to obtain specific hybridomas. Monoclonal antibodies have thus been produced against protein antigens and a hapten, such as human and sperm whale myoglobin, pig insulin and benzo[*a*]pyrene as well as against bacterial surface antigens (*Pseudomonas fragi*) using in *in vitro* immunized BALB/c cells and Sp2/0-Ag14 myeloma cells as fusion partners. Bovine serum albumin (BSA) was used as a protein carrier for benzo[*a*]pyrene at a ratio of 1:8. BSA-benzo[*a*]-pyrene, human myoglobin and pig insulin were used at a concentration of 50–100 μg/ml during the immunization period. The specific efficiency ((no. of culture plate wells producing specific antibodies/no. culture plate wells exhibiting cell growth) ×100) of hybridization experiments using spleen cells immunized in culture by these antigens was in the range of 20–30 %, which is comparable to when cells immunized *in vivo* were used. Monoclonal antibodies expressing both γ and μ isotypes could be isolated. To evaluate the relationship between the amount of antigen used in the immunization and the specific efficiency of each fusion, concentrations from 1 ng/ml up to 10 μg/ml of sperm whale myoglobin were tested in the *in vitro* immunization. The immune spleen cells were then immortalized and the antigen specific response was recorded after three weeks when the hybrid cells were tested. A positive dose-response relation was recorded ranging from a specific efficiency from 3 up to 15 %, depending on the antigen concentration used in the immunization step. This means that at a concentration as low as 1 ng antigen/ml of culture medium 3 % of the culture wells that are exhibiting hybrid cell growth are also producing antigen specific antibodies.

The effect of TCRF on the outcome of the *in vitro* immunization was also tested. *Pseudomonas fragi* cells were used as immunogen (the ratio of spleen cells to bacterial cell was 1:8) and the immunizations were performed in medium supplemented with TCRF or medium lacking any exogeneous TCRF. These two cell populations were then used as fusion partners in hybridization experiments with myeloma cells and a total dependence on the lymphokine preparation for the production of cell hybrids that produced specific antibodies was recorded. The specific efficiency of the hybrid cells produced using

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spleen cells immunized in the absence of TCRF was 0 % whereas the presence of TCRF during the immunization procedure yielded hybrid cells exhibiting a specific efficiency of 12 %. Cells that were immunized without the support of TCRF were hence unable to produce any specific immunoglobulins in the subsequent immortalization step illustrating the important role that lymphokines play during the immunization.

In summary, immunization in culture is an important technique which offers several advantages: (1) only a few nanograms of antigen are sometimes needed; (2) defined amounts of antigen and lymphokines are present during the immunization; (3) the immunization takes five days to perform. Furthermore, the normal cellular control of the immune response seems not to function in culture which results in the possibility of producing antibodies to highly conserved antigens.

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A Quick and Simple Bioassay System for Effectors of Cell Metabolism Using Biospecifically Immobilized Cells: Assay of Thiamine and Amphotericin Using Yeast Cells *

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Bioassays have usually been performed in plate cultures of microbial cells.[1] Such assays are very specific and often very sensitive.[2] But a drawback of these bioassays is that they are substantially slower than modern chromatographic and immunologic techniques. If they could be speeded up, the inherent characteristics of the bioassay systems would make bioassays an attractive alternative when analysing many biomolecules.

In a recent paper[3] we described that reversible biospecific immobilization can be used in assays of the number of viable cells in a sample. The sample is passed over a bed of biospecific sorbent and the number of cells captured is estimated from their metabolic activity when treated under standardized conditions with a defined substrate. This paper reports the use of affinity immobilization of cells prior to bioanalysis of effectors of cell metabolism, a positive – thiamine, and a negative – amphotericin-B.

Materials and Methods. Chemicals used: Amphotericin-B was a generous gift from Squibb and Sons, London, UK., Thiamine, thiamine pyrophosphate and acid phosphatase (from potato E.C. 3.1.3.2. grade II) were purchased from Sigma Chemical Co., St. Louis, Mo, USA, neutral red and bromophenol blue from E. Merck, Darmstadt, Concanavalin A Sepharose (Con A Sepharose) from Pharmacia Fine Chemicals AB, Uppsala, Sweden, and thiamine assay medium was obtained from Difco, Detroit, Mi, USA. All other chemicals used were of analytical grade.

Yeast cells were obtained from a local source.

Cultivation of yeast cells (Saccharomyces cerevisiae) for thiamine assay: One hundred ml of sterile thiamine assay medium (8.5 % w/v) were inoculated with 2 mg of a lyophilized yeast preparation and then incubated on a shaker at 37 °C for 36 hrs. The cells were collected by centrifugation and washed three times with water giving 1 g of yeast cells (wet paste).

Immobilization procedure. The cells were biospecifically bound to Con A Sepharose according to a standardized procedure. Aliquots of Con A Sepharose (0.5 ml sedimented gel) were filled into disposable plastic syringes equipped with a nylon net (25μ mesh) at its outlet.

The cells were suspended in 0.1 M acetate buffer containing 137 mM NaCl, 0.95 mM $CaCl_2$, 5.35 mM KCl, 0.80 mM $MgSO_4$, 1.0 mM $MnCl_2$, pH 4.5, and a fixed volume of the buffer was sucked into each of the syringes. As earlier, capture is very fast[3], but for practical reasons a defined incubation time of 20 min was used. The sorbent was washed by filling and emptying each syringe 4 times with the above described acetate buffer prior to use in the bioassays.

Exposure to thiamine – containing solutions. Standard solutions of thiamine were prepared in the above described acetate buffer. To improve the uptake of thiamine by the cells glucose (to 1 mM) was added to the medium. The same buffer was used when real samples were analyzed.

Exposure to amphotericin-B. The fungicide, which is poorly soluble in water, was first dissolved in *N,N*-dimethylformamide (to 6.66 mg/ml) and then added to the above acetate buffer. Final concentration of *N,N*-dimethylformamide was maximally 0.5 %. After exposure to amphotericin the metabolic activity of the immobilized cells was measured.

Assay of metabolic activity. After having been exposed to the substance to be quantified the immobilized cells were carefully washed with the buffer making up the assay medium, except for glucose and the indicator, before incubation in a glucose-rich buffer containing an indicator, either a redox or a pH-indicator.

The buffer containing the redox indicator consisted of: 50 mM glucose in the above described acetate buffer. pH was set at 4.5. 100 ml of this buffer was mixed with 1.0 ml of a solution obtained by dissolving 0.1 g of brom phenol blue in 14.9 ml 10 mM NaOH, which then was diluted to 265 ml.

The other assay solution was based on the pH-indicator neutral red. It consisted of a buffer containing 0.004 mM KH_2PO_4, 0.041 mM $NaHCO_3$, 0.055 mM Tris, 0.95 mM $CaCl_2$, 5.35 mM KCl, 137 mM NaCl, 0.80 mM $MgSO_4$ and 50 mM glucose, pH 7.4. To 100 ml of this glucose-containing buffer was added 4.0 ml of a solution of neutral red obtained by dissolving 10 mg of indicator in 50 ml of ethanol and then diluting the

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mixture with 50 ml of water.

The cells were incubated in their respective assay media by filling the syringes with a fixed volume (1.0 ml). The incubation time was usually 2 hrs, except when low concentrations of amphotericin were quantified when 4 hrs incubation was used.

After exposure the solvent was pressed out of the syringe into a cuvette and assayed spectrophotometrically at 585 nm (bromophenol blue) and at 528 nm (neutral red).

Results and discussion. The yeast cells were immobilized by affinity interactions between mannans on the cell surface and the immobilized lectin, concanavalin A bound to Sepharose 4B. The substrate used in this assay was glucose dissolved in buffer and the presence of an indicator. The uptake of the vitamin is pH-dependent and the yield of thiamine in the final assay step was improved by addition of glucose to the thiamine-containing medium.

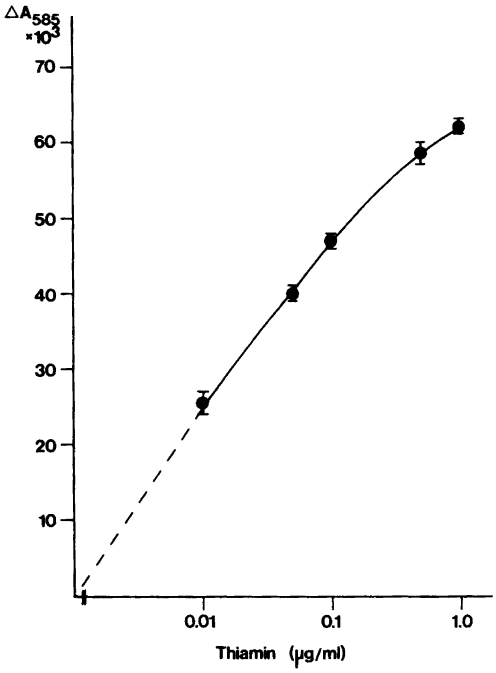

Fig. 1. Calibration curve for thiamine. The metabolic response registered as a function of thiamine concentration. All measurements were performed in triplicate. The bars in the figure denote the range of variation of the results of the assays. The syringes used were all preexposed to 6×10^6 cells. The figures are corrected for blank reactions registered in the blank sample. Experimental details as given in the text.

Furthermore, the duration of exposure of the cells to the vitamin solution is important. The metabolic stimulation from the preceeding step was maximal when the exposure lasted 30 min. In a typical assay 6×10^6 cells were added to each syringe. Fig. 1. shows a typical standard curve. All the measurements were made in triplicate and the bars in the figure denote the range of variation of the assays. Some food samples were analysed as well, and compared to results from the conventional procedures using fluorescence assay.[4] It was, however, found that the analytical results differed markedly, but those from the bioassay agreed well to those obtained with a yeast electrode.[5] The difference between the analytical results of the present method and those of the conventional spectrofluorimetric may be ascribed to the fact that the bioassay method is extremely specific, whereas a chemical method is less so. Some recovery experiments were performed to check that the response reflected a thiamine-related activity. Recovery was approximately 90 %.

The effect of thiamine pyrophosphate (TPP) on the system was showed. TPP as such has no effect on the yeast cells but it lowers the response on thiamine. This could be interpreted in terms of specific inhibition of vitamin uptake. Treatment of TPP with acid phosphatase, showed that the inhibiting effect disappeared together with a concomitant increased recovery of the added TPP in the analysis.

The sensitivities in the thiamin – assay are well within the range of interest in *e.g.* food analysis, and are better than those reported for bioassays based on microbe electrodes.[6]

Analysis of amphotericin-B. In a similar way as the effect of a stimulus may be registered it should also be possible to assess the effect of inhibitors. Since the effect of amphotericin is said to be due to its effect on biological membranes, one could expect that at low concentrations the damage to the cell membranes would be small and consequently only marginally influence the metabolism.

Earlier work on yeast cells has shown that pH-indicators[3] can be used when quantifying the metabolic activity of the cells. Using a redox indicator showed a large change whereas no effect was observed on neutral red.

The change in the assay with bromphenol blue must be due to factors other than changes in metabolism, unless the indicators influence the cells in markedly different ways – which has not been observed in former studies.

The amphotericin molecules dissolve constituents of the membrane and thereby make it easy for brom phenol blue to pass through into the

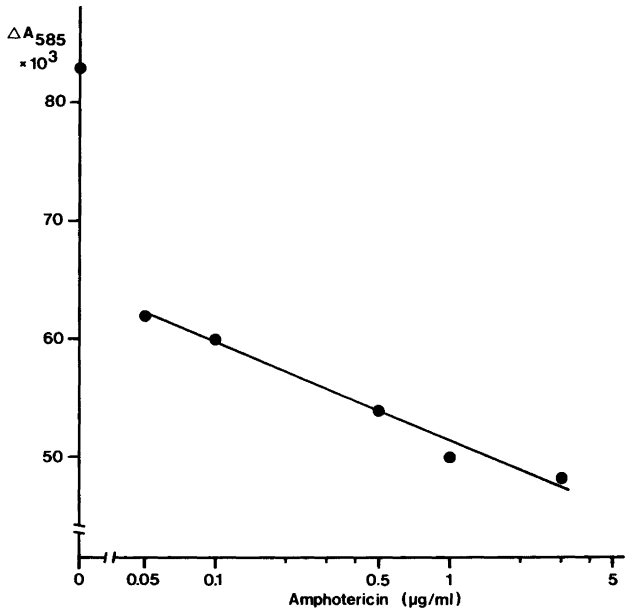

Fig. 2. Response of affinity bound *Saccharomyces cerevisiae* to treatment with amphotericin according to the procedure given in the text.

membrane. The difference between the results of the experiments with the different indicators can then be ascribed to differences in their affinity for the membrane.

A calibration curve towards amphotericin is shown in Fig. 2. The concentration range in which the assay reported in this paper operates is well within that of clinical interest.

The use of redox indicators instead of pH-indicators eliminates the need of very weak buffers in the incubation step. In the experiments reported here 0.1 M buffer was used throughout. Using such a strong buffer gives a better pH-stability and reduces the need of washings between the exposure of the cells to the systems to be quantified and the final registration of the metabolic activity.

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Tobacco Chemistry. 60.* Five New Hydroperoxycembratrienediols from Tobacco

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Five new diterpenoids of the cembrane class have been isolated from flowers of Greek tobacco and shown to be (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*1*, *2*) and (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*3*–*5*) by spectral and chemical methods. The biogenesis of these compounds is discussed.

The cembranic diterpenoids, which are present in the gummy exudate of the leaf and flower of most tobacco varieties, include as the major components the (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols (*6*, *7*). These two compounds are the postulated precursors of the majority of the other tobacco cembranoids.[2] We now report the isolation and biomimetic synthesis of five new cembranoids, which are likely to arise in tobacco by oxidation of the 4*S*,6*R*- and 4*R*,6*R*-diols (*6*, *7*).

Results. The first two compounds (*1*, *2*) gave rise to ^{1}H and ^{13}C NMR spectra consistent with the presence of an isopropyl group, a methyl group linked to a fully substituted, oxygen-carrying carbon atom, a vinylic methyl group and three double bonds. Of these, one is *E*-disubstituted, one is trisubstituted and one extends to an exocyclic methylene group. Two protons, resonating at δ 4.28 and 4.66 for *1* and at δ 4.30 and 4.68 for *2*, are evidently attached to oxygen-bearing carbon atoms. These results suggest that the new compounds (*1*, *2*) are structurally related to the 2,7,12(20)-cembratriene-4,6,11-triols, which have previously been isolated from tobacco.[1,3]

A comparison of the ^{13}C NMR data confirmed this and revealed that the C-1 to C-9 and C-14 to C-19 signals are present at virtually invariant positions in the spectra of *1* and (1*S*,2*E*,4*S*,6*R*,

Table 1. ^{13}C NMR chemical shifts and assignments for compounds *1*–*5* and *8*–*12*.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
1	48.7	127.9	139.7	74.2	46.2	68.5	128.5	137.5	30.2	34.6	88.1	147.1	29.6	29.0	32.2	19.2	20.8	33.1	15.9	113.5
2	47.6	129.8	137.0	72.3	52.1	64.7	129.8	138.7	31.3	35.0	86.2	148.6	30.2	28.6	31.9	18.8	20.5	31.6	16.6	111.8
3	50.7	127.9	138.4	74.2	46.7	69.4	127.9	135.2	41.0	126.9	133.9	85.0	35.8	26.5	30.2	17.9	21.6	31.6	18.3	24.4
4	50.8	128.1	138.5	73.9	47.0	69.3	127.6	135.4	41.1	130.0	134.0	86.4	35.5	27.4	30.2	18.0	21.6	31.8	18.1	20.9
5	48.3	129.4	136.7	72.0	52.1	64.9	129.4	138.3	41.7	128.0	133.6	85.0	35.9	25.9	29.8	17.9	21.2	30.4	18.2	23.2
8	49.0	127.4	139.8	74.1	46.5	68.5	128.5	137.8	29.8	32.9	74.8	151.6	34.6	29.3	32.2	19.2	20.8	33.4	16.0	111.2
9	47.6	129.7[b]	137.1	72.1	51.8	64.7	129.6[b]	139.1	30.3	32.5	73.4	152.4	34.9	30.3	31.9	18.7	20.6	31.4	16.6	110.3
10	50.8	127.3	138.3	74.3[b]	47.2	69.4	128.4	134.5	40.7	124.6	138.9	74.0[b]	40.1	26.5	30.1	17.8	21.8	31.6	18.0	30.0
11	49.9	127.6	138.4	74.4	47.1	69.2	128.0	135.7	41.0	126.1	138.1	73.9	40.2	28.0	29.8	17.9	21.6	31.7	17.9	27.1
12	49.3	128.8	137.2	71.9	52.3	64.8	128.8	138.9	40.9	124.8	138.0	73.8	39.7	25.9	29.6	17.6	21.5	30.4	18.2	29.8

[a] δ-Values in $CDCl_3$ relative to TMS. [b] Assignment may be reversed.

* For part 59 see Ref. 1.

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0302-4369/83 $2.50

7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*8*,[3] *cf.* Table 1). However, since the shieldings of C-11 were found to be markedly different, δ 88.1 for *1* as against δ 74.8 for *8*, and since the ^{1}H NMR spectrum of *1* exhibits a broad singlet at δ 8.05,[4] *1* was provisionally formulated as a (1*S*, 2*E*,4*S*,6*R*,7*E*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol. Analogous findings obtained by a comparison of the ^{13}C NMR spectra of *2* and (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*9*)[1] allowed the identification of *2* as a (1*S*,2*E*,4*R*,6*R*,7*E*,11ξ)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol. These assignments were in harmony with the mass spectra of *1* and *2*, which displayed a peak at *m/z* 320 (M-18) due to a $C_{20}H_{32}O_3$ ion.

The remaining three new compounds (*3–5*) were also hydroperoxides [broad singlets at δ 8.22, 7.33 and 7.43 in the ^{1}H NMR spectra of *3–5*, respectively; ^{13}C NMR signals at δ 85.0 (*3*, s), δ 86.4 (*4*, s) and δ 85.0 (*5*, s)]. In contrast to hydroperoxides *1* and *2*, however, compounds *3–5* contain two *E*-disubstituted and one trisubstituted double bond as well as three methyl groups, of which one is vinylic and two attached to (a) fully substituted carbon atom(s).

These results and a comparison of the ^{13}C NMR spectra with those of the (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triols (*10–12*)[1,3] led to a tentative formulation of *3* and *4* as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diols and of *5* as a (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12ξ)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol.

Conclusive evidence of the structures of hydroperoxides *1–5* was obtained *via* biomimetic syntheses. These involved sensitized photo-oxygenation of the 4*S*,6*R*- and 4*R*,6*R*-diols (*6*, *7*) and resulted, in each case, in the formation of a mixture of ene products,[1] which was subjected to repeated HPLC. The three major products of the 4*S*-series, which were identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)- and 1*S*,2*E*,4*S*,6*R*,77*E*,10*E*,12*R*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol by reduction to the known triols *8*, *10* and *11*[1,3] using triethyl phosphite, proved to be indistinquishable from hydroperoxides *1*, *3* and *4*, respectively.

The two major photo-oxygenation products of the 4*R*-series, which were correlated with triols *9* and *12* by reduction, were found to be identical to hydroperoxides *2* and *5*, respectively. The latter are hence fully characterized as (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*2*) and (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*5*).

With the structures of these new compounds at hand, it can be inferred from the ^{1}H and ^{13}C NMR results that the conformational difference with respect to the orientation about the 5,6 bond, previously noted between triols of the 4*S*- and the 4*R*-series (*e.g. 8, 10* and *11 vs. 9* and *12*),[1] is retained among the hydroperoxides.

Discussion. The present results provide experimental support for the view that the 4,6,11- and 4,6,12-triols, which are fairly abundant in the cuticular wax of the tobacco leaf and flower, are formed from the 4,6-diols (*6–7*) through the intermediacy of hydroperoxides.[1,2] They do not, however, imply the exclusive operation of singlet oxygen reactions, since enzyme-catalyzed oxidations would also be expected to proceed *via* hydroperoxide intermediates.

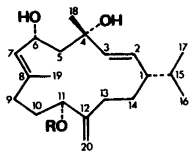
These new compounds (*1–5*), which are the only hydroperoxides found in tobacco so far,* add to the small but growing group of terpenic hydroperoxides. Among these are neoconcinndiol hydroperoxide from *Laurencia snyderiæ*,[4] crispolide from *Tanacetum vulgare*[5] as well as 4(15),5,10(14)-germacratriene-1β-hydroperoxide from *Senecio glanduloso-pilosus*.[6]

Experimental. With the exception of accurate mass measurements, which were carried out on a Kratos MS 50 Stereo DS 55 SM/DS 55 S mass spectrometer-computer system and the NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 7 were used.

An extract (83 g) obtained by immersing flowers of Greek *Nicotiana tabacum* (Basma Drama) in chloroform, was initially separated into five fractions, A (12.7 g), B (4.7 g), C (8.0 g), D (30 g) and E (3.6 g) by flash chromatography using a column packed with silica gel and gradients of hexane–ethyl acetate–methanol as eluent.

Fraction C was further separated by flash chromatography over silica gel into five fractions C1 (250 mg), C2 (485 mg), C3 (3.0 g), C4 (3.5 g) and C5 (465 mg). Repeated HPLC of fraction C4 using columns packed with Spherisorb and Spherisorb 5 Nitrile led to the isolation of 21 mg

* An analysis by TLC using ferrous thiocyanate as the spraying reagent of an extract, obtained by immersing fresh green leaves of Burley tobacco in cold chloroform (0 °C), revealed the presence of a peroxide-positive zone, whose R_F value agreed with that of hydroperoxides *1–5*. Moreover, since the 4,6-diols (*6,7*) fail to produce hydroperoxides even on prolonged exposure to triplet oxygen, it can be concluded that hydroperoxides *1–5*, now isolated from flowers of Greek tobacco, are not artefacts but genuine tobacco constituents.



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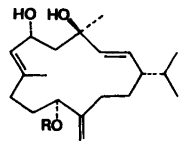
8 R=H



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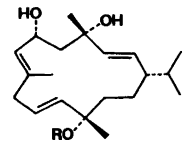

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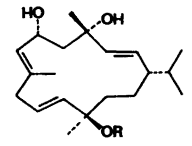

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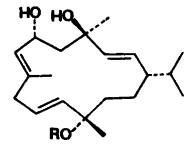
11 R=H



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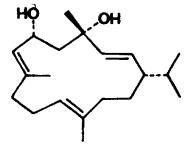
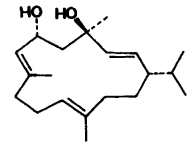
12 R=H



6



7

of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*1*), 28 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*3*) and 5 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*4*).

Fraction C5 was further separated by HPLC using columns packed with Spherisorb and Spherisorb 5 Nitrile to give 7 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*2*) and 5 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*5*).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-Hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*1*) had m.p. 132–134 °C, $[\alpha]_D$ +53° (*c* 0.47, $CHCl_3$) (Found: $[M-18]^+$ 320.2337. Calc. for $C_{20}H_{32}O_3$: 320.2352); IR ($CHCl_3$) bands at: 3603, 3464, 3080, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (d, J=6.7 Hz)/0.89 (d, J=6.7 Hz) (H-16/H-17), 1.28 (s, H-18), 1.66 (d, J=1.4 Hz, H-19), 1.77 (dd, J=2.4 and –14.4 Hz, H-5a), 2.23 (dd, J=5.6 and –14.7 Hz, H-5b), 4.28 (dd, J=4.5 and 9.1 Hz, H-11), 4.66 (ddd, J=2.4, 5.6 and 9.3 Hz, H-6), 5.12 (q, J=1.6 Hz, H-20a), 5.15 (broad s, H-20b), 5.46 (d, J=15.6 Hz, H-3), 5.54 (dd, J=8.3 and 15.6 Hz, H-2), 5.61 (broad d, J=9.3 Hz, H-7) and 8.05 (broad s, OO*H*); MS [*m*/*z* (%)]: 320 (0.2), 302 (0.3), 259 (0.8), 243 (1), 217 (0.9), 203 (1), 135 (8), 121 (13), 109 (16), 95 (20), 81 (20), 69 (21), 55 (25) and 43 (100).

(1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-Hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*2*) was an oil, which had $[\alpha]_D$ +34° (*c* 0.54, $CHCl_3$) (Found: $[M-18]^+$ 320.2358. Calc. for $C_{20}H_{32}O_3$: 320.2352); IR ($CHCl_3$) bands at: 3601, 3401, 3080, 1384 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, J=6.4 Hz)/0.85 (d, J=6.2 Hz) (H-16/H-17), 1.36 (s, H-18), 1.72 (d, J=1.3 Hz, H-19), 1.96 (dd, J=7.7 and –14.1 Hz, H-5a), 2.05 (dd, J=2.8 and –14.1 Hz, H-5b), 4.30 (t, J=6 Hz, H-11), 4.68 (ddd, J=2.8, 7.7 and 9.0 Hz, H-6), 5.06 (q, J=1.4 Hz, H-20a), 5.13 (broad s, H-20b), 5.28 (broad d, J=9.0 Hz, H-7), 5.37 (dd, J=8.3 and 15.8 Hz, H-2), 5.47 (d, J=15.8 Hz, H-3) and 7.90 (broad s, OO*H*); MS [*m*/*z* (%)]: 320 (0.2), 302 (0.5), 259 (0.8), 243 (1), 215 (1), 203 (2), 135 (8), 121 (14), 109 (16), 95 (24), 81 (24), 69 (24), 55 (27) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-12-Hydroperoxy-2,7,10-cembratriene-4,6-diol (*3*) was an oil, which had $[\alpha]_D$ +91° (*c* 0.50, $CHCl_3$) (Found: $[M-18]^+$ 320.2345. Calc. for $C_{20}H_{32}O_3$: 320.2352); IR ($CHCl_3$) bands at: 3602, 3384, 1664, 1384 and 1369 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, J=6.7 Hz)/(0.86 (d, J=6.6 Hz) (H-16/H-17), 1.21 (s)/1.37 (s) (H-18/H-20), 1.67 (broad s, H-19), 1.73 (dd, J=1.8 and –14.4 Hz, H-5a), 2.20 (dd, J=6.2 and –14.4 Hz, H-5b), 2.65 (ddd, J=0.6, 7.0 and –17.6 Hz, H-9a), 2.75 (ddd, J=0.6, 7.0 and –17.6 Hz, H-9b), 4.76 (ddd, J=1.8, 6.2 and 8.4 Hz, H-6), 5.39 (dt, J=0.6 and 16.1 Hz, H-11), 5.41 (d, J=15.4 Hz, H-3), 5.56 (broad d, J=8.4 Hz, H-7), 5.57 (dd, J=8.5 and 15.4 Hz, H-2), 5.63 (dt, J=7.0 and 16.1 Hz, H-10) and 8.22 (broad s, OO*H*); MS [*m*/*z* (%)]: 320 (0.1), 302 (0.2), 287 (0.4), 259 (0.4), 243 (0.8), 219 (0.8), 203 (1), 135 (8), 121 (10), 109 (16), 95 (18), 81 (17), 71 (21), 55 (21) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-12-Hydroperoxy-2,7,10-cembratriene-4,6-diol (*4*) was an oil, which had $[\alpha]_D$ +124° (*c* 0.23, $CHCl_3$) (Found: $[M-36]^+$ 302.2270. Calc. for $C_{20}H_{30}O_2$: 302.2246); IR ($CHCl_3$) bands at: 3602, 3472, 1667, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (d, J=6.7 Hz)/0.86 (d, J=6.7 Hz) (H-16/H-17), 1.23 (s, H-20), 1.35 (s, H-18), 1.68 (broad s, H-19), 1.74 (dd, J=2.2 and –14.3 Hz, H-5a), 2.20 (dd, J=6.4 and –14.3 Hz, H-5b), 2.75 (dd, J=0.8

and 6.8 Hz, H-9a and H-9b), 4.79 (ddd, J=2.2, 6.4 and 8.6 Hz, H-6), 5.44 (d, J=15.4 Hz, H-3), 5.45 (dt, 0.8 and 16.0 Hz, H-11), 5.48 (broad d, J=8.6 Hz, H-7), 5.55 (dd, J=8.3 and 15.4 Hz, H-2), 5.67 (dt, J=6.8 and 16.0 Hz, H-10) and 7.33 (broad s, OO*H*); MS [*m/z* (%)]: 302 (0.1), 287 (0.6), 259 (0.5), 243 (0.8), 217 (1), 203 (1), 135 (5), 121 (9), 109 (18), 95 (18), 81 (17), 69 (18), 55 (20) and 43 (100).

(1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-Hydroperoxy-2,7,10-cembratriene-4,6-diol (*5*) was an oil, which had $[\alpha]_D$ +64° (*c* 0.09, $CHCl_3$) (Found: $[M-18]^{+\cdot}$ 320.2343. Calc. for $C_{20}H_{32}O_3$: 320.2352); IR ($CHCl_3$) bands at 3602, 3381, 1662, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.76 (d, J=6.6 Hz)/0.85 (d, J=6.5 Hz) (H-16/H-17), 1.36 (s)/1.37 (s) (H-18/H-20), 1.79 (d, J=1.3 Hz, H-19), 1.83 (dd, J=9.3 and –13.8 Hz, H-5a), 2.05 (dd, J=2.9 and –13.8 Hz, H-5b), 2.61 (dd, J=7.5 and –14.9 Hz, H-9a), 2.83 (dd, J=6.7 and –14.9 Hz, H-9b), 4.69 (ddd, J=2.9, 8.8 and 9.3 Hz, H-6), 5.27 (broad d, J=8.8 Hz, H-7), 5.35 (dd, J=8.3 and 15.8 Hz, H-2), 5.45 (d, J=15.6 Hz, H-11), 5.45 (d, J=15.8 Hz, H-3), 5.72 (ddd, J=6.7, 7.5 and 15.6 Hz, H-10) and 7.43 (broad s, OO*H*); MS [*m/z* (%)]: 320 (0.1), 302 (0.2), 287 (0.2), 259 (0.4), 243 (0.7), 221 (1), 203 (1), 136 (6), 121 (11), 109 (15), 95 (20), 81 (19), 69 (21), 55 (23) and 43 (100).

*Sensitized photo-oxygenation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol* (*6*, *7*). A solution of 1.1 g of *6* and 34 mg of Rose Bengal in 20 ml of methanol, in a tube cooled by a water jacket, was irradiated with a 400 W sodium high pressure lamp placed outside the tube, while oxygen was bubbled through the reaction mixture. After 1.5 h the mixture was taken to dryness and the residue filtered through alumina. Repeated HPLC using a column packed with Spherisorb 5 Nitrile allowed the isolation of 210 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*1*), 140 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*3*) and 12 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*4*).

A solution of 200 mg of *7* and 10 mg of Rose Bengal in 25 ml of methanol was treated with singlet oxygen for 1 h using the apparatus described above. Separation by repeated HPLC using a column packed with Spherisorb 5 Nitrile afforded 80 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*2*) and 40 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*5*).

Of these, the (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diols were identical (optical rotation, IR, ^1H NMR and MS) to compounds *1* and *2* and the (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diols to compounds *3*, *4* and *5*, respectively.

Conversion of hydroperoxides 1–5 *to corresponding triols* (8–12). A solution of 6.9 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*1*) and 10 µl of triethyl phosphite in 1 ml of methanol was kept at room temperature for 5 min. Removal of the solvent and separation by HPLC using a column packed with Spherisorb and hexane–ethyl acetate (20:80) as an eluent afforded 6.0 mg of a product, whose IR, ^1H NMR and mass spectra were identical to those of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*8*).[3]

Using the same method, (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (*2*), (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (*3–5*) were converted to compounds, which were indistinguishable from (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*9*),[1] (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triol (*10–12*),[1,3] respectively.

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Some Comparative Studies of Phosphoglycerate Kinase from Spinach, Wheat and Yeast *

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Phosphoglycerate kinase (ATP: 3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) catalyzes the energy transforming reaction of glycolysis, gluconeogenesis and photosynthesis

$$\text{3-P-Glycerate} + \text{ATP} \overset{Me^{2+}}{\rightleftharpoons} \text{1,3-P}_2\text{-Glycerate} + \text{ADP}$$

In vitro experiments [1] for the yeast enzyme show that Me^{2+} stands for Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+} or Cd^{2+} and that the function of Me^{2+} in the catalytic reaction is to form complex with the nucleotide substrate. The two substrates bind independently to the enzyme.[2] Anions[3] act as activators of the reaction at low concentrations and inhibitors at higher concentrations. The effects are most pronounced with SO_4^{2-}.[3,4] Phosphoglycerate kinase has been isolated from a variety of sources. Until now most studies have been performed on the yeast enzyme, however. Very little is known about the enzyme from plants, disregarding some miscellaneous molecular and kinetic properties.[5,6] For the yeast enzyme it is evident[2,7] that multiple binding sites exist for both the substrates. ADP[8] and 1,3-P_2-glycerate[9] appear preferentially to bind to sites outside the binding sites for the corresponding substrates $MgATP^{2-}$ and 3-*P*-glycerate (*cf.* also ref. 10).

Experimental. Spinach phosphoglycerate kinase was purified from fresh leaves (cultivar Dominant) by affinity chromatography on ATP-ribosyl-adipoyl-dihydrazo-Sepharose according to Kuntz *et al.*[11] The same method was also used for the enzyme from fresh wheat leaves (cultivar Swedish Amy). The yeast enzyme was prepared as described earlier.[12] The kinetic experiments were performed as described before[2,8] in 50 mM Tris-HCl (pH 7.8, 25 °C) containing 0.10 M NaCl. The total Mg^{2+} and ATP concentrations were 1 mM if not stated otherwise.

Results. The spinach enzyme was eluted from the ATP-ribosyl-dihydrazo-Sepharose column with MgATP after unspecifically bound proteins had been removed with 0.7 M NaCl as described by Kuntz *et al.*[11] The wheat enzyme, and the earlier purified (*cf.* ref. 12) yeast enzyme (form 2), were eluted already with 0.3–0.7 M NaCl. For wheat a second peak appeared after elution with MgATP.

Kinetic studies of $MgATP^{2-}$ and 3-P-glycerate at various fixed concentrations of 3-*P*-glycerate and $MgATP^{2-}$, respectively, show for the spinach enzyme that K_m for one of the substrates is independent of the concentration of the second substrate. The K_m value for $MgATP^{2-}$ and 3-*P*-glycerate was 0.24 mM and 0.63 mM, respectively (mean of five experiments in each case). Similar results were earlier[2] obtained for the yeast enzyme. After the spinach enzyme had been kept frozen for some months K_m for 3-*P*-glycerate had increased to 1.1 mM (mean of four experiments). Attempts to make the transformation of this K_m value reversible were not successful. Kuntz and Krietsch reported[5] that K_m for 3-*P*-glycerate is 1.1 mM, a value close to that obtained for the yeast enzyme under certain conditions (*cf.* ref. 2).

Inhibition of the spinach enzyme by ADP was linearly noncompetitive with either substrate, $MgATP^{2-}$ or 3-*P*-glycerate. A competitive contribution was found in both cases. The results with both the spinach and the yeast[8] enzyme give a strong indication for an ADP binding site outside the binding site for the nucleotide substrate. The kinetics appeared to fit to eqn. (1). The symbols used represent: A, $MgATP^{2-}$; B, 3-P-glycerate; I, ADP; K_A and K_B, the respective Michaelis constants; K_i, K_i' and K_i'' the dissociation constant for the complex between I and the enzyme, the enzyme-MgATP and the enzyme-(3-*P*-glycerate) complex, respectively; v, the initial velocity; V, v at saturating conditions of both the substrates. K_m (3-*P*-glycerate) was 0.63 mM (*cf.* above) K_i was estimated to 0.1 mM, K_i' to 0.2 mM and K_i'' to 0.3 mM.

Inhibition of the spinach enzyme by AMP was

$$v = \frac{V}{1+\left(1+\frac{[I]}{K_i''}\right)\frac{K_A}{[A]}+\left(1+\frac{[I]}{K_i'}\right)\frac{K_B}{[B]}+\left(1+\frac{[I]}{K_i}\right)\frac{K_A \cdot K_B}{[A][B]}} \quad (1)$$

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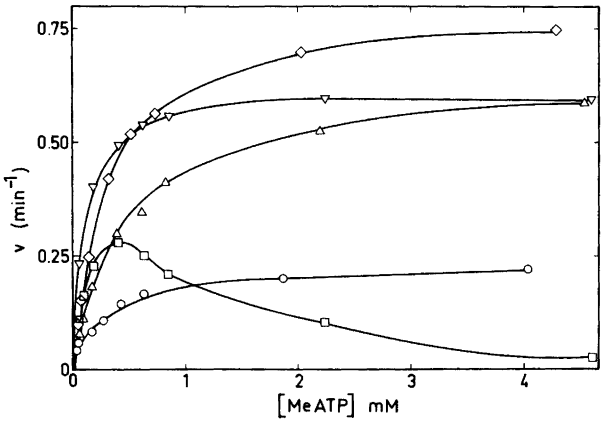

Fig. 1. The activity of spinach phosphoglycerate kinase with variable ATP-metal ion concentrations. MeATP stands for $MgATP^{2-}$ (◇), $MnATP^{2-}$ (▽), $CoATP^{2-}$ (△), $CaATP^{2-}$ (○) and $ZnATP^{2-}$ (□). For the complex constants used see ref. 1. The total metal ion and ATP concentrations were 1:1; the 3-P-glycerate was 2 mM. The deviations from hyperbolic kinetics are probably due to the increasing free metal ion concentrations (see ref. 1).

linearily competitive with $MgATP^{2-}$ and linearily noncompetitive (a competitive contribution occurred) with 3-P-glycerate up to AMP concentrations of 3 mM. The kinetics appeared to fit into eqn. (2):

$$v = \frac{V}{1+\left(1+\frac{[I]}{K_i'}\right)\frac{K_A}{[A]}+\frac{K_B}{[B]}+\left(1+\frac{[I]}{K_i}\right)\frac{K_AK_B}{[A][B]}} \quad (2)$$

the symbols as for Eqn. 1 but I stands for AMP and K_i and K_i' for the dissociation constant for the complex between I and the enzyme and the enzyme-(3-*P*-glycerate) complex, respectively. K_M (3-*P*-glycerate) was 1.1 mM (*cf.* above) K_i was estimated to 0.2 mM and K_i' to 0.4 mM. Very similar results were earlier[8] obtained for the yeast enzyme. From the K_i values it is evident that the spinach enzyme has a higher affinity for AMP than the yeast enzyme has.

The metal ion specificity of the spinach enzyme was tested. The ATP and metal ion concentrations (molar ratio 1/1) were varied in separate experiments with Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+}, respectively. The rate profiles, Fig. 1, appeared very close to those of the yeast enzyme (Fig. 1 in ref. 1). The most significant differences were observed for $CaATP^{2-}$. Compared to the yeast enzyme and $MgATP^{2-}$, $CaATP^{2-}$ is only

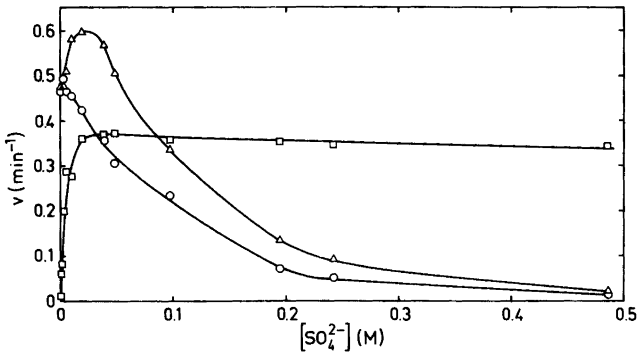

Fig. 2. The effect of SO_4^{2-} on the activity of phosphoglycerate kinase from yeast (△), spinach (○) and wheat (□), (the wheat enzyme eluted with 0.3–0.7 M NaCl). The total ATP concentration was 1 mM, Mg^{2+} and 3-*P*-glycerate 2 mM.

about half as active as a substrate for the spinach enzyme. The K_m values are within 0.1 and 0.4 mM, increasing in the following order

$$MnATP^{2-} < ZnATP^{2-} < MgATP^{2-} < CaATPu22^{-} < CoATP^{2-}$$

similar to the yeast enzyme.

The SO_4^{2-} effects on the enzymes were very different (Fig. 2): For the yeast enzyme activation occurred at low and inhibition at high SO_4^{2-} concentrations; for the spinach enzyme SO_4^{2-} inhibition was quite obvious, possible activation can be suspected only; and for the wheat enzyme, eluted from the affinity column with NaCl, only activation showed up. If the SO_4^{2-} activation is related to the fact that the enzyme was eluted from the column by fairly low NaCl concentrations is still an open question. The wheat enzyme fraction eluted with MgATP (see above) showed a SO_4^{2-} rate profile (not shown in Fig. 2) very similar to the yeast enzyme, even if the activation was only one fifth of the value obtained with the yeast enzyme.

Conclusions. Phosphoglycerate kinase from various sources appear to have many molecular and kinetic properties in common. Some differences between the spinach, wheat and yeast enzymes have been observed, however: 1. in behaviour on an affinity column labelled with a substrate homologue, note that two forms of the wheat enzyme were obtained (*cf.* Refs. 12 and 13); 2. in sensitivity to sulfate ion; 3. in the affinity for AMP and 4. in the relative efficiency of the Ca^{2+} activation.

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Steric Effects in S_N2 Reactions. Determination of Transition State Structures for the Quaternization of 2-Alkylpyridines and -thiazoles by a Combined Experimental and Molecular Mechanics Procedure

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S_N2 transition-state structures for the Menschutkin reaction between 2-alkylpyridines or -thiazoles and CH_3X (X=I and SO_3F) have been calculated by the Allinger 1973 force field method. Experimentally found differences in steric energies between the quaternary iminium ions and the activated complexes are used as measures for the evaluation of the position of the transition state. Estimates of the carbon–nitrogen distance (r_{C-N}) in the transition state result in a 22±4 % extension compared to the ground state value (1.48 Å) depending upon the transition state model used. The preferred model results in r_{C-N}=1.812±0.013 Å. Consistent results were obtained with nucleophiles of different geometries (pyridines and thiazoles). A change of leaving group from iodide to fluorosulfonate leads to an extension of r_{C-N} by 0.04±0.02 Å. The results are discussed in relation to earlier work and the reactivity selectivity principle.

In the ideal case, the mechanism of a chemical reaction may be considered known if the motions of the participating atoms and the connected potential energy could be determined during the path from reactants to products. Since it is not generally possible to obtain such an intimate picture, one is reduced to obtaining information at one or more crucial stages of the reaction. The widespread use of the transition state (TS) theory has focussed the interest on the structure of the activated complex, but even this more modest purpose is not easily achieved.

Nucleophilic displacement reactions at saturated carbon have been the subject of continuous interest with respect to their mechanistic details.[1] Although numerous experimental studies have been carried out on the S_N2 reaction, little is known about the detailed geometry of the activated complexes. The experimental methods that are used are indicative rather than conclusive and give qualitative estimates of possible changes in TS structures imposed by small changes in the reactants, such as change of solvent,[2] nucleophile [3–6] and leaving group.[3,7,8] The results are often used as arguments in the ongoing controversy concerning the validity of the reactivity–selectivity principle (RSP) and related to the predictions of Hammond,[9] Thornton [10] and Harris and Kurz.[11] These approaches, however, only tell us the direction and not the magnitude of the possible changes imposed by the perturbation.

There have been several MO studies on the S_N2 reaction.[12–16] The calculations have usually been carried out on reactions with symmetrical TS of the type $[H{---}CH_3{---}H]^-$, $[F{---}CH_3{---}F]^-$ and $[H_2O{---}CH_3{---}OH_2]^+$. The CNDO [14,16] and INDO [15] methods give values of the length of the reacting bond in the TS which are 7–12 % longer than in the reactants (products) for the negatively, as well as the positively, charged TS:s. On the

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other hand, an enlargement of 29–48 % was obtained by the *ab initio* method.[12,13] The calculations were performed for reactions *in vacuo* except in one case,[14] where a small increase from 7 to 8 % was calculated for the influence of solvation (water). What we know today about the profound influence of solvation on the Menschutkin reaction (*vide infra*) gives no support to any direct comparison between gas phase data (or calculations that refer to gas phase properties) and results obtained from reaction in solution. In this work we are interested in the reaction in solution.

Recently, we proposed the use of a linear free energy relationship of the type $\log (k/k_o)_1 = \delta \log (k/k_o)_2$ to correlate steric effects with geometric parameters.[17] This idea is based upon the assumption that the steric strain might be expected to monotonously increase (or decrease) along the reaction coordinate for the reactions studied at least in the neighbourhood of the transition state. A minor perturbation, causing a shift of the position of the TS should thus imply a change of the strain, which in turn should affect the relative rate of the reaction. The utility and significance of such an approach has been demonstrated for variation in nucleophile geometries,[17] for variation in the nature of the leaving group [18] and in the study of the evaluation of the position of the TS in the Menschutkin reaction.[19] Furthermore, there is an interesting potential in this method, namely, since the geometric dependence of steric effects is approximately known, an actual estimate of distances and distance changes in the TS might be made, assuming that the proper energetics are known. This work describes an attempt to calculate, by an established force-field method, the geometries of the activated complexes for the reactions between 2-alkylpyridines or 2-alkylthiazoles and methyl substrates, using the steric parameters from Refs. 17–19 and some other work. The calculation of steric effects of alkyl groups on the rate constants for the S_N2 reaction has been the subject of several investigations since the classical work by Ingold and coworkers [20] on alkyl halides. Ingold's approach has been refined and applied to modern force-field methods.[21,22]

In this work we use the molecular mechanics method to calculate substituent strain increments in such a way that they can be related to experimental values in order to give information about the geometries of the TS. In this respect there is an obvious resemblance between our approach and those of previous work, but in this work we study non-symmetrical TS and heteroaromatic nucleophiles. We also want to emphasize that it is not attempted to be an *ad hoc* force field calculation of the S_N2 TS. Such calculations are necessarily prone to certain difficulties regarding the validity of the potential functions and of the force field for highly distorted species and regarding the problem of localization of saddle points on the potential energy surface. Furthermore, we are interested in the geometry of the TS for reaction in solution and not in gas phase. The most interesting geometrical parameters of the S_N2 TS are the bond lengths of the two bonds which are formed and broken in the reaction. They are often used as "reaction coordinates" but essentially no attempts have been reported to quantitatively estimate these values by experimental methods.

EVALUATION OF EXPERIMENTAL DATA

More than twenty years ago Brown *et al.* made extremely careful studies of the rates of reaction of 2-, 3- and 4-alkylpyridines with alkyl iodides.[23] They found that while the introduction of an alkyl group in the 3- or 4-position resulted in small increases in rate following the basicity of the nucleophiles, the introduction of such groups in the 2-position resulted in a decrease in rate being gradually more pronounced in the series methyl<ethyl<isopropyl<*t*-butyl. They proposed that this decrease in rate was due to steric strain in the activated complex and estimated this strain ($\Delta\Delta H$) to 1.7 kJ for 2-methyl-, 2.5 kJ for 2-ethyl-, 5.0 kJ for 2-isopropyl and 16.3 kJ/mol for 2-*t*-butylpyridine, in all cases related to pyridine. Furthermore, they evaluated the strain in the activated complex to amount to 65 % of the strain in the final product by considering homomorphous complexes of pyridines and Lewis acids. Our later investigations [17–19,24] are in excellent agreement with the results obtained by Brown *et al.*

In Fig. 1 a generalized energy profile for the reaction between a 2-alkylpyridine and CH_3X is shown. According to the principle of microscopic reversibility, the reverse reaction, *i.e.* the dequaternization of the pyridinium salt, should

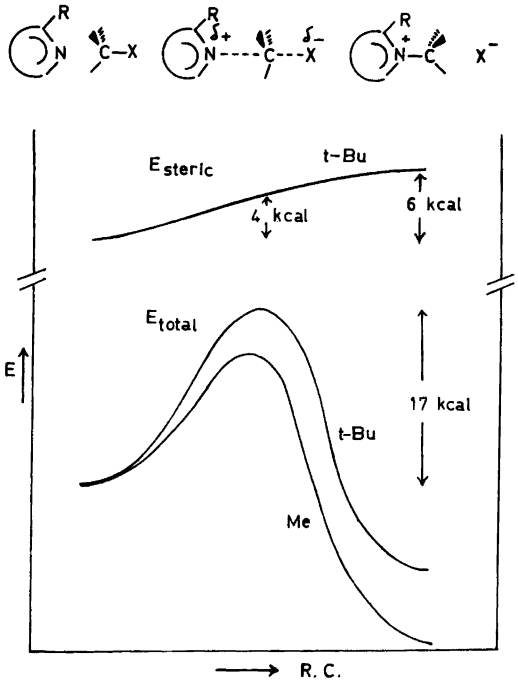
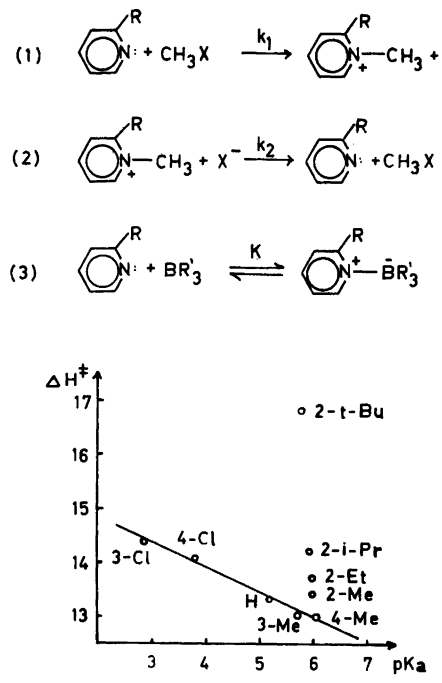


Fig. 1. A pictural potential energy profile for the quaternization of 2-methylpyridine and 2-*tert*-butylpyridine and the steric contribution to the reaction. (1 kcal=4.186 kJ).



Scheme 1. Graphical representation of the evaluation of the steric energies and the reactions for which the method was applied (1 kcal=4.186 kJ).

have the same TS and the reverse energetics as compared to the quaternization. This should also be valid for the steric part of the energy. In a recent investigation of the demethylation of 2-substituted pyridinium salts by triphenylphosphine in DMF,[19] we estimated the relief of strain in the transition state of this reaction to be 53±3 % of the steric compression in that of the methylation. This is equivalent to a strain in the activated complex amounting to 65±3 % of the strain in the salt in striking agreement with the data of Brown *et al.*[23] Experimental data are shown in Table 1. A similar quaternization/dequaternization investigation has also been carried out for 2-alkylthiazoles [25-26] (Table 2), and a strain in the activated complex amounting to 57±4 % of the strain in the salt was estimated.*

* In our earlier papers [19,25] we proposed that triphenylphosphine is the dealkylating agent. It has since been shown that the iodide counter ion is actually the nucleophile.[27] The conclusions are, however, still valid or actually reinforced by this new knowledge.

The pertinent thermodynamic parameter for comparison with calculated steric energies is the enthalpy. In the forward reaction the steric contribution of a substituent R was evaluated by the deviation from the linear relation between activation enthalpy and pK_a for 3- and 4-substituted pyridines according to eqn. (1) as illustrated in Scheme 1.

$$\Delta\Delta H_R^{\ddagger} = \Delta H_R^{\ddagger} - (\alpha \cdot pK_a + \text{const.}) \qquad (1)$$

For the reverse reaction only relative rate constants are available. We were thus forced to introduce the approximation that *the entropy of activation is constant for 2-Me, 2-Et, 2-i-Pr and 2-t-Bu pyridines in this reaction.* The observation that the relief of strain is 53 % of the compression in the methylation enables the calculation of the steric enthalpy increments of the 2-substituents in the *N*-methylpyridinium ion. Essentially the same values were obtained by Brown *et al.*[23] The experimental results for both pyridines and thiazoles are collected in Table 3.

The purpose is now to calculate the differences in strain energy (δE_s) between the pyridinium (thiazolium) ion and the TS, which reproduce the experimental data in Table 3. Furthermore, the results in Ref. 18 may be used to study the effect of a change of the leaving group on the TS geometry. We found that the strain in the TS upon methylation of 2-alkylpyridines with methyl fluorosulfonate was 69±3 % of the strain in the TS on methylation with methyl iodide. Thus, the δE_s values for methyl fluorosulfonate methylation given in Table 3 may be calculated.

METHOD OF CALCULATION AND TS MODELS

The δE_s values given in Table 3 represent differences in strain energy between the quaternary iminium salt and the activated complex.

Table 1. Kinetic data for methylation and demethylation in the pyridine series.

2-Subst.	Methylation [a] $k^{25\,°C} \cdot 10^6$ [c]	log k/k_H	$\Delta H^{\neq}$ [d]	$\Delta S^{\neq}$ [e]	Demethylation [b] log k/k_H
H	343	0	55.7	-124.7	0
Me	162	-0.32	56.1	-129.8	0.00
Et	76.4	-0.65	57.3	-131.9	0.163
i-Pr	24.5	-1.14	59.4	-134.4	0.459
t-Bu	0.080	-3.63	70.3	-144.8	1.70

[a] By CH_3I in nitrobenzene.[23a] [b] Of pyridinium iodides by Ph_3P in DMF at 153 °C.[19] [c] $M^{-1}\,s^{-1}$. [d] kJ mol^{-1}. [e] J $mol^{-1}\,K^{-1}$.

Table 2. Kinetic data for methylation and demethylation in the thiazole series.

2-Subst.	Methylation [a] $k^{25\,°C} \cdot 10^6$ [c]	log k/k_H	$\Delta H^{\neq}$ [d]	$\Delta S^{\neq}$ [e]	Demethylation [b] log k/k_H
H	3.22	0	68.6±0.8	-119.7±5.0	0
Me	4.54	0.15	66.5±1.7	-123.5±9.6	−0.22
Et	3.47	0.03	67.8±0.4	-122.6±1.2	−0.056
n-Pr	3.51	0.04	67.4±0.4	-123.5±1.2	−0.027
i-Bu	3.31	0.01	67.4±0.4	-123.9±2.9	+0.0086
neo-Pent	2.44	-0.12	68.2±0.8	-124.3±4.2	+0.20
i-Pr	1.53	-0.32	70.7±1.2	-119.3±7.1	+0.26
t-Bu	0.098	-1.52	76.6±1.7	-122.2±8.8	+1.08

[a] By CH_3OTs in nitrobenzene.[26] [b] Of thiazolium iodides by Ph_3P in DMF at 153 °C. [c] $M^{-1}\,s^{-1}$. [d] kJ mol^{-1}. [e] J $mol^{-1}\,K^{-1}$.

Table 3. Strain energies ($\Delta\Delta H$/kJ mol^{-1}) in the transition states and in the iminium ions evaluated from the methylation–demethylation data.

2-Subst.	Pyridines TS	Salt	δE_s^{I} [a]	$\delta E_s^{OSO_2F}$	Thiazoles TS	Salt	δE_s^{I} [a]
Me	1.9	2.8	0.9	1.5	0.6	1.3	0.7
Et	2.9	4.4	1.5	2.4	1.7	2.6	0.9
i-Pr	5.0	7.5	2.5	4.1	4.2	6.1	1.9
t-Bu	15.9	23.9	8.0	12.9	10.5	16.8	6.3

[a] $\delta E_s = \Delta\Delta H_{salt} - \Delta\Delta H_{TS}$.

Thus the procedure of calculation falls into two parts. In the first part the energy of the salt is calculated. This is in principle a straight-forward calculation of a molecule with ground state structure and properties. In the second part TS models are to be constructed in such a way that the difference in calculated steric energy between the salt and the activated complex reproduces experimental δE_s values. The TS models are described in detail below.

Allinger's 1973 force field [28,29] has been used throughout the calculations. The steric energy calculated by the molecular mechanic method is not the same as the experimental strain energies, but contains also contributions that depend on how the force field has been defined and on the motional restrictions that we introduce (*vide infra*). In the method to be described, differences in calculated steric energies between two species are carried out in such a way that these force-field dependent contributions cancel out.

The structures of *N*-methylthiazolium iodides with alkyl groups in the 2- and 4-positions have been determined by X-ray crystallographic methods.[30] In the pyridinium series no 2-substituted salt has been studied. Instead X-ray data on 3-substituted *N*-methylpyridinium salts make the experimental basis for the structure of the pyridinium ring in this work.[31] Since the force field is not parametrized for the pyridine and thiazole system, the ring skeleton (carbon, nitrogen and sulfur atoms) was kept rigid, and idealized geometries, taken from Refs. 30 and 31 were used (Fig. 2), the same for both salts and TS models. Apart from this restriction full relaxation was allowed, *i.e.* the ring hydrogens, the *N*-methyl and the 2-alkyl substituents were allowed to move with respect to all degrees of freedom.

The calculations satisfactorily reproduced known structures of 2-alkylpyridines and -thiazoles as well as the corresponding salts.

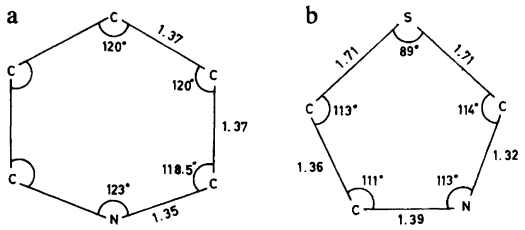


Fig. 2. Geometries for the ring skeleton, a. pyridine; b. thiazole.

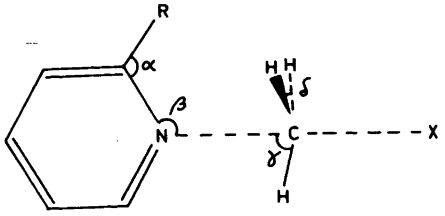


Fig. 3. Definition of the geometrical parameters of the transition state.

The TS models are defined by the internal coordinates given in Fig. 3. The main assumptions implicit in the ground state are, when possible, maintained in the TS model. The problem consists of choosing values of the angles α_o, β_o, γ_o, δ_o, the distance r_{C-N} and their corresponding force constants.*

The structures of 2-alkylpyridines or 2-alkylpyridinium salts have not been determined so that the value of α_o, the minimum energy angle, was varied within a few degrees around the one for which it symmetrically bisects the extranuclear angle. For the thiazole series α_o was chosen so as to reproduce experimental structures of the 2-alkylthiazolium salts. The same α_o was invariably used for the TS model as for the salt.

Since the bond between the central carbon and the nitrogen atom is created as the reaction progresses, the bending constant k_β is a function of the position along the reaction coordinate axis. More specifically, the value for k_β may be considered to increase from zero at the initial state to the normal value of the salt. Either of these extreme values has been used in the calculations.

The problem of choosing minimum energy values for the angles γ and δ is also connected to the degree of evaluation of the reaction at the TS. At present, there seems to be a general agreement that the TS of the Menschutkin reaction is reactant-like.[2] In the absence of detailed information we have, somewhat arbitrarily, chosen a model in which the inversion has progressed 85 % from ground-state angles to angles corresponding to a planar state (or, equivalently, to 42.5 % of the total inversion). This model was

* Subscript o, as in α_o, is denoted for minimum energy values for bond angles and bond lengths.

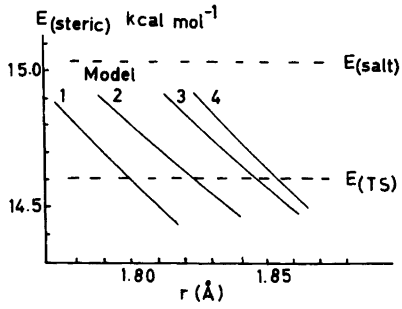


Fig. 4. Example of relations between calculated steric energy and carbon–nitrogen bond length for different transition-state models. (1 kcal=4.186 kJ).

recently used in force-field calculations on an S_N2 transition state.[32] In parallel studies, the planar model was used in order to make possible an estimation of the sensitivity of final geometries on variations in γ_o and δ_o. The bending constant k_γ was also varied from ground-state value to zero, whereas k_δ was kept at the ground-state value throughout.

The treatment of the reacting bonds in force-field calculations causes important problems, since it is highly questionable whether the normal ground-state force constants are valid for strongly distorted bonds. Moreover, it is by no means evident that the ordinary potential functions are valid in this case. Our method affords a way of getting around this problem. We perform calculations with a stiff C–N bond for varying bond lengths giving rise to an energy-bond distance relation (Fig. 4). A value of 999.9 m dyn $Å^{-1}$ was used for k_s, the stretch force constant.

The leaving group/counter ion is omitted in all calculations. Pyridinium ions form donor–acceptor complexes with iodide ions, but the latter exert little influence on the geometry of the cation,[31] so that the above approximation should cause no obvious disadvantage in the product salt calculations. In the activated complex the steric interactions between the leaving group and the rest of the atoms are obviously not negligible and these interactions are subject to strong variations during the course of the reaction. Of more importance to this investigation is, however, the difference in the development of these interactions between the 2-alkyl substituted pyridines (or thiazoles) on one hand and that of the unsubstituted heterocycles on the other hand. This difference is much smaller and if it can be neglected the leaving group may be omitted. We have made this assumption throughout the calculations. Support for this assumption was obtained in calculations on the hypothetical molecule 2-alkyl-*N*-iodopyridine with N–I bond lengths varying from 4.0 to 5.0 Å showing that the iodine atom is situated at or beyond the "van der Waals distance" between this atom and the 2-alkylpyridine moiety. Furthermore, an increase of the N–I distance was accompanied by a minimal increase in energy of 2.0 kJ $Å^{-1}$ or less.

The parameters are summarized in Tables 4 and 5.

Table 4. Force field parameters. Other parameters are found in Ref. 29.

van der Waals constants		
Atom	r^* (Å)	ε (kcal/mol)
C_{TS}	1.85	0.030
Stretching constants		
Bond	l_o (Å)	k_s (m dyn $Å^{-1}$)
C_{TS}–N	[a]	999.9
C_{TS}–H	1.09	4.6
Bending constants		
Angle	θ_o (deg)	k_b (m dyn Å rad^{-2})
$C_{sp}2$–N–$C_{sp}3$	118.5 (123.5) [b]	0.70
$C_{sp}3$–$C_{sp}2$–N	[a]	0.38
$C_{sp}3$–$C_{sp}2$–S	[a]	0.38
$C_{sp}2$–N–C_{TS}	118.5 (123.5) [b]	[a]
H–C_{TC}–H	[a]	0.19
H–C_{TS}–N	[a]	[a]

Torsional constants

Angle	V_1	V_2	V_3 (kcal/mol)
H–C_{TS}–N–$C_{sp}2$	0.0	0.0	0.0
H–$C_{sp}3$–N–$C_{sp}2$	0.0	0.0	0.0
$C_{sp}3$–$C_{sp}3$–$C_{sp}2$–N	0.0	0.0	0.30
$C_{sp}3$–$C_{sp}3$–$C_{sp}2$–S	0.0	0.0	0.30
H–$C_{sp}3$–$C_{sp}2$–N	0.0	0.0	0.0
H–$C_{sp}3$–$C_{sp}2$–S	0.0	0.0	0.0

[a] Varied in different transition state models according to Table 5. [b] Values in parentheses correspond to thiazole derivatives.

Table 5. Transition state models. Parameters are defined in Fig. 3.

Model	α_o [a]	k_α [b]	β_o [a]	k_β [b]	γ_o [a]	k_γ [b]	δ_o [a]	k_δ [b]
1	118(122) [c]	0.38	118.5(123.5)	0.70	90	0.42	120	0.19
2	118(122)	0.38	118.5(123.5)	0	90	0.42	120	0.19
3	118(122)	0.38	118.5(123.5)	0.70	87	0.42	119.25	0.19
4	118(122)	0.38	118.5(123.5)	0	87	0.42	119.25	0.19
5	120(124)	0.38	118.5(123.5)	0.70	87	0.42	119.25	0.19
6	120(124)	0.38	118.5(123.5)	0	87	0.42	119.25	0.19
7	118(122)	0.38	118.5(123.5)	0	87	0.30	119.25	0.19
8	118(122)	0.38	118.5(123.5)	0	87	0.05	119.25	0.19
9	118(122)	0.38	118.5(123.5)	0.10	87	0.20	119.25	0.19
10	118(122)	0.38	118.5(123.5)	0.20	87	0.10	119.25	0.19
11	120(124)	0.38	118.5(123.5)	0.05	87	0.25	119.25	0.19
12 [d]	120	0.38	118.5	0.04	86	0.25	119.0	0.19

[a] Degrees. [b] m dyn Å rad^{-2}. [c] Values in parentheses refer to thiazole derivatives. [d] Model for leaving group OSO_2F.

Table 6. Carbon–nitrogen bond lengths (r_{C-N}/Å) in the transition state for the models defined in Table 5.

Model	Me		Et		i-Pr		*t*-Bu	
	Pyridine	Thiazole	Pyridine	Thiazole	Pyridine	Thiazole	Pyridine	Thiazole
1	1.782	1.791	1.799	1.800	1.808	1.819	1.955	2.013
2	1.778	1.768	1.790	1.767	1.792	1.799	1.818	1.839
3	1.862	1.873	1.877	1.881	1.883	1.898	2.032	2.095
4	1.859	1.849	1.869	1.859	1.867	1.878	1.896	1.902
5	1.849	1.873	1.870	1.882	1.885	1.900	2.042	2.093
6	1.847	1.852	1.866	1.854	1.874	1.885	1.912	1.893
7	1.839	1.839	1.832	1.838	1.828	1.830	1.846	1.824
8	1.730	1.728	1.705	1.700	1.695	1.681	1.672	1.666
9	1.806	1.833	1.800	1.836	1.801	1.843	1.831	1,857
10	1.744	1.738	1.725	1.713	1.720	1.720	1.783	1.789
11	1.810	1.823	1.812	1.823	1.817	1.820	1.793	1.799

RESULTS AND DISCUSSION

Table 6 reports the carbon–nitrogen distances for 11 different TS models as defined by the force fields in Table 5. The models are chosen so as to cover different possibilities of combinations of extreme parameters as well as to make possible the choice of a "best" model as it is given by certain criteria. The total spread in r_{C-N} is 1.67–2.10 Å. Certain interesting points arise already at this level. (1) There is a striking agreement between pyridines and thiazoles throughout the series. (2) The parameters for which r_{C-N} is most sensitive are those associated with the degree of inversion around the central carbon, γ (δ) and k_γ. A variation from 35 to 50 % inversion causes a change of r_{C-N} of -0.08 ± 0.01 Å. (3) The *t*-Bu substituted compounds are much more sensitive to the parameter set than the Me, Et and i-Pr derivatives.

The question arises: can one discriminate between the models and how can that be done? We are setting up the following conditions:

(i) *The variation of* r_{C-N} *within a series Me, Et, i-Pr and* t*-Bu should not be too large.* We are here guided by the calculations for the change of leaving group from iodide to fluorosulfonate (*vide infra*). Fluorosulfonate is more reactive than iodide by a factor 10^4 whereas 2-Me-

pyridine is more reactive than 2-*t*-Bu-pyridine by a factor 10^3. Hence we anticipate that the corresponding change in r_{C-N} should not be significantly larger for a change of 2-substituent than for a change of leaving group.

(ii) *A steric perturbation in the Menschutkin reaction is associated with a change of the position of the transition state according to the Hammond postulate.* In two independent studies le Noble and coworkers claim that the most hindered and thus slowest reaction possesses the latest transition state and *vice versa.*[6,33] Their conclusion is based upon activation volume measurements [6] and kinetic isotope effects [33] for the methylation of 2,6-dialkylpyridines.

(iii) *The implications inherent in the technique of evaluating the experimental energy differences must be valid for nucleophiles with no* ortho *substituent.* Since the experimental values represent substituent strain increments the corresponding values for unsubstituted heterocycle should be zero or very close to zero for a realistic TS *i.e.* for an r_{C-N} distance fitting the pattern for the 2-alkyl derivatives. It has to be emphasized that we have no data that allow us to determine the TS structure for unsubstituted compounds in the same way as for the 2-alkyl derivatives.

Following these principles it turned out to be quite easy to determine an acceptable TS force field. Interestingly enough, this could be achieved for an essentially unique set of parameters. A limited variation of k_β from 0 to 0.10 m dyn Å rad^{-2} could be compensated by a variation of k_γ from 0.30 to 0.20 m dyn Å rad^{-2} resulting in a few equally significant models. We have chosen model 11 as the "best" model since we would like to avoid k_β=0. The r_{C-N} distances for which the energy of the iminium ion is the same as for the TS are 1.793 Å for *N*-methylpyiridinium and 1.791 Å for *N*-methylthiazolium (by model 11) which were considered to account for the condition (iii). Some interesting geometrical parameters are shown in Table 7. The conformations of the 1-methyl-2-alkyl groups are illustrated in Fig. 5.

Carbon–nitrogen distance. The length of the carbon–nitrogen bond in the TS is 1.793–1.823 Å in model 11 corresponding to an extension of the normal value (1.482–1.485 Å) by 21–23 %. It is obvious that these values are model dependent, but considering the results from calculations with extreme parameters one might estimate the extension to be 22±4 % or r_{C-N} to lie in the interval 1.75–1.87 Å including all eight compounds.

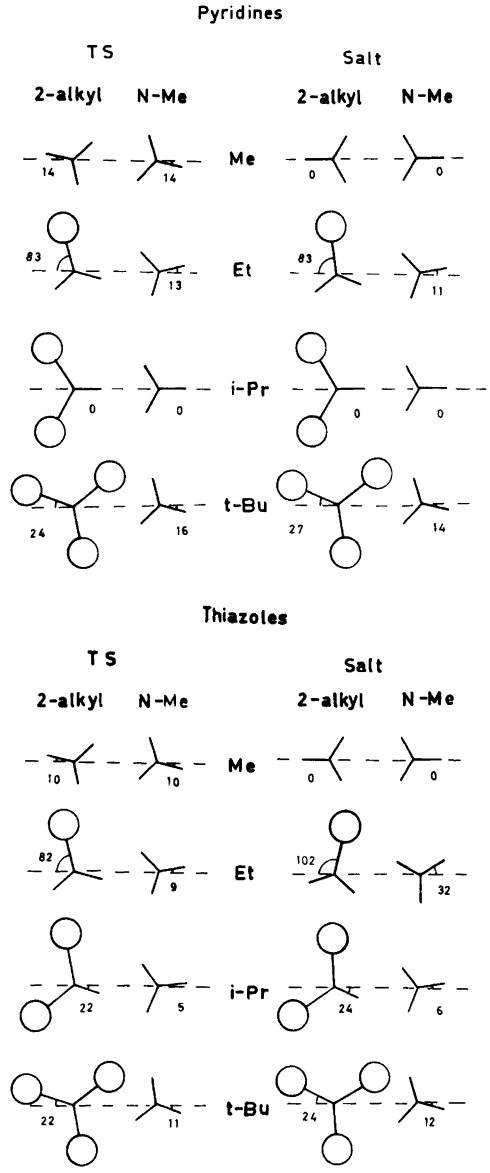


Fig. 5. Conformations of the 1-methyl-2-alkyl groups in the salt and in the transition state. The numbers indicate the dihedral angle between the sp^2-framework and the indicated bond.

In the Br^-+RBr halide exchange reactions Abraham *et al.* calculated C–Br extensions in the

Table 7. Transition state geometries according to model 11.

	Me		Et		i-Pr		*t*-Bu	
	Pyridine	Thiazole	Pyridine	Thiazole	Pyridine	Thiazole	Pyridine	Thiazole
r_{C-N}/Å	1.810	1.823	1.812	1.823	1.816	1.820	1.793	1.809
α/degrees	122.3	125.1	123.7	125.8	123.6	126.0	124.5	126.9
β/degrees	120.8	125.1	122.0	125.3	123.6	126.7	130.3	131.9
$\bar{\gamma}$/degrees [a]	93.2	92.2	93.3	92.0	93.6	93.1	95.4	94.6

[a] Mean value.

Table 8. Effects of changing the leaving group from iodide to fluorosulfonate.

Leaving group	Model		r_{C-N}			
			Me	Et	i-Pr	*t*-Bu
I^-	11		1.810	1.812	1.816	1.793
OSO_2F^-	11		1.832	1.839	1.849	1.851
		Δr_{C-N}	0.022	0.027	0.033	0.058
OSO_2F^-	12		1.851	1.849	1.850	1.858
		Δr_{C-N}	0.041	0.037	0.034	0.065

symmetrical transition state of 13–15 % [21] and De Tar *et al.* obtained 14–38 %.[22] Both of these groups allowed the C–Br bond to relax thus giving rise to energy minima. Julian and Taylor used kinetic isotope effects to evaluate transition state geometries for the reaction $PhS^- + n$-BuCl.[32] They found extensions of 10 % for the C–S distance and 2 % (!) for the C–Cl distance, the latter value not being much more than the amplitude of a normal vibration.

Effect of the leaving group. The effects of changing the substrate from methyl iodide to methyl fluorosulfonate may be treated by using the values from Table 3. Clearly all the model calculations result in an earlier TS for methyl fluorosulfonate than for methyl iodide. Adopting the same model (model 11) for both methyl iodide and methyl fluorosulfonate the change in the position of the TS, $\Delta r_{C-N} = r^F_{C-N} - r^I_{C-N}$, is 0.022 to 0.058 Å (Table 8).

It might be argued that since the position of the TS is believed to be different upon a change of leaving group, one cannot use the same parameter set. For this reason we have slightly modified the parameters, γ, k_γ and δ as shown in model 12 leading to the new Δr_{C-N} values 0.034 to 0.065 Å.

Mechanism. Recently Arnett and Reich reported a careful kinetic and thermodynamic investigation of the quaternization reaction of 3- and 4-substituted pyridines.[34] The work presents as up-to-date view of the mechanism of the Menschutkin reaction. The authors concluded that the degree of charge development in the TS is about 0.3 of that in the quaternary salt, slightly lower than the value 0.4 proposed by Abraham *et al.*[2] In geometrical terms the carbon–nitrogen bond was estimated to be about 30 % developed and the bond from the carbon to the leaving group to be nearly completely ruptured. The authors emphasized the crucial role of the solvent as manifested by the entropy parameters. The activation process for the forward reaction is determined to about 40 % by the entropy term and 60 % by the enthalpy term at room temperature. The entropy of activation has nearly the same large negative value (~ -125 J mol^{-1} K^{-1}) as for the complete reaction whereas the reverse

reaction showed a small positive entropy of activation.*

Olmstead and Brauman studied gas phase displacement reactions with anionic nucleophiles and deduced a mechanism which deviated significantly from reactions in solution.[35,36] This study also pointed at the decisive role of solvation in the S_N2 reaction.

How does our work fit with these interpretations? A question we have to consider is whether the steric effects observed in the Menschutkin reaction are actually diverse steric effects on solvation of the ground state compared to the TS. There is good reason that this is not the case.

(1) Although the rate of quaternization reactions is enormously dependent on the solvent there is virtually no dependence of the *ortho* steric effects on the solvent provided that the solvent is aprotic.[24]

(2) There is a good correlation between pK_a values and proton affinity in the gas phase also for 2-substituted pyridines.[37]

(3) Since only 2-alkylsubstituted pyridines are studied the electronic effects are virtually constant.

(4) Solvent reorganization is mainly taking place around the iodide atom [34] relatively far away from the place of substitution.

(5) The experimental steric energies are evaluated from $\Delta H^{\neq}$ values whereas solvent effects are largely reflected in $\Delta S^{\neq}$.

(6) The present calculations show that there is an increasing steric interaction between the nucleophile and the substrate when the 2-substituent is changed from methyl to *t*-butyl for any TS model tested.

Although the mechanism of Arnett and Reich is qualitatively in agreement with our TS model, there are very few details open to quantitative comparison. For example, how may their estimation that the carbon–nitrogen bond is 30 % developed in the TS be compared with ours that the bond is elongated 22±4 % in the TS?

We consider the result that the entropy changes from the TS to the products is small as a support to our approach of performing calculations on the salt and the TS.

The finding that steric effects are present as rate-retarding in the forward reaction and accelerating in the reverse reaction seems to be in line with a pentavalent TS in which the C–N bond is formed (or broken) in the rate determining step as in the classical S_N2 mechanism. Other authors have reached the same conclusion for the Menschutkin reaction.[2]

Effects of nucleophile geometry. Deady *et al.* have discussed the steric effects in the forward and reverse Menschutkin reactions of pyridines and thiazoles.[38] They noted that di-*ortho* substituted pyridines such as 2,6-lutidine did not follow the general trend observed for mono-*ortho* azines, for which the ratio of hindrance in the forward reaction to acceleration in the reverse reaction is approximately 2:1. Instead they found a ratio of 3:1 for the disubstituted compounds. Furthermore, they found no steric effect in the demethylation of thiazolium salts in contrast to our finding.[25] The reason for this discrepancy might be found in the following: Firstly, the sensitivity to steric effects is less important for thiazoles than for pyridines also in our hands. Secondly, we observed that the demethylation of the thiazolium series was less clean than the reaction in the pyridinium series and we observed a poorer correlation too.[25] Besides, Deady *et al.* studied only four thiazole compounds.

We thus face a situation where both the type of heterocyclic system and the substitution pattern influence the ratio of steric effects in the forward and reverse reactions. The trend is that the more susceptible to steric effects the system the larger this ratio, *i.e.* 2-substituted thiazoles 4:3, mono-*ortho* pyridines 2:1 and di-*ortho* pyridines 3:1.

As a check of the consistency of the calculations we have performed calculations for 2,6-lutidine. The strain in the TS of the methylation of 2,6-lutidine with methyl iodide can be evaluated to be 7.5 kJ mol^{-1}.[23b,38] A 3:1 ratio in methylation–demethylation leads to a strain in 1,2,6-trimethylpyridinium of 10.0 kJ mol^{-1}. Using model 11 an r_{C-N} distance in the transition state

* An interesting comparison may be found in a monomolecular process with similar charge developments; rotation around the carbon–carbon double bond in polarized ethylenes. Berg, U. and Sjöstrand, U. *Org. Magn. Reson. 11* (1978) 555. The rotational barrier around the formal carbon–carbon bond in two types of polarized ethylenes was studied: planar systems in which charge is created on going from ground state to TS and twisted systems with opposite charge development. Large negative entropy parameters were found on creation of charge and a small positive entropy term was found on extinction of charge and the effects were interpreted in terms of solvent reorganization. That study offers a case in which translational entropy contributions do not interfere.

of 1.863 Å was calculated. This value is somewhat high (by *ca.* 0.05 Å) but still within the interval of confidence. Assuming instead a ratio of 2:1 another r_{C-N} distance of 1.890 Å is obtained, a value which clearly falls outside the interval.

The reactivity–selectivity principle (RSP). Lately the RSP has attained remarkable interest as shown by the appearance of quite a few review articles in this domain.[39–42] The current controversy on the RSP is also illustrated by several recent papers.[43–60] The mere fact that there are so many violations to the RSP suggests that it can hardly be looked upon as a *general* principle in chemistry. In our opinion this does not mean that no information of interest can be obtained from reactivity–selectivity considerations. It is also clear that we need to know more of the reasons for adherence, or lack of it, to the RSP.

An interesting example in this connection is the important disparity between our results [18] and those of Arnett and Reich [43] on the effect of changing the leaving group from iodide to fluorosulfonate. Arnett and Reich found that 3- and 4-substituted pyridines did *not* obey the RSP in contrast to our results. Is this behaviour in agreement with our proposal that the position of the TS is changed 0.04±0.02 Å when the leaving group is changed from iodide to fluorosulfonate? If our hypothesis is correct that the selectivity is determined by steric effects in this reaction, the answer is yes. This is shown by calculations on unsubstituted pyridine. When the "fluorosulfonate model" (model 12) is compared to the "iodide model" (model 11) identical values in steric energy are obtained when r_{C-N} is 0.02 Å longer in the fluorosulfonate model. In other words, a shortening of the C–N distance by 0.02 Å is compensated by a change in pyramidalization with respect to the steric energy for pyridines unsubstituted in the *ortho* positions.

These observations lead to the question: what is the role of steric effects in reactivity–selectivity relationships? The general opinion seems to be that steric effects are the source of many violations of the RSP [51] and that they can usually not be quantified. We are not prepared at this time to speculate on the extent to which differential steric effects may influence the interpretability of reactivity–selectivity relationships in general. However, we believe that this study has shown that steric effects do not always obscure the interpretation but may be used, in certain cases, to gain information on mechanism and TS behaviour.

CONCLUSIONS

1. Kinetic data for the forward and reverse Menschutkin reaction of 2-Me-, 2-Et-, 2-i-Pr- and 2-*t*-Bu-pyridine and -thiazole may be used to evaluate the influence of *ortho* alkyl substituents on energies of the transition states (TS) and the product ions.

2. The ratio of the steric hindrance in the forward reaction to the acceleration in the reverse reaction increases with the susceptibility to steric effects of the system; this ratio being 4:3 for 2-substituted thiazoles, 2:1 for mono-*ortho* substituted pyridines and 3:1 for di-*ortho* substituted pyridines.

3. Force field (Allinger's MMI) calculations on the iminium ions and on TS models may be used to semi-quantitatively estimate the geometries of the transition state. Consistent results are obtained including all eight compounds.

4. The most interesting geometrical parameter, the carbon–nitrogen bond length, is 1.793–1.823 Å in the transition state employing the "best" model. Considering the model dependency and the various approximations introduced, an extension of 22±4 % from the ground state value seems realistic.

5. The studies are complementary to other work on the mechanism of the Menschutkin reaction and give support to the classical S_N2 mechanism operating in this reaction.

6. The effects of changing the leaving group from iodide to fluorosulfonate were treated and it was found that the carbon–nitrogen bond length in the TS was 0.04±0.02 Å longer for attack on methyl fluorosulfonate than on methyl iodide in accordance with the Hammond postulate. The magnitude of the geometrical variation with a perturbation appears to be small.

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Electrosynthesis of Medium- and Large-sized Rings. Part II.* Mechanism of the Anodic Cyclization of Bis(3,4-dimethoxyphenyl)alkanes and -alkenes

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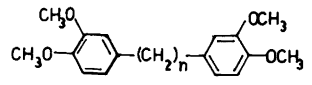
The anodic oxidation of 1,*n*-bis(3,4-dimethoxyphenyl)alkanes with n=6, 8, 10, 11 or 12 and of 1,6-bis(3,4-dimethoxyphenyl)trans-3-hexene in acetonitrile has been studied. In all cases a moderate yield of cyclic dimer was obtained. When n=11 or 12 intramolecular cyclization also occurred. HPLC-analysis at intervals during electrolysis revealed that the cyclic dimer is formed in a consecutive reaction sequence, with an open chain dimer as a stable and isolable intermediate.

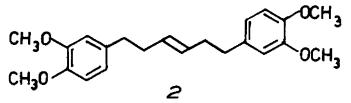
The anodic oxidation of the bis(3,4-dimethoxyphenyl)alkanes, 1-*n* (n=1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 16) in trifluoroacetic acid (TFA)–dichloromethane (DCM) solution has been described in a short communication.[1] We found that when n<6 only intramolecular cyclization occurred. When n was equal to 6 or 7 polymers and a very low yield of the cyclic dimers, *3*–6 or *3*–7, were obtained. When n was equal to 8, 9, or 10 an approximately 40 % yield of the cyclic dimers, *3*–8, *3*–9, or *3*–10, was obtained along with polymers. When n was equal to 16, both intramolecular cyclization with formation of *5*–16 and formation of the cyclic dimer, *3*–16, took place. In this communication we speculated that formation of the cyclic dimers, *3-n*, involved dimerization of an anodically generated dication diradical with a positive charge and a free electron on each aryl group. However, the results presented in this paper show that this assumption is incorrect. Furthermore, we have found that identical products are obtained in the same yield as in TFA/DCM when the electrolyses are carried out in acetonitrile. As the latter solvent is much more convenient for electrochemical studies this medium has been used throughout this work.

RESULTS AND DISCUSSION

The dependence of the products and yields on the amount of current consumed in the anodic



1-n



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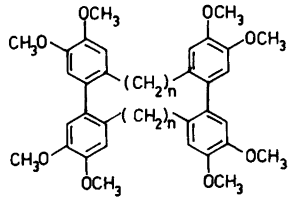

3-n

* Part I, see Ref. 1.

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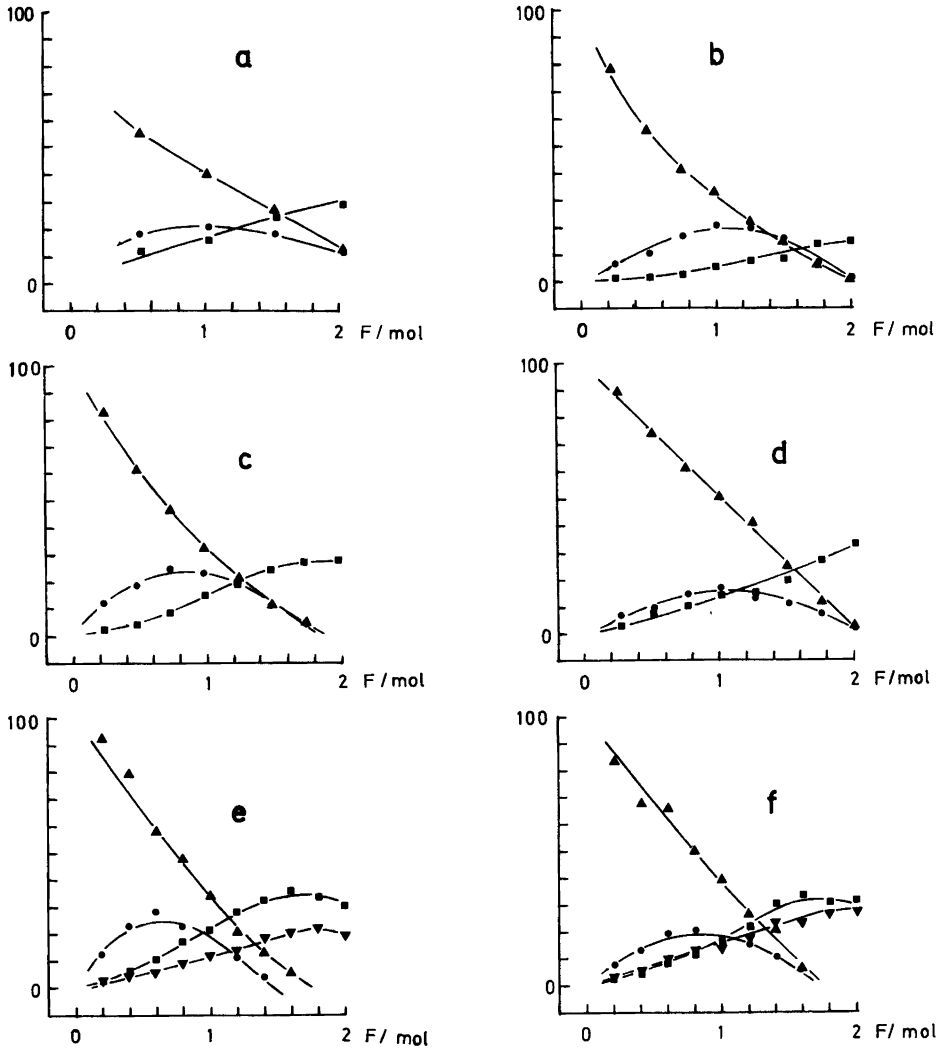
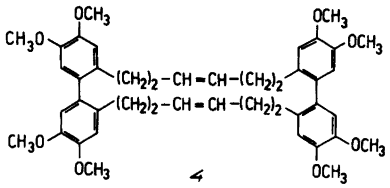


Fig. 1. Products and yields as a function of the amount of current (in Faradays per mol, F/mol) used for the anodic oxidation of compounds *1-n* (*n*=6, 8, 10, 11, 12) and *2*. Electrolyte: Acetonitrile/LiBF4. Concentration of starting compound: 67 mmol. Current density: 2 mA/cm^2. ▲ Starting compound. ● "Open dimer" (*6-n* or *7*). ■ "Cyclized dimer" (*3-n* or *4*). ▼ Cyclized product (*5-n*). (a) Oxidation of compound *2*. (b) Oxidation of compound *1*–6. (c) Oxidation of compound *1*–8. (d) Oxidation of compound *1*–10. (e) Oxidation of compound *1*–11. (f) Oxidation of compound *1*–12.

oxidation of *1-n* (*n*=6, 8, 10, 11, or 12) or *2* is shown in Figs. 1a–f. The yields in these experiments were determined by HPLC analysis. The yields of the products actually isolated in pure form were, of course, lower than the HPLC-yields (see Experimental). Changes in current density did not result in any significant changes in products or yields. However, working at lower substrate concentrations than in the experiments summarized in Fig. 1 resulted – as expected – in improved yields of the cyclic dimer. For example, reduction of the concentration of *2* from 16.7 mmolar to 8.3 mmolar resulted in a 10 % increase in the yield of *4*. The amount of charge



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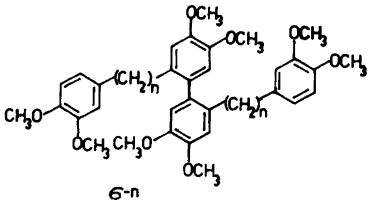
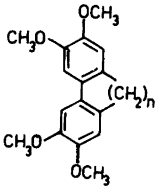
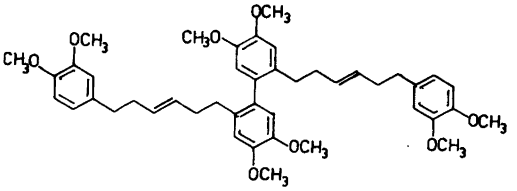


6-n



5-n



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F/mol used to oxidize the substrate was crucial.

For the compounds *1-n* the yield of the cyclic dimer *3-n* decreased rapidly if more than 2 F/mol of charge was used for the oxidation. If, for example, 3 F/mol was used to oxidize *1*–8 the yield of *3*–8 was 16.0 %, whereas the yield when 2 F/mol of charge was used was 28.8 %. As can be seen from Fig. 1 the current yield in the oxidations of compounds *1-n* is virtually 100 %, as 2 F/mol of charge suffices to achieve complete oxidation of the substrates. For compound *2* the current yield is less than 100 % (Fig. 1f), but increasing the amount of charge above 2 F/mol does not increase the yield of *4* very much. For example, oxidizing *2* with 3 F/mol leads to complete conversion of *2* with a 33.5 % yield of *4*.

In all the electrolyses of the compounds *1-n* and *2* the concentration of the open dimer *16-n* or *7* goes through a maximum at 1 F/mol of charge and at 2 F/mol of charge the concentration of the open dimer is virtually zero. The sum of the concentrations of the cyclic dimer (*3-n* or *4*) and the intramolecularly cyclized product (*5-n*) increases steadily until 2 F/mol has been consumed. If more charge is passed through the electrolyte, destructive oxidation of these products occurs and the total yield starts to decrease. It is noteworthy that intramolecular cyclization only occurs when $n \leq 5$ or $n \geq 11$.

The changes in the concentrations of starting compounds and the various products observed (Fig. 1) clearly indicates that the open dimers (*6-n* and *7*) are intermediates in the formation of the cyclic dimers (*3-n* and *4*). The efficiency of the intramolecular cyclization of *6-n* or *7* to *3-n* or *4* is very high and can only be explained by assuming an ordered structure for the open chain dimer in which the two aliphatic chains are aligned parallel to each other and bound by van der Waals forces in a similar fashion as the lipophilic chains of surfactants in micelles. In such a structure the two veratryl units at the ends of the open dimer become favourably oriented for coupling. In a similar way we can explain why intramolecular coupling only occurs when $n \leq 5$ or $n \geq 11$. In the first case the two veratryl units are close enough for coupling to occur in most of the possible conformations of the molecule. In the intermediate cases ($6 \leq n \leq 10$) the hydrocarbon chain between the veratryl units is too short to give any appreciable stabilization by folding (through van der Waals interaction between the two halves of the hydrocarbon chain). This however appears to be the case when $n \geq 11$ and intramolecular coupling competes efficiently with the dimerization leading initially to the open chain dimer (*3-n*).

EXPERIMENTAL

General procedures and apparatus used for voltammetry and coulometry were conventional and have been described previously.[1] The acetonitrile was analytical grade. The NMR spectra were recorded in deuteriochloroform solution with Me_4Si as internal reference.

The diarylalkanes *1-n* (n=6, 8, or 10 and *2* were prepared in a two-step synthesis involving Friedel Crafts acylation of 2 mol of 1,2-dimethoxybenzene with the acid chlorides derived from hexanedioic, octanedioic, decanedioic, dodecanedioic and trans-3-hexenedioic acid, respectively, followed by Clemmensen reduction of the resulting diketone.

1,6-Bis(3,4-dimethoxyphenyl)hexane (1-6). M.p. 76–78 °C (lit[2] M.p. 76.5–77.5 °C). NMR δ 6.8 (6H, s), 3.93 (12H, s), 2.57 (4H, t, (J=6 Hz), 1.43 (8H, bs) p.p.m.

1,8-Bis(3,4-dimethoxyphenyl)octane (1-8). M.p. 73–75 °C (EtOH). NMR δ 6.73 (6H, s), 3.83 (12H, s), 2.55 (4H, broad t (J=6 Hz)), and 1.25 (12H, bs) p.p.m.

1,10-Bis(3,4-dimethoxyphenyl)decane (1-10). M.p. 82–84 °C (lit[3] M.p. 82–83 °C). NMR δ 6.76 (6H, s), 3.9 (12H, s), 2.55 (4H, broad t (J=6 Hz)), 1.25 (12H, bs) p.p.m.

1,12-Bis(3,4-dimethoxyphenyl)dodecane (1-12). 1,10-Bis(3,4-dimethoxybenzoyl)decane was prepared as described and subjected to catalytic hydrogenation over a Pd (5 %) on carbon catalyst in ethanol: TFA (50:1) solvent to give a virtually quantitative yield of *1*–12. M.p. 86–89 °C. NMR δ 6.7 (6H, s), 3.83 (12H, s), 2.6 (4H, broad t (J=7 Hz)), and 1.3 (20H, bs) p.p.m.

1,11-Bis(3,4-dimethoxyphenyl)undecane (1–11). 1,9-Dibromononane, 20 ml, dissolved in anhydrous ether, 50 ml, was added to magnesium turnings, 6 g, contained in anhydrous ether, 50 ml, at a rate sufficient to keep the ether boiling. After the addition, reflux was continued for 1 h. The mixture then was cooled and 3,4-dimethoxybenzaldehyde, 34 g, dissolved in ether, 200 ml, was added with stirring. After the addition the mixture was refluxed for 1 h before it was poured on crushed ice, 100 g, mixed with concentrated hydrochloric acid, 100 ml. The organic phase was separated and washed with sodium bisulfite solution (to remove excess aldehyde) and water. Evaporation of the ether yielded an oil which was directly subjected to catalytic hydrogenation (4 atm.) in ethanol solution (300 ml) with 5 % palladium on carbon (1 g) as catalyst. During the hydrogenation a crystalline precipitate formed, 18.9 g, m.p. 66–68 °C, identified as *1*–11 by their NMR δ 6.75 (6H, s), 3.87 (12H, s), 2.57 (4H, broad t (J=7 Hz)), and 1.3 (18H, bs) p.p.m; and M^+ (m/e) 428.

1,6-Bis(3,4-dimethoxyphenyl)trans-3-hexene (2). M.p. 80–82 °C. NMR: 6.73 (6H, m) 5.50 (2H, m), 3.87 (12H, s) and 2.50 (8H, m).

Electrolyses. General. The substrate to be oxidized, 1 mmol, was dissolved in acetonitrile, 60 ml, containing lithium tetrafluoroborate, 0.5 g, and subjected to constant current (100 mA) electrolysis in an open one-compartment cell, fitted with a platinum cylinder anode (50 cm^2) and a nickel coil cathode. The reaction mixture was stirred magnetically and its temperature was kept at 10 °C by external cooling. When the desired amount of charge had been passed through the cell, the reaction mixture was worked up and analyzed by HPLC. For two compounds, *1*–11 and *1*–12, a different procedure was followed. At intervals, samples, 0.5 ml, of the reaction mixture were taken out, diluted with 5 ml of the HPLC solvent, washed with bicarbonate solution and water and injected on the HPLC-column. A straight phase column with cyclohexane–chloroform mixture as eluent and UV-detector was used. When all of the starting material had been consumed (which normally required 2F/mol) only the cyclic dimer (*3-n*) and the cyclized compound (*5-n*) were obtained and the separation and purification of the products were without complications. In the electrolysis experiments aimed at the isolation of the "open" dimers *6-n* and *7* only 0.7 F/mol of charge was passed through the cell. Column chromatography of the product gave starting material (*1-n* or *2*), the cyclized compound (*5-n*) and an inseparable mixture of the "open" dimer (*6-n* or *7*) and the cyclic dimer (*3-n* or 4). Crystallization of the latter mixture from pentane–diethyl ether gave the cyclic dimer as pure crystals whereas the "open" dimer was concentrated in the mother liquor. Repeated crystallizations of the mother liquor followed by column chromatography on silica gel (dichloromethane–diethyl ether eluent) eventually gave the open dimer as a pure compound. The pure compounds obtained in this way were used as internal standards in the HPLC analysis of the various electrolysis mixtures (see Fig. 1).

Electrolysis of 2. 1,1'-Bis(6-(3,4-dimethoxyphenyl)-hex-3-enyl)-3,3',4,4'-tetramethoxybi phenyl (7). M.p. 92–94°C, NMR δ 6.73 (10H, m), 5.4 (4H, bs), 3.93 (6H, s), 3.83 (18H, s), and 2.3 (16H, bs) p.p.m. M^+ 710-3819 (*m/e*). M^+ calculated for $C_{44}H_{54}O_8$: 710.3818.

[1,2],[3,4],[11,12],[13,14]-Tetra(4,5-dimethoxybenzo)cycloeicosa-trans,trans-7,17-diene (4). M.p. 190–192 °C. NMR: 6.73 (4H, s), 6.57 (4H, s), 5.2 (4H, bs), 3.87 (12H, s), 3.75 (12H, s) and 2.2 (16H, s) p.p.m. M^+ 708.3723 (*m/e*). M^+ calculated for $C_{44}H_{52}O_8$: 708.3662.

Electrolysis of 1-6. 1,1'-Bis(6-(3,4-dimethoxyphenyl)hexyl)-3,3',4,4'-tetramethoxybiphenyl (6-6). Oil. NMR δ 6.73 (10H, m), 3.93 (6H, s), 3.83 (18H, s), 2.4 (8H, bs) and 1.3 (16H, bs) p.p.m. M^+ 714.4193 (*m/e*). Calculated M^+ for $C_{44}H_{58}O_8$: 714.4132.

[1,2],[3,4],[11,12],[13,14]-Tetra(4,5-dimeth-

oxybenzo)cycloeicosane (3-6). M.p. 185–189 °C. NMR δ 6.75 (4H, s), 6.6 (4H, s), 3.93 (12H, s), 3.81 (12H, s), 2.3 (8H, bs), and 1.1 (16H, bs) p.p.m. M^+ 712.4026 (*m/e*). Calculated for $C_{44}H_{56}O_8$: 712.3875.

Electrolysis of 1-*8. 1,1'-Bis(8-(3,4-dimethoxyphenyl)octyl)-3,3',4,4'-tetramethoxybiphenyl (6-8).* Oil. NMR δ 6.75 (10H, m), 3.93 (6H, s), 3.87 (18H, s), 2.4 (8H, bs) and 1.23 (24H, bs) p.p.m. M^+ 770–4791 (*m/e*). Calculated M^+ for $C_{48}H_{66}O_8$: 770.4758.

[1,2],[3,4],[13,14],[15,16]-Tetra(4,5-dimethoxybenzo)cyclotetracosane (3-8). M.p. 159–161 °C. NMR δ 6.76 (4H, s), 6.6 (4H, s), 3.9 (12H, s), 3.8 (12H, s), 2.3 (8H, bs), and 1.2 (24H, bs) p.p.m. M^+ 768.4636 (*m/e*). Calculated M^+ for $C_{48}H_{64}O_8$: 768.4601.

Electrolysis of 1-*10. 1,1'-Bis(10-(3,4-dimethoxyphenyl)decyl)-3,3',4,4'-tetramethoxybiphenyl (6-10).* Oil. NMR δ 6.75 (10H, m), 3.9 (6H, s), 3.87 (18H, s), 2.4 (8H, bs), and 1.2 (32H, bs) p.p.m. M^+ 826.5374 (*m/e*). Calculated M^+ for $C_{52}H_{74}O_8$: 826.5384.

[1,2],[3,4],[15,16],[17,18]-Tetra(4,5-dimethoxybenzo)cyclooctacosane (3-10). M.p. 137–140 °C. NMR δ 6.83 (4H, s), 6.63 (4H, s), 3.93 (12H, s), 3.83 (12H, s), 2.4 (8H, bs), and 1.17 (32H, bs) p.p.m. M^+ 824.5191 (*m/e*). Calculated M^+ for $C_{52}H_{72}O_8$: 824.5227.

Electrolysis of 1-*11. 1,1'-Bis[11-(3,4-dimethoxyphenyl)-undecyl]-3,3',4,4'-teteramethoxybiphenyl (6–11).* Oil. NMR δ 6.8 (10H, m), 3.93 (6H, s), 3.87 (6H, s), 3.85 (6H, s), 2.4 (8H, bs), and 1.2 (36H, bs) p.p.m. M^+ 854.5717 (*m/e*). Calculated M^+ for $C_{54}H_{78}O_8$: 854.5697.

[1,2],[3,4],[16,17],[18,19]-Tetra(4,5-dimethoxybenzo)cyclotricontane (3-11). M.p. 133–136 °C. NMR δ 6.8 (4H, s), 6.6 (4H, s), 3.93 (12H, s), 3.83 (12H, s),2.43 (8H, bs), and 1.17 (36H, bs) p.p.m. M^+ 852.5518 (*m/e*). Calculated M^+ for $C_{54}H_{76}O_8$: 852.5540.

[1,2],[3,4]-Di-(4,5-dimethoxybenzo)cyclopentadecane (5-11). M.p. 98–102 °C. NMR δ 6.85 (2H, s), 6.6 (2H, s), 3.93 (6H, s), 3.83 (6H, s), 2.43 (4H, bs), and 1.23 (18H, bs) p.p.m. M^+ 426.2770 (*m/e*). Calculated M^+ for $C_{27}H_{38}O_8$: 426.2770.

Electrolysis of 1-*12. 1,1'-Bis(12-(3,4-dimethoxyphenyl)dodecyl)-3,3',4,4'-tetramethoxybiphenyl (6-12).* Oil. NMR δ 6.8 (10H, m), 3.9 (6H, s), 3.87 (18H, s), 2.4 (8H, bs), and 1.2 (40H, bs) p.p.m. M^+ 882.5929 (*m/e*). Calculated M^+ for $C_{56}H_{82}O_8$: 882.6010.

[1,2],[3,4],[18,19],[20,21]-Tetra(4,5-dimethoxybenzo)coclodotracontane (3-12). M.p. 93–97 °C. NMR δ 6.83 (4H, s), 6.63 (4H, s), 3.97 (12H, s), 3.85 (12H, s), 2.36 (8H, bs), and 1.2 (40H, bs) p.p.m. M^+ 880.5887 (*m/e*). Calculated M^+ for $C_{56}H_{80}O_8$: 880.5853.

[1,2],[3,4]-Di(4,5-dimethoxybenzo)cyclohexadecane (5-12). M.p. 99–102 °C. NMR δ 6.87 (2H, s), 6.63 (2H, s), 3.93 (6H, s), 3.83 (6H, s), 2.43 (4H, bs), and 1.23 (20H, bs) p.p.m. M^+ 440.2927 (*m/e*). Calculated M^+ for $C_{28}H_{40}O_4$: 440.2927.

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Chemistry of *gem*-Dihalocyclopropanes. XIX. Formation of 3-Oxabicyclo[3.1.0]hexane Derivatives by Intramolecular Insertion of Cyclopropylidenes

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Acetals of *gem*-dibromocyclopropanecarbaldehydes react with methyllithium at room temperature to give the corresponding derivatives of 3-oxabicyclo[3.1.0]hexane in good yields. These products result most probably from insertion of intermediate cyclopropylidenes into 5,6-related CH bonds adjacent to oxygen. A significant degree of stereoselectivity is observed. Small amounts of acetals of monobromocyclopropanecarbaldehydes are formed as well, and traces of allenes were detected in two of the reactions. Products derived from insertion into 1,3-related CH bonds adjacent to oxygen were not encountered. The regioselectivity is explained in terms of the coordinating effect of the oxygen atoms.

The intramolecular insertion reaction of cyclopropylidenes, generated from *gem*-dibromocyclopropanes and alkyllithium, may be influenced by the presence of heteroatoms in the molecule. In monocyclic ethers,[1,2] sulfides [3,4] and amines [5] of the general structure *1* insertion occurs exclusively at the CH bond adjacent to the heteroatom and 5,6-related to the electron deficient carbon (1,5-insertion) to give compounds *2*; compounds having available for insertion only 1,3-related CH bonds (1,3-insertion) preferred other reactions *e.g.* ring-opening to allenes. On the other hand, primary *gem*-dibromocyclopropanecarbinols (*1*, X=O, R=H) reacted with methyllithium to products derived from intermediate bicyclo[1.1.0]butanolates resulting from 1,3-insertion.[2]

Previous results indicate that carbenes may insert into acetal CH bonds.[6] A few *gem*-dibromocyclopropanes containing acetal functions have been treated with alkyllithium,[7–10] but 1,3-insertion into a CH bond adjacent to oxygen was structurally feasible in only three cases. In the first example [8] it was observed that allenes were not formed, but the products of this reaction were not described. In the two other examples,[9,10] the corresponding monobromides were obtained in high yields as the only product. We decided to investigate reactions of acetals of the general structure *3* with methyllithium hoping for 1,3-insertion to give the acetals of the highly interesting bicyclo[1.1.0]butanones. The results of this study are reported here.

RESULTS

The acetals *3* were prepared in 76–84 % yields from the corresponding carbonyl compounds [11,12] by conventional methods.[13] Reactions with methyllithium were carried out by adding the organometallic reagent to an ethereal solution of the acetal kept at −78 °C followed by a reaction period of several hours at room temperature. The crude reaction mixture was in each case analyzed by gas liquid chromatography (GLC) and spectroscopy. The products were isolated by distillation and when necessary by preparative GLC. Distillations were accompanied by polymerization to some extent and the product compositions refer to GLC analyses of the crude reaction mixtures.

The IR spectra of the crude products from acetals *3c* and *3d* exhibited weak bands at 1965 cm^{-1} indicating that allenes were present, but the

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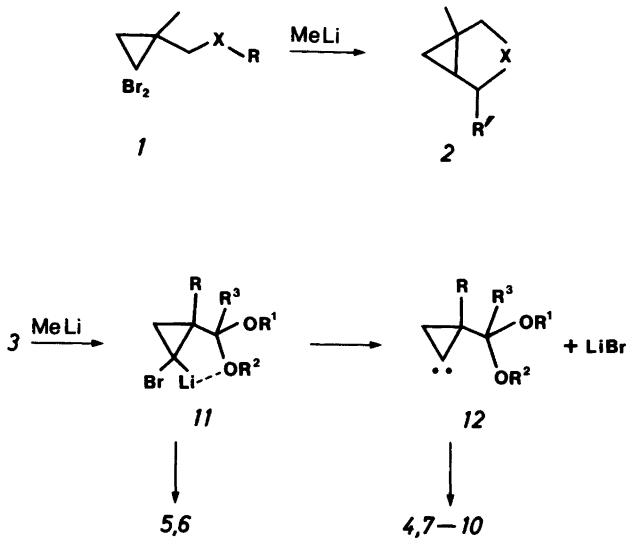
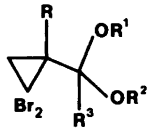


Scheme 1.

Table 1. Volatile products from reactions of acetals *3* with methyllithium.



3

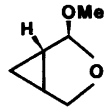
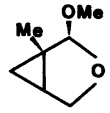
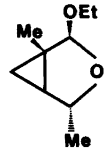
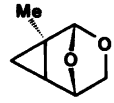
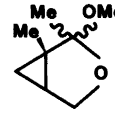
a R=R³=H,R¹=R²=Me

b R=R¹=R²=Me,R³=H

c R=Me,R¹=R²=Et,R³=H

d R=Me,R¹=R²=$-CH_2-CH_2-$,R³=H

e R=R¹=R²=R³=Me

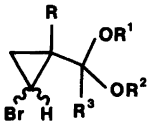
PRODUCTS	YIELDS %
 *4*	66
 *7*	73
 *8*	66
 *9*	81
 *10*	75

amounts were too small for separation by preparative GLC. The major products obtained from the reactions are recorded in Table 1. These were all isolated by distillation. In addition, small amounts of monobromocyclopropane derivatives were formed. The compounds were characterized on the basis of spectroscopic data, particularly the NMR spectra. Analyses by GLC indicated that the products recorded in Table 1 were mixtures of stereoisomers which were not separable on a preparative scale. The structural assignments had to rely on spectra recorded on the mixtures, but this did not complicate the interpretation significantly since each mixture contained 90 % or more of one isomer.

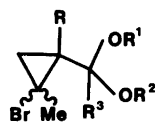
The reaction product from *3a* consisted of a mixture of four compounds in a ratio of 30:3:3:1. The first two of these, which also were the most volatile, were shown to be the stereoisomeric 2-methoxy-3-oxabicyclo[3.1.0]hexanes (*4*). Since very little spectral information on this type of compounds was available, the stereochemical assignment was not straight forward. The ^{1}H NMR spectrum of the parent compound 3-oxabiyclo[3.1.0]hexane was known [14] and surprisingly the methylene protons adjacent to oxygen are only slightly coupled to the cyclopropyl methine proton. According to the microwave spectra, both 3-oxabicyclo[3.1.0]hexane [15] and bicyclo[3.1.0]hexane [16] strongly prefer boat conformations and the replacement of the methylene group with oxygen gives rise to only minor conformational changes. This is significant since a large number of bicyclo[3.1.0]hexane derivatives have been thoroughly studied by ^{1}H NMR [17] and a comparison of these data with those of our products (Table 1) is then justified. Important in this respect is the general observation in the bicyclo[3.1.0]hexane series of a weak coupling (3–5 Hz) between the *exo* proton at C–2 and the cyclopropyl methine proton while the *endo* proton does not couple. Furthermore, the former resonates at a lower field than the latter. The acetal proton of the major isomer of *4* appears as a sharp singlet at δ 4.60 and based on the information above we assign to it the *endo* configuration, *i.e.* the methoxy group is *anti* to the cyclopropane ring.

The minor higher-boiling components of the product mixture were obtained sufficiently pure for identification. The most abundant of them was shown to be the monobromide *5a*, and the appearance of the proton adjacent to bromine as a multiplet centered at δ 2.83 may suggest a *cis* configuration.[18] The other compound was identified as one of the stereoisomers of 2-bromo-2-methylcyclopropanecarbaldehyde dimethylacetal (*6a*); the methyl group adjacent to bromine gives rise to a resonance at δ 1.72 characteristic for such compounds.[19]

The major product from the reaction of *3b* was shown to consist of a 12:1 mixture of stereoisomeric 2-methoxy-1-methyl-3-oxabicyclo[3.1.0]hexane (*7*); the acetal protons of the major and minor isomers appear as singlets at δ 4.59 and 4.80, respectively, suggesting the *exo* configuration for the former. Support for this assignment was provided by the observation of a strong nuclear Overhauser enhancement of the resonance at δ 4.59 of the major isomer when the *endo* cyclopropyl proton at C–6 was irradiated to saturation. Two minor higher-boiling products were formed in this reaction as well, but we were unable to obtain pure samples. The GLC retention times suggest they are the monobromides *5b* and *6b*.



5



6

The major product from the reaction of *3c* consisted of a 9:1 mixture of stereoisomeric 1,4-dimethyl-2-ethoxy-3-oxabicyclo[3.1.0]hexanes (*8*). The ^{1}H NMR spectrum reveals that the proton at C–4 is coupled to the adjacent cyclopropyl methine proton with J=2.9 Hz. It strongly suggests a *syn* relationship between the methyl group at C–4 and the cyclopropane ring. Furthermore, the acetal proton is present as a singlet at δ 4.54 and, by analogy,[17] the chemical shift is in accordance with an *endo* proton. Hence, we assign the *exo*-2-ethoxy, *endo*-4-methyl configuration to the major isomer. A small amount of a third compound was present in the crude product, but not sufficient for isolation; the GLC retention time indicates again that the compound is a monobromide.

The reaction of *3d* gave essentially one stereoisomer of 2-methyl-7,8-dioxatricyclo-

[3.2.1.$0^{2,4}$]octane (*9*). Only negligible coupling is observed between the protons of C-4 and C-5 in the ^{1}H NMR spectrum of *9*. In the related ether 8-oxatricyclo[3.2.1.$0^{2,4}$]oct-6-ene [20] the same protons are coupled (J=6.0 Hz) only in the *endo* isomer, while in the case of tricyclo[3.2.1.$0^{2,4}$]-octane these protons are coupled very weakly in both isomers. Hence, we cannot assign the stereochemistry of *9* on the basis of the proton coupling data alone. However, the observed shielding effects of oxygen and methylene bridges in the ^{13}C NMR spectra of norbornane derivatives [21] appear to be quite similar. Consequently we expected the cyclopropyl methylene group of *exo-9* to show an upfield shift compared with that of norcarane, while the opposite should be true for the *endo* isomer. This particular carbon resonance of *9* appears at δ 7.9, as compared with δ 10.5. for norcarane.[22] We thus favour the *exo* configuration for *9*. A small amount of higher-boiling material was also formed. When the reaction temperature was kept at −78 °C during the entire reaction period this material became practically the sole product. It was isolated in 91 % yield and identified as a 6:1 mixture of stereoisomeric 2-bromo-1-methylcyclopropanecarbaldehyde ethyleneacetal (*5d*). Based on the chemical shift of the proton adjacent to bromine the major isomer has the *trans* configuration.[18]

The product from *3e* consisted mainly of 2-methoxy-1,2-dimethyl-3-oxabicyclo[3.1.0]hexane (*10*) as a 10:1 mixture of stereoisomers. Regarding the configurational assignment the available spectral data are inconclusive.

DISCUSSION

Reactions of *gem*-dibromocyclopropane derivatives and methyllithium proceed by rapid bromine lithium exchange forming the respective α-bromocyclopropyllithium intermediate *11* (Scheme 1). Subsequently *11* eliminates lithium bromide producing a second intermediate *12* with carbene-like properties which for simplicity is regarded as a cyclopropylidene. Without stabilizing substituents *11* is converted to *12* even at −100 °C, and at −78 °C products derived from the carbene will be observed only. However, the stabilizing effect on intermediates like *11* by neighbouring oxygen atoms has been amply demonstrated [23] and it is not surprising that the monobromides, which derive from *11*, were observed in the present reactions, particularly at low temperature. More interesting, however, is the quite selective intramolecular 1,5-insertion of the cyclopropylidenes. In the present study it is practically the sole reaction of this intermediate, while ring opening to allenes was a significant path in similar reactions of ethers, amines and sulfides. Our results substantiate the notion that intramolecular 1,3-insertion into CH bonds adjacent to heteroatoms is not a favoured reaction of cyclopropylidenes. The apparent 1,5-selectivity must be attributed to the intramolecular coordinating effect of the heteroatoms with the lithium atom of *11*. The effect will probably cause *11* to attain a conformation that brings the prospective electron deficient carbon and the 5,6-related CH bond in close proximity; conversely, the 1,3-related hydrogen will point away from the said carbon, reducing the probability of insertion at that position. A prerequisite for the validity of this explanation is that insertion follows elimination of lithium bromide before significant conformational changes occur. In this connection the preference for intramolecular 1,5-insertion in arylcarbenes observed by Crow and McNab [24] is of considerable interest.

Further studies on the insertion reaction directed towards its use in synthesis are in progress.

EXPERIMENTAL

NMR spectra were recorded on Varian EM 360 A, Jeol JNM FX60 and Brucker WM-400 spectrometers.

Starting materials. The acetals were prepared from the respective carbonyl compounds [12,13] by conventional methods.[13]

2,2-Dibromocyclopropanecarbaldehyde dimethylacetal (3a), b.p. 86−88 °C (9 mmHg), 77 % yield; *2,2-dibromo-1-methylcyclopropanecarbaldehyde dimethylacetal (3b)*, b.p. 46−48 °C (0.45 mmHg), 78 % yield; *2,2-dibromo-1-methylcyclopropanecarbaldehyde diethylacetal (3c)*, b.p. 64−65 °C (0.01 mmHg), 76 % yield; *2,2-dibromo-1-methylcyclopropanecarbaldehyde diethyleneacetal (3d)*, b.p. 70−72 °C (0.01 mmHg), 79 % yield; *1-acetyl-2,2-dibromo-1-methylcyclopropane dimethylketal (3e)*, 69−70 °C (0.4 mmHg), 84 % yield.

The compounds exhibited spectral data in accordance with the assigned structures.

Reactions with methyllithium. General method. To a stirred solution of the acetal (10 mmol) in 25 ml of dry ether, kept at −78 °C, methyllithium (12 mmol, 1.5 M solution in ether) was added dropwise. After 1 h at this temperature the reaction mixture was stirred at room temperature for 6–18 h. Water was added, the ether layer separated and washed with brine. The extract was dried ($MgSO_4$) and analyzed by GLC. Subsequently the products were isolated by distillation and/or preparative GLC.

Reaction of 3a *with methyllithium.* Distillation of the crude product gave 2-methoxy-3-oxabicyclo[3.1.0]hexane (*4*), as a mixture of stereoisomers, b.p. 50–51 °C (25 mmHg). Partial decomposition occurred during distillation. *exo-4* (major isomer): ^{1}H NMR (CCl_4) δ 0.1–0.6 (2H, compl. abs.) 1.53 (2H, dq) 3.23 (3H, s), 3.70 (2H, AB J=8 Hz), 4.60 (1H, s); ^{13}C NMR (CCl_4) δ 6.5 (cyclopropyl CH_2), 14.8, 21.3 (cyclopropyl CH), 53.8 (OCH_3), 67.0 (OCH_2), 104.9 (O–CH–O).

Distillation gave also a small amount of 2-bromocyclopropanecarbaldehyde dimethylacetal (*5a*), as a mixture of stereoisomers; b.p. 70–72 °C (10 mmHg). ^{1}H NMR (CCl_4) δ 0.9–1.6 (3H, compl. abs.) 2.83 (1H, m) 3.22, 3.23 (6H, s) 4.42 (1H, d).

The second minor component 2-methyl-2-bromocyclopropanecarbaldehyde dimethylacetal (*6a*) was isolated by prep. GLC (2 m, SE-30, 15 %) as a mixture of stereoisomers.

^{1}H NMR (CCl_4) δ 0.6–1.6 (3H, compl. abs.) 1.72 (3H, s), 3.21 (3H, s), 3.23, 3.30 (3H, s), 4.10 (1H, d).

Reaction of 3b *with methyllithium.* Analysis of the crude product by GLC revealed that essentially four products had been formed in a ratio of 84:7:2:6, respectively. The 84:7 products had the same volatility and could be separated by distillation to give 2-methoxy-1-methyl-3-oxabicyclo-[3.1.0]hexane (*7*), b.p. 57–59 °C (40 mmHg). The NMR and IR spectras were recorded on this mixture. In the NMR spectra the minor isomer appears as satellites to the major resonance peaks. The 2 % and 6 % products had nearly the same retention times on GLC as the monobromides *5a* and *6a*. Attempted isolation by preparative GLC was unsuccessful.

Exo-7 (major isomer): ^{1}H NMR ($CDCl_3$) 0.42 (2H, d, J=5 Hz), 1.26 (3H, s), 1.35 (1H, d, J=5 Hz), 3.35 (3H, s), 3.38–4.05 (2H, m), 4.59 (1H, s). ^{13}C NMR ($CDCl_3$) 14.45 (cycloprop. CH_2), 14.64 ($-CH_3$), 21.98 (cycloprop. CH), 27.32 (cycloprop. C), 54.68 (CH_3-O), 67.95 ($-CH_2-O$), 106.86 (O–CH–O).

The ^{13}C NMR spectrum was sufficiently resolved in the down field part to get the resonances of *endo-7*; in the ^{1}H NMR spectrum only the acetal proton at δ 4.80 was clearly resolved. ^{13}C NMR ($CDCl_3$) δ 57.4 (CH_3O), 68.1 (CH_2O), 110.0 (OCHO).

Reaction of 3c *with methyllithium.* Distillation gave a stereoisomeric mixture of 1,4-dimethyl-2-ethoxy-3-oxabicyclo[3.1.0]hexane (*8*) b.p. 51–52 °C (9 mmHg). Major isomer: ^{1}H NMR (CCl_4) δ 0.33 (1H, q), 0.55 (1H, t, J=4.3 Hz), 1.15–1.4 (10H, compl. abs.), 3.3–3.9 (2H, compl. abs.), 4.25 (1H, dq, J=2.9 and 6.0 Hz), 4.54 (1H, s). ^{13}C NMR (CCl_4) δ 11.00 (cycloprop. CH_2), 14.90, 17.34, 30.12 (CH_3), 15.28 (cycloprop. CH), 27.30 (cycloprop. C), 62.03 (CH_2-O) 72.43 (CH–O), 105.10 (O–CH–O).

The minor isomer exhibited a singlet at δ 4.78 due to the acetal proton. The other signals were not clearly resolved.

Reaction of 3d *with methyllithium.* Analysis of the reaction product by GLC revealed that essentially one product was present and distillation afforded 2-methyl-7,8-dioxatricyclo-[3.2.1.0^{2,4}]octane (*9*), b.p. 42–43 °C (7 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.02 (1H, m), 0.60 (2H, m), 1.27 (3H, s), 3.47 (2H, d, J=1.5 Hz), 4.57 (1H, br.s), 5.23 (1H, s); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 7.9 (cyclopropyl CH_2), 13.6 (CH_3), 18.9 (cyclopropyl CH), 23.4 (cyclopropyl C), 67.6 (CH_2O), 76.8 (O–CH), 103.7 (O–CH–O).

When the reaction was carried out by stirring the reaction mixture at −78 °C for 1 h, before decomposing with water, *9* was not formed; distillation gave 2-bromo-1-methylcyclopropanecarbaldehyde ethyleneacetal (*5d*) in 91 % yield, b.p. 85–86 °C (9 mmHg) as a mixture of stereoisomers in a ratio of 6:1; ^{1}H NMR (CCl_4) δ 0.58 (1H, q, J=4 Hz) 1.28 (3H, s) 1.28 (1H, m) 3.02 (1H, q, J=4 Hz) 3.83 (4H, m) 4.63 (1H, s); ^{13}C NMR (CCl_4) δ 16.8 (CH_3) 18.5 (cyclopropyl CH_2) 24.5 (cyclopropyl C) 31.8 (CHBr) 64.7 (CH_2O) 104.9 (O–CH–O).

Reaction of 3e *with methyllithium.* Distillation of the product gave 2-methoxy-1,2-dimethyl-3-oxabicyclo[3.1.0]hexane (*10*) as a mixture of stereoisomers, b.p. 48–50 °C (18 mmHg); ^{1}H NMR (CCl_4) δ 0.28 (1H, br. s), 0.37 (1H, s), 1.17 (3H, s), 1.2 (1H, m), 1.23 (3H, s), 3.13 (3H, s), 3.62 (2H, ABX J_{AB}=7.5 Hz J_{AX}=2 Hz), ^{13}C NMR (CCl_4) δ 14.6 (cyclopropyl CH_2), 14.6, 16.4 (CH_3), 22.9 (cyclopropyl CH), 31.3 (cyclopropyl C), 47.8 (OCH_3), 67.0 (OCH_2), 106.9 (O–CH–O).

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1,3-Dithiolan-2-ylium and 1,3-Dithian-2-ylium Tetrafluoroborates from Carboxylic Acids

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A general method is given for the synthesis of acylium synthons masked as 2-substituted-1,3-dithiolan-2-ylium and -1,3-dithian-2-ylium tetrafluoroborates; acid chlorides are reacted with the respective dithiol in ethereal tetrafluoroboric acid. Ready conversions of the salts into dithioacetals and ketene dithioacetals are described. In the mass spectrometer the salts either undergo a thermal redox process with radical formation or suffer deprotonation to yield the volatile species.

Organometallic derivatives of 1,3-dithiolanes and 1,3-dithianes are important sources of anionic carbon in organic synthesis.[1] As a source of cationic carbon, however, these heterocycles have been relatively little exploited. Triphenylmethyl tetrafluoroborate has been used to prepare the cation from 1,3-dithiane,[2] and also to prepare 2-*p*-tolyl-1,3-dithiolan-2-ylium tetrafluoroborate in low yield by the same procedure.[3] 1,3-Dithiolan-2-ylium tetrafluoroborate has been prepared by the reaction of 2-methylthio-1,3-dithiolane with tetrafluoroboric acid,[4] and very recently it was reported that 2-methyl-1,3-dithiolan-2-ylium and -1,3-dithian-2-ylium perchlorate are formed from the respective dithiol and acetic anhydride in the presence of perchloric acid.[5]

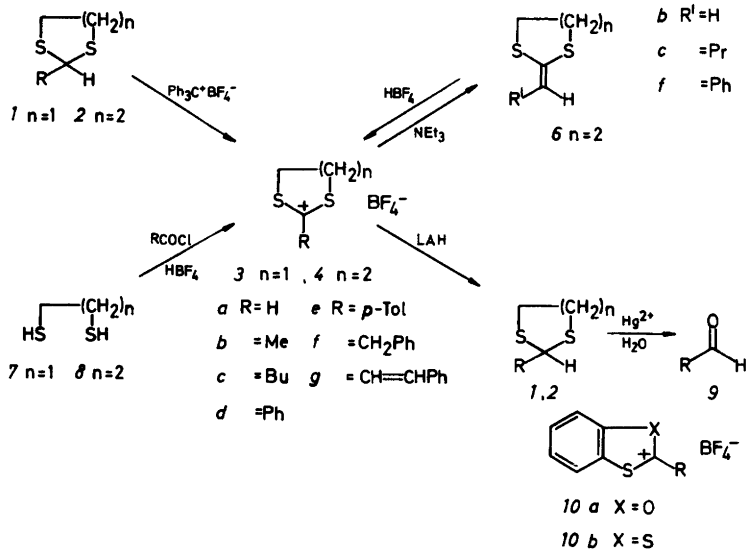
A carbenium ion which is stabilized by two adjacent heteroatoms, corresponds to a masked acylium ion. Because of the potential usefulness of such systems in organic synthesis, we became interested in developing routes for the preparation of 1,3-dithiolan-2-ylium *3* and 1,3-dithian-2-ylium *4* salts. In our first approach to the synthesis of *4*, 2-methyl- (*2b*),[6] 2-benzyl- (*2f*)[7] and 2-phenyl-1,3-dithiane (*2d*)[8] were treated with triphenylmethyl tetrafluoroborate.[9] In agreement with other findings,[10] however, the yields of the dithianylium salts were unsatisfactory, although the parent compound *4a* is readily available by this method. The reaction is slow at room temperature, especially for the 2-phenyl derivative, and the increase in rate on heating is accompanied by decomposition of the thermolabile product.

In another approach for the synthesis of *4f*, 2-benzylidene-1,3-dithiane was treated with ethereal tetrafluoroboric acid to form the salt *4f*. The alkenyl derivatives *6* are available from the reaction between 2-lithium-2-trimethylsilyl-1,3-dithiane and aldehydes.[11] This approach excludes the preparation of salts in which the α-carbon of the 2-substituent carries no hydrogen, *e.g.* the aryl derivatives (*3d, 3e, 4d, 4e*). We have found, however, a general and a very simple method for the preparation of the salts *3* and *4* where the nature of simple 2-substituents does not impose restrictions on the reaction.

In the new approach *3* and *4* are formed in high yields from the respective dithiols and acid chlorides in ethereal tetrafluoroboric acid. The salts should be kept cold under anhydrous conditions, and most of them could be recrystallized from ether:acetonitrile on exclusion of moisture.

It would appear that the salts *3* and *4*, especially because they are readily available, are more useful as synthons than the 2-substituted benzo[1,3]oxathiolium and benzo[1,3]dithiolium tetrafluoroborates *10* which have been widely investigated for such purposes.[12]

0302-4369/83 $02.50

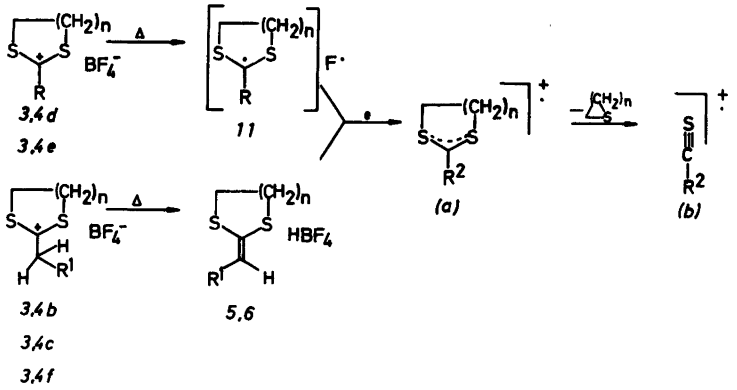


Scheme 1.

Reduction of *3* and *4* by lithium aluminium hydride in ether solution or by sodium borohydride in acetonitrile solution, gives the saturated heterocycles *1* and *2*, respectively; the latter may be hydrolyzed to aldehyde or subjected to anionic reactions.

The salts *4b*, *4c* and *4f*, in which the 2-substituent carries an α-hydrogen, are readily deprotonated by triethylamine or by pyridine in acetonitrile solution to form the respective ketene thioacetals *6b*, *6c* and *6f*.

The salts *3* and *4* are characterized by strong light adsorption; *e.g.* the long-wave band for the benzyl derivatives lies at 307 nm and the band undergoes a bathochromic shift with extended conjugation (*3d*, *3e*) into the visible region with a band at 436 nm for the styryl derivative *3g*. The cationic structures are further characterized by very low field ^{13}C-shifts for C-2 which are in the region δ 190–249 ppm. The mass spectra of *3* and *4* fall into two major classes depending on the preferred reaction pathways before the volatile compounds from the salts can be generated; salts are not volatile because of the electrostatic attraction between the oppositely charged ions. The aryl derivatives *3d,e* and *4d,e*, however, show a strong molecular ion with mass number corresponding to the mass of the cation. By analogy to our previous findings for other stable cationic systems,[13] this observation can be rationalized by a thermal redox process which occurs before volatilization generating the corresponding, volatile radical *11*. From the latter, the cation (*a*) is regenerated in the gas phase on electron bombardment, (Scheme 2). The other major group of compounds (*3f* and *4b,c,f*) are characterized by a molecular ion with mass number corresponding to the loss of a hydrogen from the cation. All these compounds have a hydrogen on the acyclic carbon adjacent to the cationic centre; the anion thus acts as a base and removes the hydrogen as a proton which results in the formation of alkylidene derivatives *5* and *6*. The latter is the volatile species as we have demonstrated for related systems.[13] The styryl derivatives (*3g* and *4g*) behave differently in that dimerization occurs to a large extent, after the primary redox process, and it is the dimeric products which are volatilized. A common feature in the fragmentation is the formation of a species corresponding to the side-chain which also includes C-2 and one of the sulfur atoms; the species is tentatively drawn as (*b*) in Scheme 2; this species is the base peak in some of the spectra. The neutral fragment expelled corresponds to a unit of thiirane or thietane. The fragmentation patterns compare with what have been published for 1,2-dithiolylium salts.[14]



Scheme 2.

EXPERIMENTAL

The mass spectra of the salts were recorded by direct insertion into the ion source at 225 °C. The data are presented as MS [70 eV; *m/z* (% rel. int.)].

All the reactions were carried out under an atmosphere of dry argon. The ether used as solvent was dried over sodium, the acetonitrile was dried over P_2O_5 before distillation and was then kept over molecular sieve (3 Å).

1,3-Dithian-2-ylium tetrafluoroborate [2] 4a *by hydride abstraction.* 1,3-Dithiane [15] was reacted with triphenylmethyl tetrafluoroborate [9] as described except that acetonitrile was found to be a superior solvent for this reaction; yield 76 %.

2-Substituted 1,3-dithiolan-2-ylium 3d–3g *and 1,3-dithian-2-ylium* 4b–4g *tetrafluoroborates from acid chlorides.* 54 % HBF_4 in ether (5 ml) was added dropwise at 0 °C to a solution of the acid chloride (10 mmol) and ethane-1,2-dithiol (10 mmol) or propane-1,3-dithiol (10 mmol) in ether (50 ml). The mixture was stirred for 15 min at this temperature, stirred for another 10 min at room temperature and finally heated under reflux for 20 min. The precipitated product, after isolation, was triturated with ether for purification; an oily product crystallized during this treatment.

2-Phenyl-1,3-dithiolan-2-ylium tetrafluoroborate 3d was obtained in 83 % yield; yellow crystalline material from MeCN–Et_2O–HBF_4 (drops), m.p. 100–103 °C. ^{1}H NMR (CD_3CN): δ 4.35 (CH_2CH_2), 7.3–8.2 (Ph). ^{13}C NMR (CD_3CN): δ 44.7 (CH_2CH_2), 130.8–140.9 (Ph), 232.7 (C-2). UV (CH_2Cl_2): 348 (log ε 3.58), 235 nm (4.28). MS: 182 (6), 181 (36, M), 171 (7), 122 (9), 121 (100), 77 (40).

2-(4-Tolyl)-1,3-dithiolan-2-ylium tetrafluoroborate 3e was obtained in 88 % yield; yellow crystalline material from MeCN–Et_2O–HBF_4 (drops), m.p. 117–120 °C. ^{1}H NMR (TFA): δ 2.60 (Me), 4.40 (CH_2CH_2), 7.3–7.5 and 7.9–8.1 (Ph). ^{13}C NMR (CD_3CN): δ 21.9 (Me), 44.3 (CH_2CH_2), 129.2–154.9 (Ph), 230.9 (C-2). UV (CH_2Cl_2): 373 (log ε 4.32), 267 (3.42), 235 nm (3.68). MS: 195 (3), 194 (14, M), 136 (10), 135 (100), 91 (28), 65 (11).

2-Benzyl-1,3-dithiolan-2-ylium tetrafluoroborate 3f was obtained in 96 % yield; off-white crystalline material from MeCN–Et_2O–HBF_4 (drops), m.p. 108–110 °C. ^{1}H NMR (CD_3CN): δ 4.30 (CH_2CH_2), 4.60 (CH_2), 7.30 (Ph). ^{13}C NMR (CD_3CN): δ 41.0 (CH_2), 46.7 (CH_2CH_2), 126.9–135.7 (Ph), 248.9 (C-2). UV (CH_2Cl_2): 307 nm (log ε 4.29), MS: 195 (12, M), 194 (100), 166 (67), 134 (54), 122 (14), 121 (29), 90 (37), 89 (23).

2-β-Styryl-1,3-dithiolan-2-ylium tetrafluoroborate 3g was obtained in 92 % yield; orange needles from MeCN–Et_2O–HBF_4 (drops), m.p. 155–158 °C. ^{1}H NMR (CD_3CN): δ 4.30 (CH_2CH_2), 7.5–7.9 (Ph), 8.00 and 8.35 (CH=CH, J_{trans} 16 Hz). ^{13}C NMR (CD_3CN): 44.5 (CH_2CH_2), 122.5 (β-CH), 132.8–136.4 (Ph), 157.4 (α-CH), 225.7 (C-2). UV (CH_2Cl_2): 436 (log ε 4.40), 244 nm (3.87). MS: 412 (100), 208 (43), 207 (12), 147 (37), 115 (24), 91 (18), 77 (13).

2-Methyl-1,3-dithian-2-ylium tetrafluoroborate 4b was obtained in 66 % yield as a yellow crystalline material which decomposed in contact with the atmosphere. ^{1}H NMR (CD_3CN): δ 2.2–2.6 (CH_2-5), 2.90 (Me), 3.5–3.8 (2 CH_2-4.6), ^{13}C NMR (CD_3CN): δ 16.9 (C-5), 32.4 (Me), 33.6 ($2CH_2$-4.6), 229.4 (C-2). UV (CH_2Cl_2): 310, 287 and 228 (log ε not given because of rapid decomposition). MS: 134 (10),

133 (12, M), 132 (100, M–H), 99 (17), 90 (10), 85 (11), 74 (21).

2-Butyl-1,3-dithian-2-ylium tetrafluoroborate 4c was obtained in 82 % yield as a yellow oily material. ^{1}H NMR (CD_2CN): δ 2.2–2.6 (CH_2-5), 3.5–3.8 ($2CH_2$-4.6), 3.2–3.4 and 1.0–2.1 (Bu). ^{13}C NMR (CD_3CN): δ 16.7 (Me), 20.2, 25.3, 37.1, 49.4 ($4CH_2$), 36.3 ($2CH_2$-4.6), 236.9 (C-2). UV (CH_2Cl_2): 312 nm (log ε 3.53). MS: 175 (3, M), 174 (27, M–H), 145 (100), 71 (51).

2-Phenyl-1,3-dithian-2-ylium tetrafluoroborate 4d was obtained in 87 % yield; white crystalline material from MeCN–Et_2O–HBF_4 (drops), m.p. 188–191 °C. ^{1}H NMR (CD_3CN): 2.3–2.5 (CH_2-5), 3.6–3.8 ($2CH_2$-4.6), 7.4–8.0 (Ph). ^{13}C NMR (CD_3CN): δ 18.2 (CH_2-5), 34.5 ($2CH_2$-4.6), 128.6–138.7 (Ph), 223.2 (C-2). UV (CH_2Cl_2): 335 (log ε 4.04), 266 (3.41), 236 nm (3.66). MS: 196 (17), 195 (95, M), 160 (13), 122 (16), 121 (100), 106 (12), 105 (48).

2-(4-Tolyl)-1,3-dithian-2-ylium tetrafluoroborate 4e was obtained in 72 % yield as a yellow oily material. ^{1}H NMR (CD_3CN): δ 2.50 (Me), 2.4–2.7 (CH_2-5), 3.7–3.9 ($2CH_2$-4.6), 7.2–7.8 (Ph). ^{13}C NMR (CD_3CN): δ 24.7 (CH_2-5), 25.1 (Me), 37.1 ($2CH_2$-4.6), 131.5–154.4 (Ph), 224.5 (C-2). UV (CH_2Cl_2): 366 (log ε 4.11), 276 (2.94), 242 nm (3.34). MS: 210 (5), 209 (17, M), 136 (12), 135 (98), 119 (100), 107 (21), 91 (38).

2-Benzyl-1,3-dithian-2-ylium tetrafluoroborate 4f was obtained in 70 % yield; white crystalline material, m.p. 94–97 °C. ^{1}H NMR (CD_3CN): δ 2.1–2.5 (CH_2-5), 3.4–3.7 ($2CH_2$-4.6), 4.40 (CH_2), 7.30 (Ph), ^{13}C NMR (CD_3CN): δ 17.2 (CH_2-5), 33.8 ($2CH_2$-4.6), 51.4 (CH_2), 130.0–134.8 (Ph), 233.0 (C-2). UV (CH_2Cl_2): 334 (log ε 3.89), 309 nm (4.19). MS: 209 (13, M), 208 (100, M–H), 161 (7), 135 (10), 134 (99), 91 (10), 90 (13), 88 (11).

2-β-Styryl-1,3-dithian-2-ylium tetrafluoroborate 4g was obtained in 93 % yield; yellow crystalline material, m.p. 126–127 °C. ^{1}H NMR ($CDCl_3$): 2.3–2.5 (CH_2-5), 3.5–3.8 ($2H_2$-4.6), 7.4–7.8 (Ph–CH=), 8.05 (CH=, J_{trans} 16 Hz). ^{13}C NMR (CD_3CN): δ 18.9 (C-5), 32.9 ($2CH_2$-4.6), 128.0–134.8 (PhCH=), 147.8 (CH=), 215 (C-2). UV (CH_2Cl_2): 431 (log ε 3.49), 303 nm (4.43). MS: 222 (4), 221 (6, M), 147 (100), 117 (9).

2-Benzyl-1,3-dithian-2-ylium tetrafluoroborate 4f *by protonation of* 6f. 54 % HBF_4 in ether (3 ml) was added dropwise with stirring at 0 °C to a solution of 2-benzylidene-1,3-dithiane [11] in ether (30 ml). After the addition was complete, the mixture was allowed to reach room temperature and the separated solid triturated with ether; yield 74 %.

1,3-Dithianes 2 *by reduction of the salts* 4. *LAH reduction.* Lithium aluminium hydride (12 mmol) was added to a suspension of the salt (10 mml) in ether (30 ml) and the mixture stirred for 2 min before it was poured into saturated aqueous NH_4Cl solution. The organic layer was separated, the aqueous phase extracted with ether, the combined ether solutions dried ($MgSO_4$), the ether distilled off, and the residue purified either by distillation or by recrystallization.

2-Methyl-1,3-dithiane [6] *2b*, b.p. 66 °C/5 mmHg; yield 64 %.

2-Butyl-1,3-dithiane [16] *2c*, b.p. 88–90 °C/0.6 mmHg; yield 82 %.

2-Phenyl-1,3-dithiane [8] *2d*, m.p. 68–70 °C; yield 97 %.

2-Benzyl-1,3-dithiane [7] *2f*, b.p. 120–121 °C/0.3 mmHg; yield 84 %.

Sodium borohydride reduction. Sodium borohydride (9 mmol) was added with stirring at 0 °C to a solution of the salt (8 mmol) in acetonitrile (30 ml). The mixture was stirred for 30 min after the addition was completed, and the reaction mixture worked up as under the LAH-reduction.

2-Phenyl-1,3-dithiane [8] *2d*, m.p. 68–70 °C (MeOH); yield 86 %.

2-(4-Tolyl)-1,3-dithiane [17] *2e*, m.p. 82–83 °C (light petroleum), yield 72 %.

2-Alkylidene-1,3-dithianes 6. Triethylamine (15 mmol) in acetonitrile (5 ml) was added dropwise to a solution of the 1,3-dithian-2-ylium tetrafluoroborate (10 mmol) in acetonitrile (25 ml) at 0 °C. The mixture was stirred for 5 min at this temperature before it was allowed to warm up to room temperature. The solvent was then allowed to distill off at reduced pressure, the residue extracted with chloroform (3×100 ml), the dried ($MgSO_4$) solution evaporated and the residue distilled.

2-Methylen-1,3-dithiane [11] *6b*, b.p. 78–80 °C/8 mmHg; yield 74 %.

2-Butylidene-1,3-dithiane [18] *6c*, b.p. 68–70 °C/0.08 mmHg; yield 88 %.

2-Benzylidene-1,3-dithiane [11] *6f*, b.p. 120–122 °C/0.05 mmHg; yield 93 %.

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[2_3]Cyclophanetrienes from Wittig Reactions and Titanium-mediated Reductive Coupling

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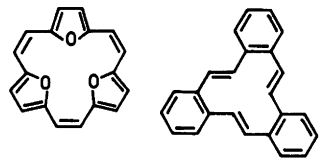
A series of new [2.2.2.]cyclophanetrienes, 4, 5, 6 and 7, has been prepared by a threefold Wittig reaction or by twofold Wittig reactions followed by titanium-mediated reductive couplings of dialdehydes. The combination of *cis*-selective Wittig reactions and intra-molecular reductive couplings is shown to be a simple and effective way to make large ring compounds containing different aromatic units. The ferrocenophane 7 in which the two five-membered rings are linked by a conjugated π-system was found by ^{1}H NMR spectroscopy to exist in two mirror image forms with opposite helicity.

It has previously been reported from this laboratory that *cis*-stereoselective multiple Wittig reactions provide convenient access to [2_4]cyclophanetetraenes, and the applicability of this one-pot synthesis of macrocycles has been demonstrated amply.[1] We now wish to report an example of the synthesis of a symmetrical [2_3]cyclophanetriene by this method. More recently, we have also employed low-valent titanium species in simple one-step syntheses of cyclophanes and annulenes *via* reductive coupling of readily available dialdehydes.[2,3] A combination of these two techniques, *i.e.* a *cis*-selective double Wittig reaction followed by intramolecular coupling of aldehyde groups has now proved to be a short and reasonably efficient synthesis of less symmetrical [2_3]cyclophanetrienes.

RESULTS AND DISCUSSION

Threefold Wittig reactions. Macrocycle synthesis employing "mixed" Wittig reagents analogous to *3* (Scheme 1) has been attempted previously. The [2_3](2,5)furanophanetriene *1* (considered to be a trioxo[18]annulene) was synthesised by Elix[4] *via* a threefold Wittig reaction (DMF, lithium ethoxide, 90 °C) while Brown and Sargent[5] have reported an analogous synthesis of Staab's [2_3]orthocyclophanetriene *2* (methanol, lithium ethoxide, reflux). However, the yield in both cases was less than one percent and in the latter case the major cyclic product (26 %) was 1,2:5.6-dibenzocyclooctatetraene.



1 *2*

In our hands, the Wittig reagent *3* which is easily prepared from benzene-1,3-dicarbaldehyde (see Experimental) underwent a threefold Wittig reaction (DMF, lithium ethoxide) to yield the all-*cis*[2_3]metacyclophanetriene *4* in 28 % yield, (Scheme 1).

Cyclisation occurred smoothly at −40 °C, the various ylid-aldehyde species involved being relatively reactive due to the absence of mesomeric effects. (Wittig reagents of type *3* in which the reactive centres are in conjugation with each

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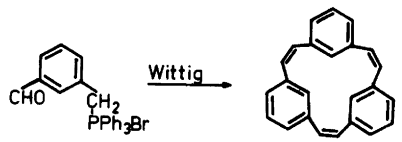
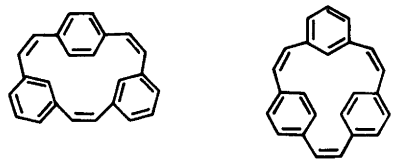


3 4

Scheme 1.

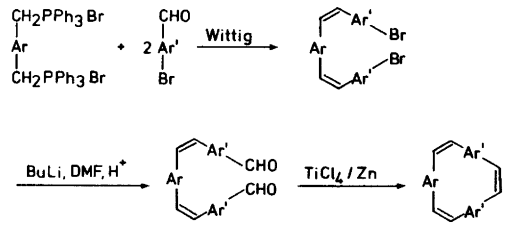
other, and thus mutually stabilised, have been observed to cyclise less readily).[6] In contrast to the reactions which gave *1* and *2*, the present reaction gave the triene *4* as the dominant ring-closed product and the yield was, in relative terms, high. As well as the enhanced reactivity of *3*, the lower reaction temperature used is undoubtedly a contributing factor, as this should have a favourable entropic effect on the ring-closure reaction. No [2.2]metacyclophanediene (or products thereof) could be detected, steric effects favouring formation of the unstrained triene *4*. However, trace amounts of two isomers of [2$_4$]metacyclophanetetraene, the all-*cis*- and the *cis,cis,cis,trans*-isomers, were observed by ^{1}H NMR spectroscopy in the crude product mixture by comparison with authentic spectra.[7]

Double Wittig reaction followed by reductive coupling. The sequence of double Wittig reaction/titanium-mediated intramolecular reductive coupling was used to synthesise cyclophanes *5*, *6* and *7* and the general method is depicted in Scheme 2.



5 6

Thus, a double Wittig reaction was carried out under *cis*-selective conditions between an aromatic bisphosphonium salt and two equivalents of an aromatic aldehyde substituted with bromine in the appropriate position. This yielded *cis,cis,*-dienes as the major products which were routinely converted to the corresponding dialdehydes. Intramolecular coupling of the carbonyl groups using the low-valent titanium species obtained by reduction of titanium tetrachloride with zinc then yielded the [2$_3$]cyclophanetrienes in reasonably good yield. In principle, aromatic dialdehydes could be used directly in the first step (this was actually done in the synthesis of *7*) but the present method avoids production of the corresponding [2$_4$]cyclophanetetraenes which are known to form easily under the conditions used.[1] The *cis,cis*-dienes isomerised partly, albeit quite slowly, upon work-up and subsequent handling and the dialdehydes were used without delay after isolation. The yields in the various steps were acceptable and the overall ease of operation and work-up makes the sequence shown in Scheme 2 an attractive one.



Scheme 2.

The presence of two *cis* double bonds in the dialdehydes used in the final step favours the desired intramolecular coupling reaction over intermolecular processes and high dilution techniques are unnecessary, a THF solution of the dialdehyde being added slowly to the pre-formed titanium reagent (THF, reflux). The desired all-*cis* trienes were the sole isolable products. This type of reductive coupling has been used previously by Kasahara *et al.*[8] in the final ring closure of the *trans,cis,trans* isomer of *7*. These workers employed a low-valent titanium species prepared from titanium trichloride and lithium aluminium hydride, which in our hands proved generally less satisfactory then the titanium tetrachloride/zinc reagent.

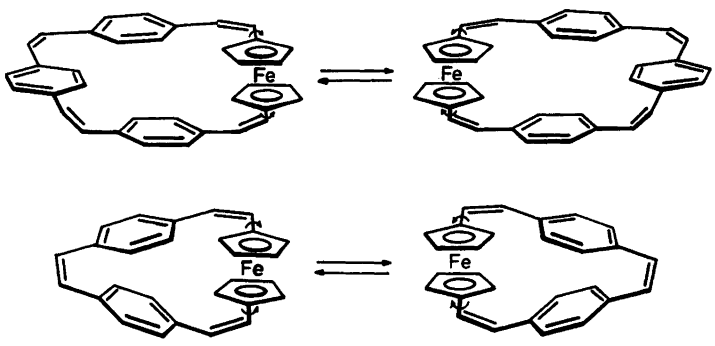
Stereochemistry. The gross structures of the cyclic products are apparent from the spectral data given in the Experimental section. From inspection of CPK models, it is immediately clear that for cyclophanes *6* and *7* the double bond formed in the final step must have the *cis* configuration, while for *5* this double bond may be either *cis* or *trans*. However, the *trans* isomer should be more strained and most of the sterically feasible conformations force the isolated protons on the metasubstituted benzene rings over the face of the parasubstituted ring. This would be

expected to result in a strong shielding of these protons in the ^{1}H NMR spectrum, but no such effects are obtained. The IR spectrum also shows the absence of *trans* double bonds. Since the NMR data are consistent with a single isomer, the all-*cis* configuration can be assigned with confidence to cyclophane *5*.

Cyclophanes *4*, *5* and *6* are expected to be relatively unstrained, conformationally mobile species and it is difficult to predict which conformations are of lowest energy. This expectation is borne out by the room-temperature ^{1}H NMR spectra which show the patterns expected for rapid equilibration between the possible conformers in each case, with low barriers to the flipping of the para- and meta-substituted benzene rings. The compounds thus resemble more closely the anlogous all-*cis*[2₄]cyclophanetetraenes [1,7] than the more strained and compact [2₂]para- and metacyclophanedienes.[9,10] The chemical shifts of the aromatic and olefinic protons are unremarkable, and no unusually strong shielding or deshielding effects are apparent. In cyclophane *6*, however, the singlet from the olefinic protons is shifted to slightly lower field than the signals from the adjacent parasubstituted benzene ring. This type of deshielding effect has been observed in other [2₃]cyclophanetrienes, particularly those incorporating parasubstituted *cis*-stilbene subunits.[11]

Stereochemically, the ferrocenophane *7* is of greater interest. We have previously synthesised the "homologous" all-*cis* [2₄] (1,1′)ferrocenophanetetraene *8* which, from the study of models and on the basis of UV and ^{1}H NMR spectroscopy was suggested to adopt a helix-like conformation which allowed extensive conjugation between the "top" and "bottom" halves of the molecule.[12] Further, DNMR studies showed that the interconversion of the two helical mirror-image conformers *via* rotation of the entire ferrocene unit through the central cavity of the cyclophane takes place over a barrier of 40 kJ mol^{-1} (see Scheme 3). In the fast exchange spectra of *8*, the ferrocene protons appear as an AA′XX′-pattern which changes to an ABCD-pattern in the "frozen" conformational mixture.

Inspection of a CPK model of the ferrocenophane *7* suggests that this molecule must also be constrained to a helix-like geometry, the helix being much "tighter" than that of *8*. The effect of the shortened bridge between the cyclopentadienyl rings in *7* should be to force the benzene rings out of conjugation with the adjacent double bonds and also to raise the barrier to the interconversion of the enantiomeric helical conformers. The former effect is apparent from comparison of the UV spectra of *7* and *8*. While *8* has an intense absorption band around 312 nm, the ferrocenophane *7* has no strong band above 230 nm. The observed difference between *7* and *8* supports our earlier conclusion that the ferrocenophane *8* does indeed possess unbroken conjugation between its two five-membered rings. This point has been discussed more recently by Katz and Pesti [13] who, in claiming the first example of this type of structure, made the observation that in *8* "adjacent double bonds are not constrained parallel". While this statement is obviously accurate, a rigidly clamped π-system is hardly a necessary condition for π-electron conjugation.



7 *8*

Scheme 3.

Concerning the dynamic stereochemistry of *7*, the room-temperature ^1H NMR spectrum is consistent with a species of C_2-symmetry in which the rotation or flipping of the benzene rings is still rapid on the NMR time-scale (as in *8*) while rotation of the ferrocene unit through the central cavity of the cyclophane is already "frozen". The protons on the benzene rings thus give rise to the expected AA'BB'-pattern centred on δ 7.08 while the cyclopentadienyl protons constitute an ABCD spin system appearing as four sharp, well-resolved, multiplets at δ 4.36, 3.99, 3.81 and 3.56. No significant broadening of the NMR signals due to rapid rotation of the ferrocene unit was observed at elevated temperatures. However, a distinct broadening and shift of the signals due to decomposition of the sample to give paramagnetic ferrocenium ions was apparent at temperatures above 100 °C in dideuterotetrachloroethane.

EXPERIMENTAL

Melting points are uncorrected. NMR spectra were obtained on a Bruker WH 270 instrument, IR spectra on a Beckman IR9, UV spectra on a Varian Cary 210 and mass spectra on an AEI MS 902 instrument.

The apparatus, reaction conditions and work-up for the Wittig reactions have been described earlier.[1]

[2$_3$]Metacyclophanetriene, 4. Benzene-1,3-dialdehyde (10 g) and sodium borohydride (750 mg) were dissolved in ethanol (200 ml) and stirred for 1 h. The solvent was removed by evaporation and the residue dissolved in hot water and then cooled. Unreacted dialdehyde (2.1 g) was collected. The aqueous solution was extracted several times with diethyl-ether. The ethereal solution was dried over $MgSO_4$ and the solvent removed to give *3-hydroxymethylbenzaldehyde* (5.7 g, 57 %, colourless oil). The crude product was treated with hydrobromic acid in acetic acid (33 %, 25 ml) and stirred for 1 h at room temperature. The mixture was poured onto ice-water and the white precipitate of *3-bromomethylbenzaldehyde* was collected and dried (6.6 g, 82 %, m.p. 36–37 °C).

The crude product above, 3-bromomethylbenzaldehyde (5.97 g) and triphenylphosphine (7.86 g) were dissolved in dry DMF (50 ml) and heated to 130 °C. The solution was cooled and a few crystals of the phosphonium salt, prepared by addition of diethyl ether to a small sample of the solution, were added. The white precipitate of the *phosphonium salt from 3-bromomethylbenzaldehyde* was collected and carefully dried (7.6 g, 55 %, m.p. 301–303 °C).

The triphenylphosphonium salt from 3-bromomethylbenzaldehyde (4 g) was dissolved in dry DMF (100 ml) and the mixture cooled under nitogen to −40 °C. Freshly prepared lithium ethoxide in ethanol was added dropwise to the stirred solution which slowly changed colour from orange to yellow. The addition was complete within a few hours when no further colour change was observed on addition of base. The mixture was warmed to room temperature and water (100 ml) was added followed by extraction with diethyl ether. The combined ethereal solutions were washed with water, dried over $MgSO_4$ and solvent removed. The white semi-solid residue was separated from triphenylphosphine oxide by column chromatography to give crude *[2$_3$]metacyclophanetriene, 4* (265 mg, 48 %). The crude product was purified by distillation at $6 \cdot 10^{-2}$ mm Hg, 160 °C, to give pure *4* (113 mg, 20 %, white solid m.p. 72–75 °C). UV (ethanol): 253 nm, ε 13 400. ^1H NMR (270 MHz, $CDCl_3$): δ 7 · 18 (3 H, t, *J* 8 Hz), 6.94 (6 H, dd, *J* 2 Hz), 6.69 (3 H, m) aromatic protons, 6.57 (6 H, s) olefinic protons. MS (70 eV): *m/e* 306 (100 %, M^+). Abs. mass 306.142±0.003; calc. for $C_{24}H_{18}$ 306.141. The distillation residue was purified by preparative TLC to give another 8 % of the cyclophane *4* and *ca.* 8 % (42 mg) of a mixture of all *cis* and *cis,cis,cis,trans* [2$_4$]metacyclophanetetraene, identified by comparison of the ^1H NMR spectrum with that from authentic material.[7]

General procedure for intramolecular reductive coupling of dialdehydes. A three-necked round-bottomed flask (250 ml) fitted with N_2 inlet, reflux condenser and Schlenk-type dropping funnel is charged with dry THF (100 ml, distilled from Na–benzophenone) and cooled to −78 °C. Titanium tetrachloride (30 mmol) is then added carefully, with stirring, followed by zinc powder (60 mg-atom) and dry pyridine (0.5 ml). The resulting black mixture is first refluxed under nitrogen for 1 h and then a solution of the dialdehyde (3 mmol) in dry THF (100 ml) is added *slowly via* the Schlenk funnel over a period of *ca* 12 h. The reaction is followed by TLC (disappearance of the dialdehyde) and finally cooled to 0 °C (ice bath) and quenched by addition of 10 % aqueous K_2CO_3. The resulting grey precipitate is filtered off and both filtercake and filtrate extracted with dichloromethane. The combined organic phases are dried ($MgSO_4$) and the solvent removed to yield a residue which is chromatographed on a short column of silica gel with dichloromethane as eluent. The products

can be recrystallised from a mixture of dichloromethane and methanol.

[2$_3$]Metametaparacyclophanetriene, 5. 3-Bromobenzaldehyde (7.4 g) and the bistriphenylphosphonium salt from 1,4-bis-(bromomethyl) benzene (15.76 g) were dissolved in dry DMF (200 ml) and cooled to −40 °C under nitrogen. Freshly prepared lithium ethoxide (40 mmol) was added slowly during 3 h to the stirred solution. The mixture was then warmed to room temperature and diluted with water (200 ml). The aqueous phase was extracted with diethyl ether two or three times. The combined ethereal fractions were washed with water, dried ($MgSO_4$) and concentrated to allow triphenyl phosphineoxide to precipitate. The remaining solution contained three isomers, the *cis,cis-*, the *cis,trans-* and the *trans,trans-*isomer of *1,4-bis-(bromostyryl)benzene* (5.7 g, 65 %).

The crude oil above was dissolved in dry ether (100 ml) under N_2 and cooled to 0 °C. Butyllithium (25 ml, 1.6 M in hexane, Merck) was added *via* syringe and the mixture was stirred for 3 h. Dry DMF (5 ml) was added and stirring continued for another hour. Finally, hydrochloric acid (100 ml, 3 M) was added and the mixture stirred for 30 min more. The ethereal solution was separated, washed with water, dried ($MgSO_4$), concentrated and cooled. A light yellow precipitate of *cis,cis-1,4-bis(3-formylstyryl)benzene* was collected (1.83 g, 42 %, m.p. 64–68 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.93 (2 H, s) aldehyde protons, 7.74 (2 H, m), 7.70 (2 H, dt), 7.49 (2 H, dt), 7.37 (2 H, t) metasubstituted ring protons, 7.07 (4 H, s) parasubstituted ring protons, 6.64–6.61 (4 H, AB-pattern, *J* 12 Hz) olefinic protons. MS (70 eV): *m/e* 338 (100 %, M^+), 310 (18), 203 (13), 202 (10), 178 (10). Abs. mass 338.131; calc. for $C_{24}H_{18}O_2$ 338.131.

The reductive coupling of *cis,cis*-1,4-bis(3-formylstyryl)-benzene as described by the general procedure above gave *[2$_3$]metametaparacyclophanetriene, 5,* as white crystals (477 mg, 52 %, m.p. 127–129 °C). UV (ethanol): 274 nm, ε 25 500. ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.43 (2 H, m), 7.23 (2 H, t), 7.17 (2 H, dt), 7.05 (2 H, dt) metasubstituted ring protons, 7.20 (4 H, s) parasubstituted ring protons, 6.70, 6.55 (4 H, AB-pattern, *J* 12.5 Hz), 6.48 (2 H, s) olefinic protons. MS (70 eV): *m/e* 306 (100 %, M^+), 290 (6), 289 (11), 276 (8). Abs. mass 306.140; calc. for $C_{24}H_{18}$ 306.141.

[2$_3$]Metaparaparacyclophanetriene, 6. 4-Bromobenzaldehyde (7.4 g) and the bistriphenylphosphonium salt from 1,3-bis-(chloromethyl) benzene (13.98 g) were dissolved in dry DMF (200 ml) under N_2 and the mixture was cooled to −40 °C. Freshly prepared lithium ethoxide (40 mmol) was then added dropwise under 3 h to the stirred solution. The resulting mixture was warmed to room temperature and diluted with water (200 ml). The aqueous solution was then extracted with two or three portions of diethyl ether. The combined ether fractions were washed with water, dried ($MgSO_4$), and the solution concentrated. The precipitated triphenylphosphine oxide was filtered off and the rest of the solvent evaporated. The crude product (5 g) was used in the subsequent reaction.

The combined products from two of the reactions above (10 g) were dissolved in dry diethyl ether (100 ml) under N_2 and cooled to 0 °C. Butyl-lithium (30 ml, 1.6 M, Merck) was added *via* syringe and the mixture was stirred for 3 h. Dry DMF (10 ml) was added and stirring was continued for another hour. Finally, hydrochloric acid (150 ml, 2 M) was added followed by stirring for 30 min more. The ethereal solution was washed with water, dried ($MgSO_4$), and the solvent evaporated. The residual oil was carefully chromatographed on silica gel with dichloromethane as eluent. The first fractions contained unreacted starting material followed by monoaldehydes. Subsequent fractions contained the desired *cis,cis 1,3-bis(4-formylstyryl)benzene* (1.20 g, 16 %, colourless oil). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.93 (2 H, s) aldehyde protons, 7.67, 7.31 (8 H, AA'BB'-pattern, *J* 8 Hz) parasubstituted ring protons, 7.11–7.03 (4 H, m) metasubstituted ring protons, 6.61, 6.54 (4 H, AB-pattern, *J* 12 Hz) olefinic protons.

The crude oil from the reaction above was used in an intramolecular reductive coupling as described above to give [2$_3$]metaparaparacyclophanetriene, *6* (275 mg, 30 %. m.p. 75–77 °C). UV (ethanol): 227 (sh), ε 23 500. ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.30 (1 H, t), 7.10 (2 H, d) 6.99 (1 H, broad s) metasubstituted ring protons, 6.86, 6.62 (8 H, AA'BB'-pattern, *J* 8 Hz) parasubstituted ring protons, 7.01 (2 H, s), 6.61, 6.50 (4 H, AB-pattern, *J* 13 Hz) olefinic protons. MS (70 eV): *m/e* 306 (100 %, M^+), 305 (9), 303 (6), 291 (6), 290 (8), 289 (13), 277 (8), 276 (15), 138 (8). Abs. mass 306.141; calc. for $C_{24}H_{18}$306.141.

[2$_3$](1,1')Ferrocenoparaparacyclophanetriene, 7. The bistriphenylphosphonium salt from 1,1'-bis(chloromethyl)-ferrocene [12] (6.1 g, 7.5 mmol) was reacted with benzene-1,2-dicarbaldehyde (2.0 g, 15 mmol) under the standard conditions for the Wittig reactions (−20 °C) described above and in Ref. 12 to give *1,1'-bis(4-formylstyryl)ferrocene* as a dark red oil (*ca.* 1.3 g, 3 mmol, 35–40 %) consisting of the *cis,cis*-isomer after purification by column chromatography

(silica gel, dichloromethane). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.99 (2 H, s) aldehyde protons, 7.78 7.46 (8 H, AA'BB'-pattern J 8 Hz) parasubstituted ring protons, 6.47, 6.33 (4 H, AB-pattern, J 12 Hz) olefinic protons, 4.89, 4.83 (8 H, m) ferrocene protons. The *cis,trans-* and *trans,trans*-isomers were also formed in the Wittig reaction but in minor amounts.

The crude *cis,cis*-1,1'-bis(4-formylstyryl)ferrocene above was used without further purification in the intramolecular reductive coupling as described above to give cis,cis,cis-*[2$_3$](1,1')ferrocenoparaparacyclophanetriene, 7,* as bright red needles (250 mg, 20 %, m.p. 189–191 °C). UV (ethanol): 475 nm (ε 500), 285 (sh), 230 (ε 36 000). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.08 (8 H, m) parasubstituted ring protons, 6.97 (2 H, s), 6.55 and 6.07 (4 H, AB-pattern, J 12 Hz) olefinic protons, 4,36 (2 H, m), 3.99 (2 H, m), 3.81 (2 H, m), 3.56 (2 H, m) ferrocene protons. MS (70 eV): *m/e* 414 (100 %, M^+) no other peaks >5 %. Abs. mass 414.107; calc. for $C_{28}H_{22}Fe$ 414.107.

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Reactions of Tributyltin Methoxide with Chloromethyl Esters

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Reactions of tributyltin methoxide with esters RCO_2R' ($R'=CH_2Cl$, $CHCl_2$ or CCl_3) have been performed to study the influence of the halogens in the alkyl component of an ester on the course of the reaction. In all reactions tributyltin chloride and methyl ester were formed whereas the subsequent reaction depended on the number of halogens in the methyl group.

The versatility of organotin alkoxides in transesterification and related reactions is well known.[1] Exchange of the acyl group in an ester can be accomplished, *e.g.*, by reactions (1) and (2).

$$Bu_3SnOCH_3+RCO_2R' \rightleftharpoons Bu_3SnOR'+RCO_2CH_3 \quad (1)$$

$$Bu_3SnOR'+R''COCl \rightarrow Bu_3SnCl+R''CO_2R' \quad (2)$$

An explanation for the ability of organotin alkoxides to promote ester exchange is the fact that an alkoxy group attached to tin is in rapid exchange between different tin sites. The rate of the exchange depends largely on the size of the alkoxy group. Thus it has been found that the rate for tributyltin methoxide at −20 °C is of the same order as that for tributyltin 2-propoxide at a temperature above 160 °C.[2] It has also been proposed that the transesterification would be an addition–elimination process[3] which leaves the most slowly exchanging group attached to tin, reaction (3).

$$Bu_3SnOCH_3+RCO_2R' \rightleftharpoons Bu_3SnO\underset{\displaystyle OCH_3}{\overset{\displaystyle R}{C}}OR' \rightleftharpoons Bu_3SnOR'+RCO_2CH_3 \quad (3)$$

Most of the known organotin alkoxides have no halogens attached to the alkoxy group. Delmond *et al.* have shown that also stable alkoxides containing 2-halogens in the alkoxy group can be prepared from tributyltin ethoxide and 2-haloalcohols.[4] These alkoxides react intramolecularly at 70–210 °C giving principally epoxides. Tributyltin 3-bromoalkoxides undergo a similar reaction at 170 °C giving oxetanes in an 80–90 % yield.[5] On the contrary, tributyltin methoxide gives with 4-chlorobutanol no isolable tributyltin alkoxide, but directly tributyltin chloride and tetrahydrofuran.[6] Yet, the reaction intermediate is found to be tributyltin 4-chlorobutoxide, reaction (4).

$$Bu_3SnOCH_3+Cl(CH_2)_4OH \rightarrow [Bu_3SnO(CH_2)_4Cl]+CH_3OH \quad (4)$$

$$[Bu_3SnO(CH_2)_4Cl] \rightarrow Bu_3SnCl+O\begin{cases} CH_2-CH_2 \\ | \\ CH_2-CH_2 \end{cases}$$

In general, however, quite little is known about reactions of organotin alkoxides with halogenated esters. We have found when attempt-

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ing to prepare chloromethyl esters *via* reactions (1) and (2) that no transesterification took place. This led us to study the effect of halogens in methyl esters on the reaction with tributyltin methoxide.

RESULTS AND DISCUSSION

Three chloromethyl esters ($CH_3CO_2CH_2Cl$, $CH_2ClCO_2CHCl_2$ and $CCl_3CO_2CCl_3$) were allowed to react with tributyltin methoxide. These esters have different acyl groups but, according to our results, the acyl group is unreactive towards tributyltin methoxide, the reaction instead taking place on the alkoxy group. This was confirmed by studying the reaction between tributyltin methoxide and ethyl chloroformate or ethyl trichloroacetate. In both cases the only reaction was transesterification to an extent of 45–50 %. No formation of tributyltin chloride, and thus no reaction of tributyltin methoxide with the halogens in the acyl group, could be detected.

The reaction of tributyltin methoxide with chloromethyl acetate gave tributyltin chloride, methyl acetate and paraformaldehyde. The formation of methyl acetate suggests that transesterification is the first stage in this reaction. Another product would then be tributyltin chloromethoxide, which decomposes rapidly to tributyltin chloride and formaldehyde, reactions (5) and (6).

$$Bu_3SnOCH_3+CH_3CO_2CH_2Cl\rightarrow [Bu_3SnOCH_2Cl]+CH_3CO_2CH_3 \quad (5)$$

$$[Bu_3SnOCH_2Cl]\rightarrow Bu_3SnCl+HCHO \quad (6)$$

We were not able to detect the presence of tributyltin chloromethoxide by the ^1H NMR spectrum obtained immediately after mixing the reagents, and it could not be trapped with trimethylchlorosilane either. Further, reactions (1) and (2) gave no positive results in attempts to synthesize other chloromethyl esters. This suggests that tributyltin chloromethoxide is a very short-lived species if it exists at all. This unstability compared with corresponding 2- and 3-haloalkoxides is, however, in accord with the observed dependence of exchange rates on the size of the alkoxy group.

The reaction of tributyltin methoxide with dichloromethyl chloroacetate (molar ratio 2:1, see experimental part) produced methanol and carbon monoxide in addition to tributyltin chloride and methyl chloroacetate. Now the first products from the reaction of equimolar amounts of starting materials appear to be, disregarding the possible existence of tributyltin dichloromethoxide, tributyltin chloride, methyl chloroacetate, and formyl chloride which further decomposes to hydrogen chloride and carbon monoxide, reactions (7) and (8). The reaction of hydrogen chloride with another mol of tributyltin methoxide finally yields tributyltin chloride and methanol, reaction (9).

$$Bu_3SnOCH_3+CH_2ClCO_2CHCl_2\rightarrow Bu_3SnCl+[HCOCl]+CH_2ClCO_2CH_3 \quad (7)$$

$$[HCOCl]\rightarrow CO+HCl \quad (8)$$

$$HCl \xrightarrow{Bu_3SnOCH_3} Bu_3SnCl+CH_3OH \quad (9)$$

Traces of water found in some experiments may then come from reaction of methanol with hydrogen chloride. Formyl chloride could, in principle, react with tributyltin methoxide too. However, no methyl formate from such a reaction was detected. This is probably due to the fact that formyl chloride decomposes much faster than it reacts with alkoxide.

Tributyltin methoxide reacted with trichloromethyl trichloroacetate (molar ratio 3:1, see experimental part) forming methyl trichloroacetate, tributyltin chloride, and dimethyl carbonate. Equimolar amounts of reagents produced some methyl chloroformate in addition to dimethyl carbonate. This indicates that the first reaction product is carbonyl chloride, which can react further with tributyltin methoxide forming tributyltin chloride and methyl chloroformate, reactions (10) and (11). According to Röse,[7] methyl chloroformate forms dimethyl carbonate and hydrogen chloride with methanol. In this case tributyltin methoxide can serve as a source of the alkoxy group, thus the final products are tributyltin chloride and dimethyl carbonate, reaction (12).

$$Bu_3SnOCH_3+CCl_3CO_2CCl_3\rightarrow Bu_3SnCl+CCl_3CO_2CH_3+COCl_2 \quad (10)$$

$$COCl_2 \xrightarrow{Bu_3SnOCH_3} Bu_3SnCl + ClCO_2CH_3 \quad (11)$$

$$ClCO_2CH_3 \xrightarrow{Bu_3SnOCH_3} Bu_3SnCl + (CH_3O)_2CO \quad (12)$$

In the light of the reactions studied it seems obvious that organotin alkoxides containing 1-halogens are not sufficiently stable to be used as a source of chloromethoxy groups. Possible explanations for this instability may be, in addition to steric reasons, the greater exchange ratio of the chloromethoxy group compared to 2- and 3-haloalkoxy groups. These considerations do not, however, explain the instability of tributyltin 4-chlorobutoxide, which indicates that also other factors have to be present.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer 180 IR spectrometer, and ^1H NMR spectra were obtained on a Jeol JNM–PMX 60 spectrometer in CCl_4.

Chloromethyl acetate,[8] dichloromethyl chloroacetate,[9] trichloromethyl trichloroacetate[8] and tributyltin methoxide[2] were prepared according to procedures described in the literature. The purity of reagents was checked by ^1H NMR and IR spectroscopy or by GLC using a 25 m silica capillary column coated with OV 101 or OV 17 liquid phase. The products formed in the reactions studied were characterized by physical constants and by comparing their IR and NMR spectra with those of authentic samples.

Reaction of tributyltin methoxide with chloromethyl acetate. Tributyltin methoxide (3.47 g, 10.8 mmol) and chloromethyl acetate (1.18 g, 10.9 mmol) were mixed. After mixing, there was no apparent change but the NMR spectrum showed that the signals of initial reagents had disappeared. Heating at 90 °C for an hour yielded a white stiff substance. Part of this was soluble in carbon tetrachloride, and the NMR spectrum revealed that it consisted of tributyltin chloride (δ 0.7–2.0) and methyl acetate (δ 1.98 and 3.65). The insoluble solid component proved to be paraformaldehyde. Distillation of the mixture at 10 Pa gave tributyltin chloride, b.p. 83–85 °C, and methyl acetate and paraformaldehyde, which were collected in the cold trap. The reaction took place in a similar way when chloromethyl acetate was replaced by chloromethyl propanoate or 2-methylpropanoate.

Reaction of tributyltin methoxide with dichloromethyl chloroacetate. A preliminary experiment with equimolar amounts of starting materials showed that dichloromethyl chloroacetate reacted only partially. It was consumed totally when the amount of tributyltin methoxide was doubled.

The ester (1.50 g, 8.46 mmol) was placed in a flask equipped with a stirrer, a pressure-equalizing dropping funnel and a gas-collecting device. Tributyltin methoxide (5.42 g, 16.9 mmol) was added slowly from the dropping funnel. The subsequent reaction was exothermic and the mixture frothed vigorously. The IR spectrum of the collected gas showed absorption at 2140 cm^{-1} which was assigned to carbon monoxide. Collected volume (NTP) was 184 cm^3 (97 %). The NMR spectrum of the remaining liquid showed a broad absorption at δ 0.7–2.0 assigned to tributyltin chloride, and singlets δ 2.05, 2.35 (methanol), 3.77 and 3.97 (methyl chloroacetate). The samples obtained by fractional distillation were further characterized by methods described above. A trace of water was occasionally present in duplicate experiments.

Reaction of tributyltin methoxide with trichloromethyl trichloroacetate. When equimolar amounts of tributyltin methoxide and trichloromethyl trichloroacetate were mixed, the analysis of the resulted mixture revealed that it consisted of tributyltin chloride, methyl trichloroacetate, methyl chloroformate and unreacted trichloromethyl trichloroacetate. Hence, the amount of tributyltin methoxide was increased until all starting ester was consumed. In a second experiment, tributyltin methoxide (7.52 g, 23.4 mmol) was added during 15 min to the melted ester (2.20 g, 7.82 mmol). After the exothermic reaction had ceased, NMR analysis of the mixture showed that it consisted of tributyltin chloride (δ 0.7–2.0), dimethyl carbonate (δ 3.73), and methyl trichloroacetate (δ 3.99). Examination of the samples obtained by fractional distillation confirmed the analysis.

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Preparation of 2-Chlorovinyl Esters

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The 2-chlorovinyl esters of the three chloroacetic acids have been prepared and their *E* and *Z* isomers separated with preparative GLC. 2-Chlorovinyl acetate was prepared from 2,2-dichlorovinyl acetate by reduction with tributyltin hydride, whereas the other acetates were prepared by ester exchange from 2-chlorovinyl acetate using tributyltin methoxide as a transfer agent.

The only known method for synthesis of 2-halovinyl esters is that of Mylo,[1] who prepared 2-bromovinyl acetate from acetyl bromide and dibromoacetaldehyde by dehalogenation with copper. Later, after modifications of the method, it proved usable for preparation of 2-bromovinyl chloroacetate as well as 2-chlorovinyl acetate.[2] However, this is not a general method and attempts to prepare other esters have failed. Yet, it was possible to develop a more general method by using selective reduction with organotin hydride[3] followed by ester exchange,[4] *i.e.* first 2,2-dichlorovinyl acetate is reduced with tributyltin hydride to 2-chlorovinyl acetate which is then treated with tributyltin methoxide, reactions (1) and (2). The resulting tributyl-2-chlorovinyloxytin yields the desired ester after reaction with acyl halide, (3). Using mild reaction conditions the hydrostannolysis of the alkyl–oxygen bond or addition of tributyltin hydride to the vinyl double bond can be avoided.

$$RCO_2CH{=}CCl_2 + Bu_3SnH \rightarrow RCO_2CH{=}CHCl + Bu_3SnCl \quad (1)$$

$$RCO_2CH{=}CHCl + Bu_3SnOCH_3 \rightarrow RCO_2CH_3 + Bu_3SnOCH{=}CHCl \quad (2)$$

$$Bu_3SnOCH{=}CHCl + R'COCl \rightarrow Bu_3SnCl + R'CO_2CH{=}CHCl \quad (3)$$

2-Chlorovinyl esters consist of a pair of *E* and *Z* isomers. The *E*:*Z* ratio of isomers was found to vary from 1:2.4 to 1:3.9 in different experiments. The configuration of the ester was not retained, when the ester exchange reactions (1) and (2) were carried out using pure *E* or *Z* isomer as starting material. The resulting mixture contained *E* and *Z* isomers in the same ratio as mentioned above. Hence, the separation of isomers must be performed after reaction (3) if an isomerically pure ester is required.

In the cases of enolic acetates, some investigators have proposed that a metallotropic tautomerism between species *1* and *2* in (4) may result in a change of configuration, but no detailed mechanistic study concerning this kind of tautomerism has been carried out.[5] On the other hand, French authors[6] suggest that the existence of an enolate-type intermediate in the transfer of the alkoxy group can explain the change of the configuration, eqn. (5). In the case of tributyl-2-chlorovinyloxytin species *2* was not detected, thus the existence of the enolate-type intermediate seems to be a more likely explanation for the change of configuration.

$$\underset{1}{Bu_3Sn{-}OCR{=}CR'_2} \rightleftharpoons \underset{2}{Bu_3Sn{-}CR'_2{-}C(R){=}O} \quad (4)$$

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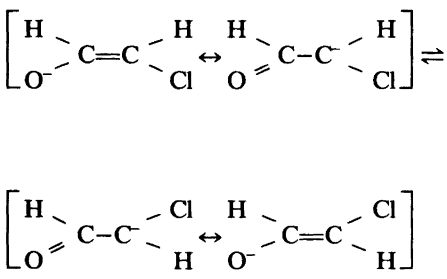

(5)

The isomeric purity of 2-chlorovinyl acetate was not retained during storage. Initially pure *E* and *Z* isomers changed slowly to a mixture of both isomers. This was probably caused by the presence of a trace of moisture which hydrolyzes the ester partly to acetic acid. Acetic acid is further capable of promoting an exchange of alkoxy groups,[9] which then leads to a mixture of isomers through the intermediate presented above.

EXPERIMENTAL

Apparatus. Infrared spectra were recorded on a Perkin-Elmer 180 IR spectrophotometer, and nuclear magnetic resonance spectra on a Jeol JNM–PMX 60 and a Jeol FX-60 spectrometer at 60 MHz (^{1}H NMR spectra) and at 15.03 MHz (^{13}C NMR spectra), respectively. Separation of *E* and *Z* isomers was carried out with a Perkin-Elmer F 21 preparative gas chromatograph using 0.5 cm^2×600 cm columns filled with 8 % Antarox CO-990 (for 2-chlorovinyl acetate) or with 10 % Silicone XE-60 (for other 2-chlorovinyl esters) on Chromosorb W. A Philips 125 W mercury lamp was used to provide UV light in reductions.

Reagents. Zinc powder (G.R., Merck AG) and zinc chloride (G. R., Merck AG) were used as received. The quality and dryness of these reagents seem to be crucial in regard to the yield in the synthesis of 2,2-dichlorovinyl acetate. Tributyltin hydride[7] and tributyltin methoxide[8] were prepared according to procedures described in the literature. 2,2-Dichlorovinyl acetate was prepared according to the method of Euranto *et al.*[4] with a slight modification to increase the original low yield (see below). Acyl halides were commercial products (*purum*, Fluka AG) and they were purified by distillation before use. All reactions involving tributyltin hydride were carried out under dry nitrogen.

2,2-Dichlorovinyl acetate. Chloral (195 g, 1.32 mol), acetyl chloride (133 g, 1.69 mol) and some zinc chloride were refluxed for 25 h. The resulting 1,2,2,2-tetrachloroethyl acetate was distilled at 80 °C/1.9 kPa. Yield 171 g (57 %).

1,2,2,2-Tetrachloroethyl acetate (171 g, 0.76 mol), zinc powder (149 g, 2.28 mol) and diethyl ether (140 ml) were refluxed for 19 h and the ether was evaporated. 2,2-Dichlorovinyl acetate was then collected from the reaction mixture at 0.1–0.2 kPa (low pressure and temperature are necessary to prevent polymerization) and redistilled, b.p. 61 °C/3.2 kPa. Yield 82 g (70 %).

2-Chlorovinyl acetate. Tributyltin hydride (33.25 g, 0.114 mol) was added under stirring during 1 h to 2,2-dichlorovinyl acetate (17.5 g, 0.113 mol) at 0 °C under influence of UV light. Stirring was continued for 0.5 h in room temperature after the addition. A fraction of 12.6 g, which contained (by GLC) 2-chlorovinyl acetate (80 %), 2,2-dichlorovinyl acetate (18 %), and vinyl acetate (2 %), was evaporated from the reaction mixture at 0.1–0.2 kPa. Distillation through a fractionating column gave pure 2-chlorovinyl acetate, b.p. 71–72 °C/13 kPa, 130–132 °C/101 kPa. IR (film), *E* isomer: 1770 cm^{-1} (C=O), *Z* isomer: 1767 cm^{-1} (C=O), 1651 cm^{-1} (C=C). ^{1}H NMR (CCl_4), *E* isomer: δ 2.10 (3H, s), 6.06 (1H, d, *J* 11.6 Hz), 7.42 (1H, d, *J* 11.6 Hz), *Z* isomer: δ 2.21 (3H, s), 5.52 (1H, d, *J* 4.8 Hz), 7.39 (1H, d, *J* 4.8 Hz). ^{13}C NMR ($CDCl_3$), *E* isomer: δ 20.5 (CH_3), 167.1 (C=O), 138.5 (CH), 108.4 (CHCl), *Z* isomer: δ 20.5 (CH_3), 167.1 (C=O), 135.3 (CH), 103.2 (CHCl).

When AIBN (2,2'-azobis(2-methylpropionitrile)) was used in one of the experiments as a reaction promoter instead of UV light, an uncontrolled, vigorous reaction took place. This was probably caused by the fast polymerization of the starting ester.

2-Chlorovinyl dichloroacetate. Tributyltin methoxide (27.8 g, 86.5 mmol) and 2-chlorovinyl acetate (10.0 g, 83.0 mmol) were mixed and heated at 60 °C for 20 min. Methyl acetate was then evaporated off at 3.0 kPa. Dichloroacetyl chloride (12.75 g, 86.5 mmol) was added slowly to the resulting tributyl-2-chlorovinyloxytin. Distillation of the reaction mixture gave 7.2 g (44 %) pure 2-chlorovinyl dichloroacetate, b.p. 83–86 °C/2.1 kPa. IR (film), *E* isomer: 1767 cm^{-1} (C=O), *Z* isomer: 1783 cm^{-1} (C=O), 1652 cm^{-1} (C=C).

^{1}H NMR, *E* isomer ($CDCl_3$): δ 5.96 (1H, s), 6.33 (1H, d, *J* 11.4 Hz), 7.41 (1H, d, *J* 11.4 Hz), *Z* isomer (CCl_4): δ 6.10 (1H, s), 5.82 (1H, d, *J* 4.2 Hz), 7.41 (1H, d, *J* 4.2 Hz).

^{13}C NMR ($CDCl_3$), *E* isomer: δ 63.3 ($CHCl_2$), 160.8 (C=O), 137.9 (CH), 111.6 (CHCl), *Z* isomer: δ 63.4 ($CHCl_2$), 161.0 (C=O), 135.1

(CH), 107.1 (CHCl).

2-Chlorovinyl chloroacetate. Prepared as in the case of dichloroacetate. Yield 42 %, b.p. 80 °C/ 2.0 kPa. Melting point of the *E* isomer was found to be 31 °C. IR (film), *E* isomer: 1777 cm^{-1} (C=O), *Z* isomer: 1782 cm^{-1} (C=O), 1651 cm^{-1} (C=C). ^1H NMR ($CDCl_3$), *E* isomer: δ 4.13 (2H, s), 6.23 (1H, d, *J* 11.2 Hz), 7.44 (1H, d, *J* 11.2 Hz), *Z* isomer: δ 4.24 (2H, s), 5.72, d, *J* 4.4 Hz), 7.43 (1H, d, *J* 4.4 Hz). ^{13}C NMR ($CDCl_3$), *E* isomer: δ 40.1 (CH_2Cl), 163.6 (C=O), 138.0 (CH), 110.2 (CHCl), *Z* isomer: δ 40.3 (CH_2Cl), 163.9 (C=O), 135.1 (CH), 105.3 (CHCl).

2-Chlorovinyl trichloroacetate. Prepared as in the case of dichloroacetate. Yield 41 %, b.p. 63–65 °C/0.55 kPa. IR (film), *Z* isomer: 1783 cm^{-1} (C=O), 1651 cm^{-1} (C=C). ^1H NMR (CCl_4), *E* isomer: δ 6.46 (1H, d, *J* 11.8 Hz), 7.47 (1H, d, *J* 11.8 Hz), *Z* isomer: δ 5.88 (1H, d, *J* 4.4 Hz), 7.42 (1H, d, *J* 4.4 Hz). ^{13}C NMR ($CDCl_3$), *E* isomer: δ 88.6 (CCl_3), 158.4 (C=O), 138.3 (CH), 112.4 (CHCl), *Z* isomer: δ 88.6 (CCl_3), 158.5 (C=O), 135.7 (CH), 108.1 (CHCl).

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Crystal Structures of *N,N,N',N'*-Bis(tetramethylene)thiuram Disulfide and Tetramethylthiuram Disulfide

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Both compounds crystallize with four molecules in a monoclinic unit cell with dimensions *1*: $[S_2CN(CH_2)_4]_2$, a=9.432(1) Å, b=10.432(1) Å, c=13.851(1) Å, β=97.08(1)°, V=1353(1) Å^3, *2*: $[S_2CN(CH_3)_2]_2$, a=9.672(1) Å, b=9.931(2) Å, c=11.882(8) Å, β=99.43(3)°, V=1120(5) Å^3. The space groups are *1*: *A2/a*, *2*: *C2/c*. Both structures were determined with direct methods from 1081(1) and 883(2) reflections, collected with a four-circle X-ray single-crystal diffractometer at room temperature. The structures were refined by the method of least-squares rendering a final R=0.041 for both. The structures are similar and consist of van der Waals packed $(S_2CNR_2)_2$-units. The geometry of the S_2CNC_2-units in thiuram disulfides and ionic dithiocarbamates is compared. The only significant differences are found in the CS_2-moiety.

This investigation was made in connection with a project where the geometry of substituted dithiocarbamate ions, $^-S_2CNR_2$ [R_2=$(CH_2)_4$, $(CH_3)_2$, $(C_2H_5)_2$, $(CH(CH_3)_2)_2$] is studied in ionic environments.[1–4] Recrystallization of $NaS_2CN(CH_3)_2 \cdot 2H_2O$ in aqueous–ethanolic solutions resulted in the formation of an unknown phase, which was analyzed by X-ray crystallography. For organic compounds this method provides a rapid, definite and fairly cheap analysis.[5] The compound proved to be tetramethylthiuram disulfide, the X-ray structure of which was reported already in 1965.[6] That study is, however, of low accuracy since it is based on two-dimensional film data. It is interesting to compare the geometry of thiuram disulfides and dithiocarbamates. In thiuram disulfides the $>NCS_2$-group is covalently bonded to another S-atom, while in alkali–metal dithiocarbamates it is involved in a hydrogen bond system as well as ionic metal coordination. Besides $[S_2CN(CH_3)_2]_2$ (denoted *2* below) only one crystal structure determination (for compounds of the type $[S_2CNR_2]_2$) has been reported, $[S_2CN(C_2H_5)_2]_2$.[7] Therefore the crystal structure of $[S_2CH(CN_2)_4]_2$ (denoted 1) was also determined. Compounds *1* and *2* are illustrated in Fig. 1.

EXPERIMENTAL

It is known that dithiocarbamates may be oxidized to thiuram disulfides in acetonitrile according to eqn. (1).[8]

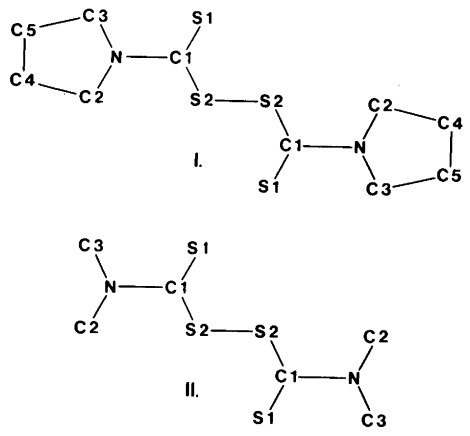

Fig. 1. Atomic numbering of compounds *1* and *2*. Multiple bonds are not included.

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$$2S_2CNR_2^- \rightarrow [S_2CNR_2]_2 + 2e^-, \quad e^\circ = +0.05\ V\ (R_2 = (C_2H_5)_2) \qquad (1)$$

In mixed water–ethanol solutions of $NaS_2CN(CH_3)_2 \cdot 2H_2O$ the reaction (2) occurs.

$$2S_2CN(CH_3)_2^- + H_2O + \tfrac{1}{2}O_2 \rightarrow [S_2CN(CH_3)_2]_2 + 2OH^- \qquad (2)$$

Bubbling air through an aqueous-ethanol solution of $NaS_2CN(CH_2)_4 \cdot 2H_2O$ gave crystals of $[S_2CN(CH_2)_4]_2$ in high yield according to this reaction. For both compounds the crystals are colourless, irregular and hydrophobic.

Data collected on a CAD4 diffractometer revealed the Laue class 2/m and the systematic extinctions *hkl*, $h+k$ odd and *h0l*, *l* odd, for both compounds, consistent with the space group *C2/c*. For 1 the space group *A2/a* was chosen. The different sizes of the molecules are reflected in the length of the *c*-axis. Intensity data for all independent reflections in the interval $3<\theta\leq 70°$ (1, Cu$K\alpha$ radiation) and $3<\theta\leq 30°$ (2, Mo$K\alpha$) were measured on the CAD4 using graphite monochromator. Table 1 gives information concerning the data collection, the data reduction and the least-squares refinements. The values of I and $\sigma_c(I)$ were corrected for Lorentz, polarization and absorption effects ($\sigma_c(I)$ is based on counting statistics).

STRUCTURE DETERMINATIONS AND REFINEMENTS

The non-hydrogen atoms were located with MULTAN[9] and the hydrogen atoms in succeeding difference Fourier calculations. The structures were refined using full-matrix least-squares minimizing $\Sigma w(|F_o|-|F_c|)^2$ with weights $w=[\sigma_c^2/4|F_o|^2+(C_1 \cdot |F_o|)^2+C_2]^{-1}$, so that constant values of $<w(|F_o|-|F_c|)^2>$ were obtained in different F_o and $\sin\theta$-intervals. The presence of extinction effects in the data sets were investigated by including an isotropic extinction parameter[10] in the calculations. Ten (1) and two (2) reflections had extinction corrections larger than 10 %, with

Table 1. Crystal data, collection and reduction of intensity data and least-squares refinements.

	$[S_2CN(CH_2)_4]_2$	$[S_2CN(CH_3]_2]_2$
Crystal size (mm)	0.21×0.19×0.19	0.50×0.20×0.30
Space group	*A2/a*	*C2/c*
a (Å)	9.432(1)	9.672(1)
b (Å)	10.432(1)	9.931(2)
c (Å)	13.851(1)	11.882(8)
β (°)	97.08(1)	99.43(3)
V (Å^3)	1353(1)	1120(5)
D_x(Mg m^{-3})	1.436	1.426
Wavelength (Å)	1.5418 (Cu$K\alpha$)	0.71069 (Mo$K\alpha$)
θ-interval (°)	3–70	3–30
ω-2θ scan width (°)	0.80+0.5tanθ	0.80+0.6tanθ
Maximum recording time(s)	120	180
$\sigma_c(I)/I$ requested in a scan	0.03	0.03
μ (mm^{-1})	5.99	0.77
Range of transmission factors	0.35–0.49	0.74–0.84
Total number of reflections	1449	1786
Number of reflections with zero weight ($I<3\sigma$)	368	868
Number of reflections in final LS-cycle ($I\geq 3\sigma$), *m*	1081	883
Number of parameters refined, *n*	106	80
$R=\Sigma\|\Delta F\|/\Sigma\|F_o\|$	0.041	0.041
$R_w=[\Sigma w(\Delta F)^2/\Sigma w(F_o)^2]^{1/2}$	0.061	0.053
$S=[\Sigma w(\Delta F)^2/(m-n)]^{1/2}$	1.43	0.907
C(1) (weighting function)	0.03	0.05
C(2) (weighting function)	0.25	0.10
g·10^{-4} (extinction)	0.63(8)	0.29(8)

Table 2. Positional and isotropic thermal parameters with e.s.d.s for (a): $[S_2CN(CH_2)_4]_2$ and (b): $[S_2CN(CH_3]_2$ · $B_{eq}=\frac{4}{3}\Sigma\Sigma\beta_{ij}a_i \cdot a_j$ is given for the non-H atoms. Positional parameters for non-hydrogen atoms have been multiplied by 10^4 and for hydrogen atoms by 10^3.

(a)				
S(1)	6155(1)	2267(1)	1033(1)	4.3(0)
S(2)	8197(1)	130(1)	607(1)	4.3(0)
N	8165(3)	1212(2)	2289(2)	3.4(1)
C(1)	7475(3)	1280(3)	1392(2)	3.1(1)
C(2)	9381(4)	357(3)	2610(2)	4.2(1)
C(3)	7771(4)	2001(4)	3095(2)	4.5(1)
C(4)	9909(4)	822(5)	3623(3)	5.4(1)
C(5)	8605(5)	1343(7)	3980(3)	6.0(1)
H1C(2)	593(4)	446(4)	240(3)	4.7(8)
H2C(2)	491(5)	543(5)	285(4)	6.9(11)
H1C(3)	666(5)	200(4)	308(3)	5.8(9)
H2C(3)	797(5)	288(5)	300(4)	6.6(12)
H1C(4)	462(6)	513(6)	101(4)	8.4(14)
H2C(4)	433(6)	665(6)	140(4)	8.9(14)
H1C(5)	887(4)	214(4)	402(3)	3.7(8)
H2C(5)	809(6)	60(7)	424(5)	10.0(17)
(b)				
S(1)	3047(1)	3916(1)	2968(1)	4.5(0)
S(2)	5287(1)	1738(1)	3356(1)	5.4(0)
C(1)	4182(3)	3007(3)	3845(2)	3.6(1)
N	4394(3)	3106(3)	4985(2)	4.4(1)
C(2)	5409(5)	2311(6)	5750(4)	6.7(1)
C(3)	3618(5)	4057(5)	5566(3)	5.7(1)
HC(2)	528(4)	136(4)	566(3)	8.3(9)
HC(2)	579(4)	280(3)	643(4)	9.8(8)
HC(2)	619(4)	232(4)	552(3)	8.7(9)
HC(3)	331(4)	348(4)	613(4)	8.6(8)
HC(3)	442(4)	460(4)	622(3)	10.0(7)
HC(3)	329(4)	470(4)	519(3)	8.5(9)

maximum corrections of 41 % (1) for $22\bar{2}$ and 14 % (2) for $22\bar{1}$. Atomic scattering factors with corrections for anomalous dispersion were taken from *International Tables for X-Ray Crystallography.*[11] δR-plots comparing observed and calculated structure amplitudes[12] resulted in approximately straight lines with slopes 1.295(5) (1) and 0.952(5) (2), intercepts 0.105(5) (1) and 0.071(5) (2) and correlation coefficients 0.992 (1) and 0.990 (2).

DISCUSSION

Final positional and isotropic thermal parameters are given in Table 2* and selected distances and angles together with those for $[S_2CN(C_2H_5)_2]$ [7] in Table 3. The structures are depicted in Fig. 2. Both structures consists of van der Waals packed $[S_2CNR_2]_2$ molecules. The packing is similar in the two compounds, but in 1 there is a non-parallel arrangement of the organic ends compared to 2 where they are packed in a parallel manner. The $[S_2CNR_2]_2$ molecules are located with the S–S bonds across 2-fold axes parallel to *b*. Some short van der Waals distances are 1: C(1)···H2C(4)=2.78(6), H2C(5)···H2C (5′)=2.50(13) Å, 2: C(1)···HC(3)=2.74(4), HC(2)···HC(3)=2.96(6) Å. The geometries in relevant parts of the molecules are similar to that observed in $[S_2CN(C_2H_5)_2]_2$ (Table 3).

The influence from the lone pairs on the sulfur atoms gives torsion angles C(1)–S(2)–S(2′)–

* Lists of structure factors and anisotropic thermal parameters may be obtained from the author.

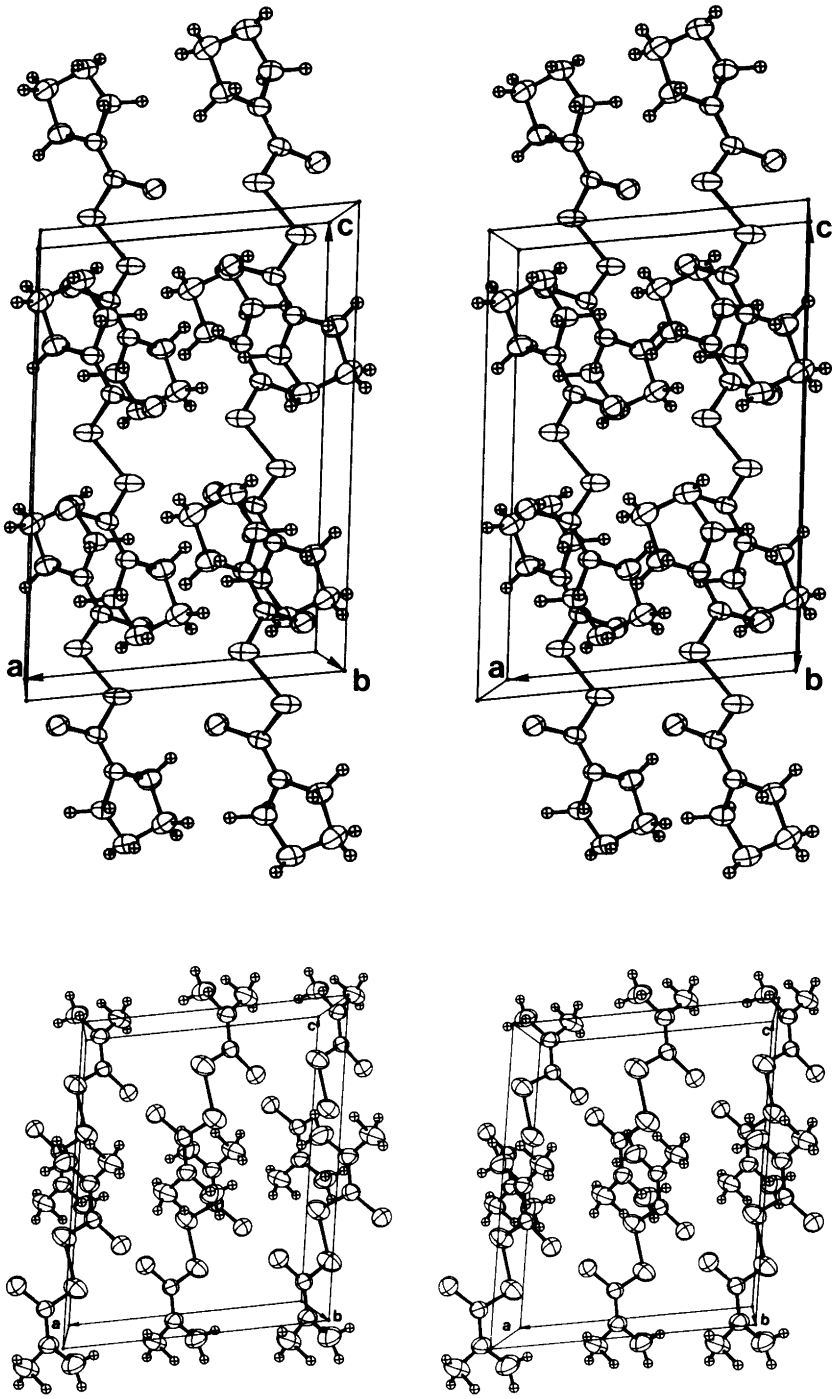

Fig. 2. Stereoscopic views of the unit-cells for compounds *1* (a) and *2* (b).

Table 3. Selected distances (Å) and angles (°) with e.s.d.s.

	$[S_2CN(CH_2)_4]_2$	$[S_2CN(CH_3)_2]_2$	$[S_2CN(C_2H_5)_2]_2$
S(2)–S(2′)	2.003(2)	2.009(2)	1.999(5)
S(1)–C(1)	1.644(3)	1.649(3)	1.634(11)
	–	–	1.662(11)
S(2)–C(1)	1.807(3)	1.807(3)	1.818(13)
	–	–	1.813(14)
C(1)–N	1.332(4)	1.334(4)	1.362(14)
	–	–	1.334(15)
N–C(2)	1.477(4)	1.454(6)	1.482(13)
	–	–	1.492(14)
N–C(3)	1.471(4)	1.447(5)	1.465(16)
	–	–	1.453(18)
C(2)–C(4)	1.510(5)	–	1.515(18)
	–	–	1.530(17)
C(3)–C(5)	1.535(6)	–	1.520(20)
	–	–	1.544(18)
C(4)–C(5)	1.484(6)	–	–
S(2)–S(2′)–C(1)	104.4(1)	104.0(1)	103.4(4)
	–	–	103.6(4)
S(1)–C(1)–S(2)	124.1(2)	123.2(2)	122.2(7)
	–	–	122.0(7)
S(1)–C(1)–N	125.5(2)	124.8(2)	125.6(9)
	–	–	124.6(10)
S(2)–C(1)–N	110.4(2)	112.0(2)	112.1(8)
	–	–	113.4(8)
C(1)–N–C(2)	125.8(3)	124.4(3)	122.6(10)
	–	–	122.0(10)
C(1)–N–C(3)	122.5(3)	121.5(3)	120.2(9)
	–	–	122.0(9)
C(2)–N–C(3)	111.7(2)	114.1(3)	116.9(9)
	–	–	115.9(9)
N–C(2)–C(4)	103.9(3)	–	110.8(9)
	–	–	110.7(9)
N–C(3)–C(5)	101.8(3)	–	110.8(9)
	–	–	109.5(9)
C(2)–C(4)–C(5)	103.6(3)	–	–
C(3)–C(5)–C(4)	105.1(3)	–	–
C(1)–S(2)–S(2′)–C(1′)	93.6(1)	88.2(1)	90.2(6)
S(1)–C(1)–N–C(2)	−177.9(2)	−179.5(3)	−174.6(9)
	–	–	−175.6(9)
S(1)–C(1)–N–C(3)	2.8(4)	0.0(4)	2.7(16)
	–	–	−2.6(16)
S(2)–C(1)–N–C(2)	1.7(4)	0.8(4)	6.5(14)
	–	–	8.3(14)
S(2)–C(1)–N–C(3)	−177.5(2)	−179.7(3)	−176.3(8)
	–	–	−178.7(8)
C(1)–N–C(2)–C(4)	170.6(3)	–	89.4(13)
	–	–	85.9(13)
C(1)–N–C(3)–C(5)	166.7(3)	–	88.5(13)
	–	–	87.8(13)
N–C(2)–C(4)–C(5)	29.4(4)	–	–
N–C(3)–C(5)–C(4)	31.2(4)	–	–
C(2)–C(4)–C(5)–C(3)	− 38.1(4)	–	–

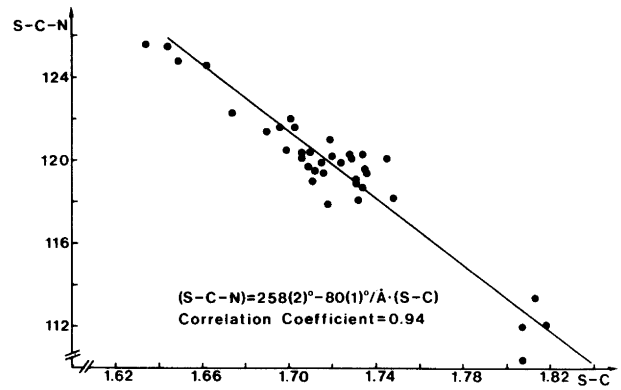

Fig. 3. The angle S–C–N as a function of the distance S–C for 13 ionic dithiocarbamates and 3 thiuram disulfides. The four extreme values above 1.80 Å and below 1.665 Å represent the thiuram disulfides.

C(1′) in the vicinity of 90° as expected. At any other angle there would be overlapping between filled orbitals.[13,14] The S(2)–S(2′) distances are of the same size as in other compounds containing –S–S– bonds *i.e.* ~2.0 Å.[15–17] The rms-deviations from the least-squares planes defined by S(1), S(2), C(1) and N are 5.17 (*1*) and 3.90 Å·10^4 (*2*). The other atoms deviate from these planes by C(2): –56(41), C(3): 119(51) (*2*) and C(2): 417(36), C(3): –540(38), C(4): 2657(43) and C(5): –3731(53) Å·10^4 (*1*). These values indicate that the S_2CNR_2-moiety in *2* is planar while the atoms C(2), C(3), C(4) and C(5) in 1 undergo conformational reorientation similar to the one found for $NaS_2CH(CH_2)_4 \cdot 2H_2O$.[1]

The geometries observed in the thiuram disulfides may be compared to those observed in 13 ionic dithiocarbamates.[1–4, 18–27] In the R_2N-parts of thiuram disulfides and ionic dithiocarbamates with equal R_2-groups no significant differences are observed. The two S–C(1) distances in ionic dithiocarbamates may be different, but their sum is often constant. This indicates different hybridization[28] on the C(1)-atom, which ought to be reflected in the angles around C(1). The angle S–C(1)–N (y) is roughly a linear function of the distance S–C(1) (x) for ionic dithiocarbamates and thiuram disulfides, with y=258(2)–80(1)°/Å·x and correlation coefficient 0.94. The thiuram disulfides represent extreme values (Fig. 3).

From a literature study of 68 S–C distances[29] the mean values 1.610(6) Å for an S=C double bond and 1.818(1) Å for a single bond are found. According to this the weighted mean value for the S(2)–C(1) bond distance [1.807(2) Å] in the thiuram disulfides represents a single bond. Similarly, the weighted mean value for the S(1)–C(1) distance 1.647(3) Å, indicates an appreciable amount of double bonding. From the 13 ionic dithiocarbamates a weighted[29] mean value of 1.722(2) Å for the S–C(1) (*i.e.* [S(1)–C(1)+S(2)–C(1)]/2) distances is found. This value is not significantly different from the value 1.726(2) Å found for the thiuram disulfides. The weighted mean values 1.334(3) Å (thiuram disulfides) and 1.332(3) Å (ionic dithiocarbamates) for the C(1)–N bond distances are not significantly different. Compared to >C=N– double bonds (1.224(10)–1.283(6) Å)[30–35] and ≻C–N≺ bonds [1.447(5)–1.492(14) Å] (Table 3) these values also indicate an appreciable amount of double bonding. The change from ionic coordination to the formation of a covalent –S–S– bond thus has no detectable effects on the C(1)–N bond.

It is concluded that the only significant difference in the comparable parts of these two types of compounds is in the CS_2-moiety. The sum of distances in S_2C–N is equal but the S–C bond order is redistributed. Furthermore, the angles around C(1) are drastically changed upon formation of the –S–S– bond.

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Ion Radicals of Organometallic Compounds. I. The Products and the Mechanism of the Decomposition of Methylbenzene Tricarbonylchromium Cation Radicals

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The electrochemically derived cation radicals of methylbenzene tricarbonylchromium complexes decompose by mechanisms of the ECE_h type to generate the methylbenzenes and carbon monoxide. First order rate constants ranging from 2500 to 0.046 s^{-1} at 298 K with corresponding activation energies of 7.8 to 17.6 kcal/mol were observed for complexes prepared from compounds with varying numbers of methyl groups ranging from benzene to hexamethylbenzene. The corresponding activation entropies ranged from −19 to −5 cal/K mol. The reversible oxidation potentials of the complexes were observed to be dependent upon the number of methyl groups but to a much lesser extent than the parent methylbenzenes. The potentials ranged from 493 to 281 mV *vs.* Ag/Ag^+ in the same order as above. All of the cation radicals are further oxidized to the dications irreversibly at about 1280 mV independent of structure.

There has been interest in the electrochemistry of organometallic compounds for a number of years.[1,2] Recently, modern techniques have been applied to study some of the redox processes in more detail.[3] The importance of the nature of the ligands on both the ease of formation and decomposition of cation radicals of some transition metal carbonyl complexes has been demonstrated.[4]

In general, organometallic compounds are readily oxidized and reduced electrochemically. Some of them, like ferrocene, are converted into stable ion radicals while others form ion radicals of limited stability which decompose to final products. The knowledge of the properties of these species is limited and it would be desirable to have a better understanding of their behaviour in solution. For example, what are the factors that govern the stability of the ion radicals? Are the reactivities related to the thermodynamic stability, *i.e.* the reversible potentials for formation either by oxidation or reduction? By what mechanisms do the ion radicals decompose? Are the kinetics of decomposition amenable to study by voltammetric techniques? What are the magnitudes of the rate constants, activation parameters, and reaction orders for the decomposition reactions? In order to obtain answers to some of these questions we have undertaken a systematic investigation of the formation and reactions of ion radicals of organometallic compounds. In this, the first stage of the investigation, we describe results obtained during the oxidation of tricarbonylchromium complexes of benzene and methyl substituted benzenes.

Some tricarbonylchromium complexes of arenes have been observed to be oxidized reversibly.[5,6] On the other hand, many of the corresponding cation radicals have been found to be highly unstable and form products which disturb electrochemical measurements by electrode filming.[5] It has recently been shown that tin

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complexes of arenetricarbonylchromium compounds can be oxidized to very stable cation radicals.[7]

Up until now, the stability of cation radicals of tricarbonylchromium complexes has only been estimated qualitatively. Thus, the mechanisms of decomposition and the related kinetic parameters have not been evaluated. The cation radicals of a number of *p*-substituted phenyltricarbonylchromium complexes have recently been reported to decompose so rapidly that it was not possible to detect the intermediates by cyclic voltammetry.[8] Our preliminary results indicated that the lifetimes of the cation radicals of a number of methylarenes complexes are sufficiently long in acetonitrile to permit the study of the formations and the decompositions by derivative cyclic voltammetry (DCV).[9]

We applied the DCV method to measure kinetic parameters for the decomposition reactions of the cation radicals of benzene and methylbenzene tricarbonylchromium complexes. In this paper we first report the general aspects of the electro-oxidation of these complexes and then the DCV measurement results. The complexes which were studied are listed below with the number in parentheses indicating the number of methyl groups which will be used later for identification. The compounds studied were tricarbonylchromium complexes of benzene (*0*), toluene (*1*), 1,4-dimethylbenzene (*2*), 1,3,5-trimethylbenzene (*3*), 1,2,4,5-tetramethylbenzene (*4*), pentamethylbenzene (*5*) and hexamethylbenzene (*6*).

EXPERIMENTAL

The solvent, acetonitrile, containing the supporting electrolyte (Bu_4NBF_4, 0.1 M) was passed through a column of activated alumina before use. The concentration of complex used was generally 2.0 mM for DCV and linear sweep voltammetry (LSV) and 10–20 mM for product studies. Highly purified nitrogen was bubbled through the solution before use.

All measurements were made at stationary platinum electrodes and the potentials refer to Ag/Ag^+ (0.01 M in acetonitrile). The DCV peak current ratios and zero current crossing potentials were the average of 5 scans which were processed after an initial scan before which the working electrode was pulsed to +3 V. The pulse was necessary to clean the surface and without it the data were not reproducible. The switching potential during DCV was set at the reversible potential +(300±2) mV. Other details of the instrumentation, cells, electrodes and data handling procedures were similar to those reported earlier.[9,10]

The temperature was controlled to ±0.1 °C from −20 to 40 °C during DCV analysis. Activation parameters were obtained by Arrhenius correlations of rate constants evaluated at 7–8 temperatures for *2*, *3* and *4* and 4–6 temperatures for *0*, *1* and *6*. Errors were estimated by the theory of least squares using standard procedures. Preparative electrolyses were carried out at ambient temperature and LSV analyses were made at 25.0(±0.2) °C.

The gas chromatographic conditions[11] were as follows: column, 10 % SE-30, carrier gas, N_2, temperature control, 80 °C for 8 min, programmed from 80 to 200 °C at 8 °C/min. Electrolysis solutions were injected without any treatment.

During the measurement of the volume of gas evolved, the solution was pre-saturated with carbon monoxide.

For mass spectroscopic detection, the evolved gas was removed using helium as a carrier.

The complexes were prepared from the parent molecules and hexacarbonylchromium by refluxing in 1,2-dimethoxyethane or with the parent molecule as solvent under an atmosphere of nitrogen.[12] The complexes were recrystallized twice from diisopropyl ether.

RESULTS

Assignment of LSV peaks. Typical LSV curves, measured for (*4*), at several scan rates ranging from 0.1 to 100 V/s are shown in Fig. 1. There are four main waves (I–IV) and several peaks following IV. Although the relative shapes of the LSV curves were similar for all complexes, the effect of changing the scan rate (v) varied. In each case v was varied over a factor of 10^3.

At high scan rates the first oxidation wave (I) was reversible and wave III was observed, while at low v wave I was irreversible and waves II and IV were observed. The fewer methyl substituents in the complexes, the higher v was required to be in order to observe reversibility. The disappearance of peak III accompanied by the appearance of peaks II and IV is a convenient measure of the reversibility. Waves II–IV were irreversible at all scan rates.

The peak current at I, normalized by dividing by $v^{1/2}$, increased in the low scan rate region to

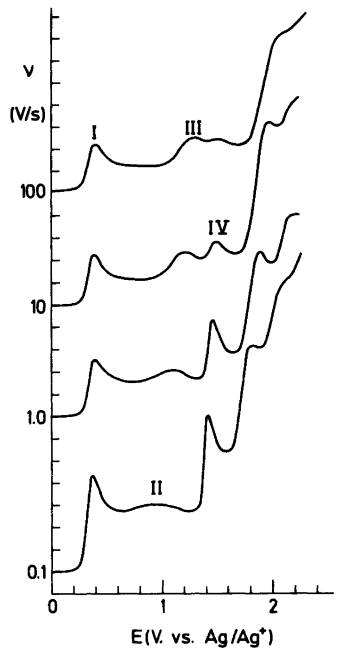

Fig. 1. Linear sweep voltammograms for the oxidation of 1,2,4,5-tetramethylbenzene tricarbonylchromium (2.0 mM) in acetonitrile containing Bu_4NBF_4 (0.1 M) at 25 °C.

about twice the diffusion current in the high *v* region during oxidation of (*2*)–(*5*). The latter indicates that I involves the transfer of 2 e^- under conditions where the wave is irreversible.[13] For complexes *0* and *1*, the scan rate necessary to observe the diffusion current was too high. Conversely, for (*6*), the current didn't reach twice the diffusion current even at the lowest sweep rate practical to make the measurement. The diffusion current was 11.2(±0.3) μA/ $(V/s)^{1/2}$ for *3*–*6* at a concentration of 2.0 mM. Under the same conditions, that for ferrocene was 13.4 μA (1 V/s) which is a reversible one-electron oxidation wave. From the above results we conclude that oxidation wave I involves two one-electron transfers with a chemical reaction occurring before the second electron transfer, *i.e.* the ECE_h mechanism (the subscript h indicates that the second electron transfer takes place in homogeneous solution rather than at the electrode).

The oxidation waves (II) were broad with peak currents and peak potentials nearly the same for all of the complexes. The broad peak (II) was observed to shift from about 910 mV at 0.1 V/s to about 1250 mV at 100 V/s. This wave is most likely due to the oxidation of a substance which is produced at the ECE_h wave (I), since it is not observed under conditions where wave I was reversible.

The peak current for wave III increased with increasing reversibility of I. This indicates that III is due to the oxidation of the primary intermediate at I, *i.e.* the cation radical. The peak potential separation between waves I and III, 800–940 mV at 100 V/s, is also in accord with what would be expected if peak III involves the oxidation of the cation radical to the dication of the complex.

The peak potentials for III measured at 100 V/s were very nearly the same, 1279(±16) mV, for complexes *2*, *6*. The cation radicals of *0* and *1* were so reactive that it was not possible to measure potentials for wave III. In any event, waves III were irreversible and the peak potentials were observed to shift about 80 mV per decade change in *v*.

On the other hand, the peak potentials of wave IV were observed to be dependent upon the structure of the complexes and were within −70 to 40 mV of the oxidation peaks for the corresponding parent compounds under the same experimental conditions. The current at this peak increased as the reversibility of wave I decreased as would be expected if peak IV is due to a product of the ECE_h reaction at I. We conclude that wave IV is due to the oxidation of the parent molecule produced during the decomposition of the cation radical of the complex. Several peaks following IV can also be assigned to oxidation products of the parent molecules.

Reversible oxidation potentials of wave I. Measurements were carried out at −10 °C in order to reach reversibility at optimum scan rates. The reversible potentials were obtained by measurement of the zero crossing points on the DCV curve.[14] Results for measurements on all of the complexes are shown in column 2 of Table 1. The difference in reversible potential for the oxidation of *6* measured at −10 and 25 °C was 0 ± 1 mV. Under the same experimental conditions a difference of −18.6 mV was observed for the oxidation of ferrocene. No attempt was made to control the reference electrode temperature.

The oxidation potentials measured in this work differed from those in the literature[5] by 251(±24)

mV. The previous values were obtained by polarographic measurements in CH_2Cl_2 and were with a different reference electrode (S.C.E.).[5] There is an approximate linear relationship between the reversible oxidation potentials of the complexes and the irreversible peak potentials for the oxidation of the parent molecules. The slope of the linear regression line was observed to be 0.20 with a correlation coefficient of 0.991. The peak potentials for the oxidation of the parent molecules were measured at 0.1 V/s.

Kinetic parameters for the decomposition of the cation radicals. The cation radicals decompose by an ECE_h mechanism at wave I. The first order rate constants (k) for these reactions were measured by DCV at temperatures ranging typically from −10 to 25 °C and were evaluated according to eqn. (1) derived from theoretical

$$k=1284(v_{1/2}/T) \quad (1)$$

data.[15] In (1) $v_{1/2}$ refers to the sweep rate necessary for the derivative peak ratio to equal 0.500. Activation energies (E_a) were determined directly from $v_{1/2}$ eqn. (2)[16] and activation entropies (ΔS^+) were evaluated using eqn. (3).[17] The results are listed in Table 1.

$$\log(v_{1/2}/T)=-E_a/(4.576\,T)+\text{constant} \quad (2)$$

$$\Delta S^+=4.576\log(k/T)+E_a/T-49.21 \quad (3)$$

The reaction orders in cation radical were determined from the dependence of $v_{1/2}$ on substrate concentration. For a first order reaction in the primary intermediate $v_{1/2}$ is expected to be a constant independent of concentration.[18] With concentrations ranging from 0.5 to 2.0 mM the reaction orders were observed to range from 0.8–1.0 indicating some deviation from theory.

Double potential step chronoamperometry (DPSC) was also applied for the measurement of the kinetic parameters for the reactions of (*2*). The DPSC measurements gave; $E_a=10.3(\pm0.1)$ kcal/mol, $\Delta S^+=-15.2(\pm0.4)$ cal/K mol, and $k_{298}=240(\pm4)$ s^{-1}. The values of the parameters obtained by DCV and DPSC agreed within experimental error with the exception that k_{298}, evaluated from the Arrhenius plots, differed by slightly more than expected. The value of $\tau_{1/2}$, the pulse width which corresponds to $v_{1/2}$ was only 1.14 ms at 298 K and this is near the limit for DPSC under the conditions of the measurements. The good agreement between the DCV and DPSC results indicates that the results in Table 1 are reliable.

Products of decomposition of the cation radicals. It has already been indicated that the parent molecules were identified as the principal products of the decomposition by the LSV analyses. This was also confirmed by gas chromatographic analysis of the solution in which complex *2* was oxidized at 400 mV, which corresponds to peak I. Coulometric results using gas chromatography to analyze for the formation of the parent substance indicate that 1.2–1.9 Faradays were consumed for the formation of 1 mol of product. Only peaks due to the complex *2*, 1,4-dimethylbenzene, and acetonitrile were observed. A peak due to hexacarbonylchromium, which was used in the

Table 1. Oxidation potentials for tricarbonylchromium complexes and kinetic parameters for the decomposition of the cation radicals.[a]

Complex[b]	E_{rev}[c]	k_{298}[d]/s^{-1}	E_a/kcal mol^{-1}	$-\Delta S^+$/cal $K^{-1}mol^{-1}$
(*0*)	493	2500(400)	7.8(0.5)	19(3)
(*1*)	441	1280(140)	8.7(0.6)	17(3)
(*2*)	403	212(4)	10.2(0.2)	15.8(0.6)
(*3*)	402	17.2(1.1)	14.1(0.5)	7.6(1.7)
(*4*)	342	6.5(0.3)	14.4(0.6)	9.6(2.0)
(*5*)	317	0.46(0.04)	16.0(1.5)	5(5)
(*6*)	281	0.046(0.019)	17.6(6.3)	5(20)

[a] In acetonitrile containing Bu_4NBF_4 (0.1 M). [b] See text for the structures. [c] The reversible peak potential *vs.* Ag/Ag^+ of wave I. [d] All errors are 95 % confidence calculated according to the theory of least squares as described in Ref. 16. The rate constants were calculated from the Arrhenius correlations.

preparation of the complex, was not observed.

Gas evolution from the anode was observed during the electrolysis of *2* at 400 mV. The gas was confirmed to be carbon monoxide by mass spectroscopy. The coulometric *n* value based on the analysis assuming the formation of 3 CO/mol was 2.6 Faradays/mol, which was associated with a high degree of error due to the small quantity of gas evolved.

Tests for the influence of other substances on the decomposition reactions. the reactions of ion radicals are often influenced by the presence of other reactants in solution, often as impurities in the solvent–electrolyte. In order to test for the influence of other reactants, kinetic experiments were carried out in the presence of water, parent substance, and carbon monoxide.

Only minor changes in rate constant for the decomposition of the cation radical of *5* were observed on changing the water concentration from nearly zero, in the presence of neutral alumina, to 200 mM. Over this range of water concentration *k* decreased by about 10 %.

In order to test for reversibility of the overall decomposition reaction, measurements were made in the presence of 1,4-dimethylbenzene and carbon monoxide during the oxidation of *2*. No change was observed in *k* with 1,4-dimethylbenzene concentrations up to about 50 mM. At higher concentrations the DCV data were not reproducible. No effect on the kinetics were observed either at −10 or 25 °C when the kinetics were carried out on solutions saturated with carbon monoxide. The experiment at −10 °C was carried out since the concentration of carbon monoxide should be higher at lower temperatures. Likewise, 1,4-dimethylbenzene (50 mM) had no effect in the CO saturated solutions.

From these experiments we conclude that the decompositions are not effected by water, present in concentrations up to 200 mM, and that the decomposition is irreversible.

DISCUSSION

Under conditions where wave I is reversible, either at high sweep rates or with the highly substituted complexes at lower sweep rates as well, the reactions can be described by eqns. (4)–(6) (Scheme 1) where M is the parent molecule.

$$MCr(CO)_3 - e^- \rightleftharpoons MCr(CO)_3^+ \quad \text{wave I} \quad (4)$$
$$MCr(CO)_3^+ - e^- \rightleftharpoons MCr(CO)_3^{2+} \quad \text{wave III} \quad (5)$$
$$MCr(CO)_3^{2+} \longrightarrow \text{products} \quad (6)$$

Scheme 1.

The products of reaction (6) are not known but the parent molecule could not be detected during LSV under conditions where the cation radicals are stable. It is possible that the decomposition of the dication gives an oxidation product of the parent molecule. Further work on the reactions of the dications are necessary.

At low sweep rates, under conditions where wave I is irreversible, the cation radicals decompose into carbon monoxide, the parent molecule and unidentified chromium compounds. The kinetic studies are indicative of an ECE_h mechanism and the product studies along with the LSV results indicate that the cation radicals completely decompose to parent molecule at wave I. The latter is evident from the voltammogram measured at 0.1 V/s (Fig. 1) where the peak heights of waves I and IV, both involving the transfer of $2e^-$, are very nearly identical. The peak height of wave IV was about 80 % of that observed for the parent substance at the same concentration as the complex. On the other hand, the gas evolution studies indicate that less than 3 CO per cation radical are evolved at wave I. This suggests that the chromium containing species after decomposition of the cation radical still contails CO. The ECE_h reaction scheme (Scheme 2) accounts for all of the results.

$$MCr(CO)_3 - e^- \rightleftharpoons MCr(CO)_3^+ \quad E \quad (7)$$
$$MCr(CO)_3^+ \longrightarrow M + Cr(CO)_n^+ + (3\text{-}n)CO \quad C \quad (8)$$
$$Cr(CO)_n^+ + MCr(CO)_3^+ \rightleftharpoons Cr(CO)_m^{2+} + MCr(CO)_3 + (n\text{-}m)CO \quad E_h \quad (9)$$

Scheme 2. ECE_h reaction scheme.

There is no evidence for the nature of the chromium containing cations written as $Cr(CO)_n^+$ and $Cr(CO)_m^{2+}$ but it is likely that they exist, probably with BF_4^- and CH_3CN as ligands. The irreversible LSV wave II is most likely due to oxidation of the chromium containing product.

The cation radicals of some arenetricarbonylchromium complexes have been generated by treatment with strong acids. The tricarbonyl chromium complex of benzyl alcohol is converted to the cation of benzyl tricarbonylchromium, by sulfuric or perchloric acid and decomposes with the release of carbon monoxide.[19,20] The electronic structure of this cation differs substantially from the cation radicals that we have studied. The positive charge is localized on the odd alternant hydrocarbon (benzyl) ligand while the cation radicals that we have been concerned with have the charge distributed mainly on the chromium atom. Since the benzyl cation complex also releases CO it is likely that a similar decomposition mechanism is involved in this case as well.

It is interesting to note that the reversible oxidation potentials of the complexes shift about 40 mV to less positive potentials for each additional methyl group. This potential shift is only about 20 % that observed for the LSV peak potentials of the parent hydrocarbons. This would seem to indicate that the highest occupied molecular orbital from which the electron is lost during oxidation is mainly associated with the chromium atom even though the orbital arises from an interaction between a chromium atom orbital with a ligand orbital. Since the reversible potentials for the oxidation of the complexes correlate well with the peak potentials for the oxidation of the parent hydrocarbons, it is most likely that the substituent effect is due to electron donation by the methyl groups. The attenuated effect then is due to the fact that the inductive donation of electrons must be transmitted to the chromium atoms.

Lloyd and co-workers[5] observed that the potential for the oxidation of the cation radicals depends more strongly on the number of methyl substituents than does the potential for the oxidation of the substrates. They report oxidation potentials ranging from 1300 to 1110 mV vs. S.C.E. for wave III. However, we cannot substantiate these observations. We find that the potential of wave III is very nearly the same for all of the complexes. Since there are three peaks (II, III and IV) after the first oxidation peak (I), one or more of these waves may have merged due to electrode filming during the longer measurement times involved in rotating disc electrode studies.[5] This then could have resulted in confunsion as to the location of the wave for the oxidation of the cation radicals.

Since there is considerable error associated with the activation entropies, we do not attempt to give a detailed treatment of the effect of structure on ΔS^+. On the other hand, there are definite trends in the activation parameters. The magnitude of E_a increases with increasing substitution while ΔS^+ becomes increasingly less negative. It is most likely that the origin of the entropy effects lies in solvation differences in the reactants and transition states.

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The Effect of Tetraalkylammonium Ions on the Heterogeneous Charge Transfer Kinetics for the Reduction of Benzonitrile and Dinitromesitylene in DMF

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The kinetics of the heterogeneous charge transfer during the reduction of benzonitrile in DMF containing different tetraalkylammonium ions have been studied at mercury electrodes. The simple Frumkin double layer correction, which assumes that electron transfer takes place at the outer Helmholtz plane, could not be applied to the data. Only the smallest tetraalkylammonium cations adsorb specifically to the electrode. For tetraalkylammonium ions containing one or more methyl groups specific adsorption was observed at negative potentials. Activation energies ranging from 20 to 40 kJ/mol were observed for the charge transfer processes on going from large to small electrolyte cations. It was shown that the simple Frumkin correction does apply to processes taking place near the potential of zero charge, for example during the reduction of dinitromesitylene in DMF. A model is discussed in which the site of charge transfer is not necessarily the outer Helmholtz plane and is different for different processes.

A number of heterogeneous charge transfer kinetic studies of the reduction of organic compounds at mercury electrodes in aprotic solvents has been carried out in order to test double layer theory on simple outer-sphere electron transfer processes.[1–4] In most cases only a single electrolyte has been used in the studies and under these conditions the theory holds quite well. The simplest version of double layer theory assumes that charge transfer takes place at the outer Helmholtz plane (OHP) and that the effect of the double layer can be accounted for by the so-called Frumkin correction.[5] However, when studying the effect of different electrolytes, deviations from the simple theory are observed. Baranski and Fawcett have shown that it cannot be assumed that the electron transfer takes place at the OHP.[6] They also proposed a model similar to the one dicussed in this paper.

In order to compare the rates of heterogeneous and homogeneous electron transfer reactions, it is necessary to have an adequate understanding of the influence of the double layer. In recent years, attempts have been made to explain discrepancies between experimental and theoretical results. Some deviations can be accounted for by considering the effect of ion-pairing, specific adsorptions and of the negatively charged electrode.

A phenomenon which has not been adequately investigated is the effect of the size of tetraalkylammonium ions on the rate of reduction of organic compounds. Several years ago it was shown that the heterogeneous rate constants for the reduction of a number of organic compounds is increased dramatically as the size of the cation is decreased.[7,8] This effect has been attributed to changes in the double layer with the different cations[9] but this has not really been demonstrated. From a practical viewpoint, this phenomenon can be of great importance. For example, it has been shown that electrode kinetic studies of the reactions of some anion radicals of

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diazo compounds can be carried out using Me_4N^+ as the electrolyte cation while such studies are not possible when the cation is Bu_4N^+ due to very slow electron transfer.[10,11]

This investigation was carried out to gain more detailed information on the effect of tetraalkylammonium ions on the rate of heterogeneous charge transfer during the reduction of some organic compounds.

EXPERIMENTAL

The instrumentation, cells, electrodes, reference electrodes, and solvent purification procedures were identical to those used previously.[12] The unsymmetrical electrolytes, Bu_3MeNI, Bu_2Me_2NI and $BuMe_3NI$ were prepared according to standard procedures. The benzonitrile was reagent grade and used without further purification. Dinitromesitylene was prepared by nitration of mesitylene.

Determination of double layer capacitances. The double layer capacitances (C_{d1}) were determined by *A.C.* voltammetry.[13] The method involves measuring the in phase i_1 and out-of-phase i_2 components of the current and C_{d1} is then calculated from eqn. (1)

$$C_{d1}=(i_1^2+i_2^2)/\omega A V i_2 \qquad (1)$$

where ω is the angular frequency, A is the electrode area, and V is the amplitude of the sine wave. A 10 mV peak-to-peak amplitude and a frequency of 1000 Hz were used. The potential of zero charge for a mercury electrode is –0.21 V *vs.* S.C.E.[14]

Determination of the heterogeneous charge transfer coefficient. The transfer coefficient (α) was determined by *A.C.* measurements. Reversible potentials were determined by the position of the maximum of the fundamental total magnitude signal for frequencies ranging from 10 to 1000 Hz. The phase angle maxima were determined with frequencies ranging from 1000 to 5000 Hz. The transfer coefficient was calculated using eqn. (2) where $(E_{dc})_{max}$ is the potential at the maximum phase angle. The error in the determination of α was less than ± 0.01.

$$(E_{dc})_{max}=E_{rev}+(RT/nF)\ln(\alpha/(1-\alpha)) \qquad (2)$$

The determination of the diffusion coefficient. At low frequencies the electron transfers of interest in this study are diffusion controlled and under these conditions the magnitude of the fundamental current (I) is proportional to the square root of the diffusion coefficient, $I(\omega)=f(D^{1/2})$. The value reported for benzonitrile[4] was taken as a standard in order to determine other values.

The determination of the electrode area. The electrode area (A) was determined by linear sweep voltammetry. At low sweep rates, where the electron transfer can be considered to be reversible, the peak current is proportional to the A. Measurements were carried out on the reduction of bensonitrile.

The determination of heterogeneous rate constants. The heterogeneous rate constants (k_s) were determined by derivative cyclic voltammetry.[14] The method has been described in detail.

RESULTS AND DISCUSSION

The differential capacitance at the mercury –*N,N*-dimethylformamide (DMF) interface has been studied by a number of researchers. Recent studies by Fawcett, Ikeda and Sellan[15] employed several tetraalkylammonium ions and led to the conclusion that ionic specific adsorption is negligible.

In order to test for an effect of the size of tetraalkylammonium ions on the differential capacitance, we systematically varied the supporting electrolyte cation from Me_4N^+ to Hx_4N^+

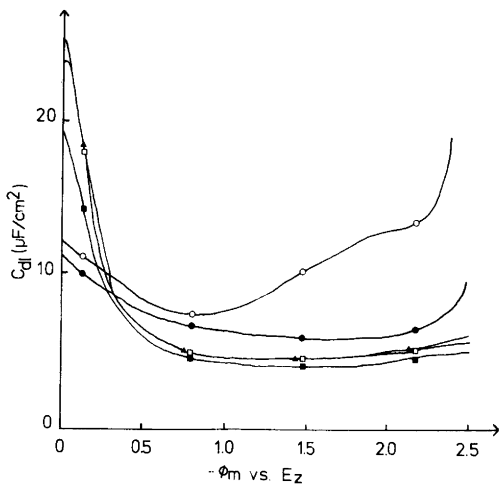

Fig. 1. The double layer capacitance as a function of the potential for some tetraalkylammonium ions. ○, 0.1 M Me_4NBF_4; □, 0.1 M Pr_4NBF_4; ●, 0.1 M Et_4NBF_4; ■, 0.1 M Bu_4NBF_4; ▲, 0.1 M Hx_4NClO_4.

(tetrahexylammonium ion). The results are illustrated in Fig. 1. With the exception where Me_4NBF_4 was the supporting electrolyte, the differential capacitance was relatively constant at potentials ranging from about –0.5 to –2.5 V *vs.* E_z, the potential of zero charge. The differential capacity does depend upon the size of the R_4N^+ and increases as the cation decreases in size.

With all of the symmetrical R_4N^+ except Me_4N^+, the differential capacitance was observed to be independent of cation concentration. This indicates that there is no specific adsorption of cations in these cases in the potential region studied. However, the situation is quite different with Me_4N^+ in which case the differential capacitance increased at negative potentials and was observed to be dependent upon the cation concentration (Fig. 2). The minima observed at E_z at the lowest concentrations indicate that specific adsorption at E_z is negligible in these cases.

Differential capacitance data using some unsymmetrical cations, with varying number of methyl and butyl groups, are shown along with that for Me_4N^+ and Bu_4N^+ in Fig. 3. The capacitance with the unsymmetrical cations increases at negative potentials as it does with Me_4N^+, indicating specific adsorption. The degree of specific adsorption is dependent upon the

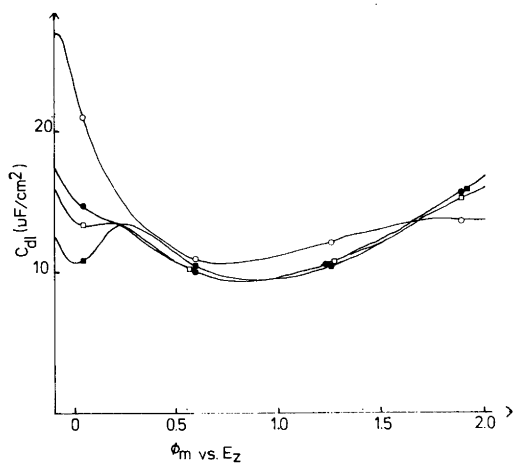

Fig. 2. The double layer capacitance as a function of the potential for different concentrations of Me_4NBF_4.○, 0.1 M Me_4NBF_4; ●, 0.05 M Me_4NBF_4; □, 0.025 M Me_4NBF_4;■, 0.01 M Me_4NBF_4.

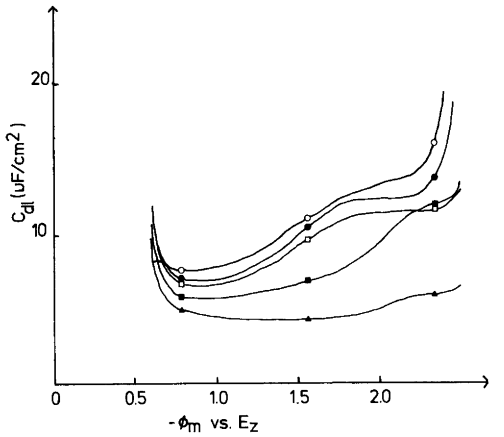

Fig. 3. The double layer capacitance as a function of the potential for some unsymmetrical alkylammonium ions.○, 0.1 M Me_4NBF_4; ●, 0.1 M Me_3BuNI: □, 0.1 M Me_2Bu_2NI;■, 0.1 M $MeBu_3NI$; ▲, 0.1 M Bu_4NI.

number of methyl groups in the cation and, at any given potential, increases with increasing number of methyl groups. The potential range studied was limited to−0.5 to −2.3 V *vs.* E_z since the iodide counter ion adsorbs at more positive potentials.

The results discussed in the preceding paragraphs suggest that it is possible to vary the double layer properties over a wide range by the variation of the nature of the tetraalkylammonium ion of the supporting electrolyte. Thus, heterogeneous kinetic studies in which significant variations in the cations are made should provide a much more stringent test of the applicability of double layer theories than those in which either a single or a limited number of cations are employed. We chose to study the kinetics of heterogeneous charge transfer to two substrates, benzonitrile and dinitromesitylene. The reason for these choices is that benzonitrile (BN) is reduced at a potential well removed from E_z where substantial double layer corrections are necessary and dinitromesitylene reduces near the E_z where double layer corrections, in the absence of specific adsorption, are small.

The most commonly used double layer correction is given by the Frumkin equation, (3), where ϕ_r is the potential of the

$$k_s^{app} = k_s^{corr} \exp[(\alpha n - z)(\phi_r F/RT)] \quad (3)$$

Table 1. The effect of electrolyte cation on the heterogeneous rate constant for the reduction of benzonitrile.

Electrolyte	$-\phi_2$/ mV	k_s^{app}/ cm s^{-1}	k_s^{corr}/ cm s^{-1}	λ^a	$-\phi_r$/ mV
Hx_4N^+	125.0	0.13	1.48	-0.45	181.2
Bu_4N^+	117.6	0.45	4.44	0	117.6
Pr_4N^+	124.8	1.10	13.2	0.42	72.4
Et_4N^+	125.4	1.20	13.8	0.46	67.7
Me_4N^+	142.8	4.5	72.4	1	0

[a] $|\phi_r|=|\phi_2|$ $(1-\lambda)$, λ is chosen so that $k_s^{corr}=4.5$ cm s^{-1}.

pre-electrode site, *i.e.* the potential at that distance from the electrode where the electron transfer takes place. In the simplest case, this potential is assumed to be that at OHP and can be calculated from eqn. (4)

$$\phi_r=2(RT/F)\sinh^{-1}(q_m/8RT\varepsilon C) \quad (4)$$

where q_m is the electrode charge, ε is the dielectric constant and C is the electrolyte concentration.

At the potential where benzonitrile is reduced, the double layer capacitance increases as the cation size decreases. This would make the Frumkin correction term larger for the smaller cations and the apparent heterogeneous rate constant, k_s^{app}, would be expected to decrease. However, the opposite trend is observed experimentally. As shown in Table 1, the corrected rate contrants, k_s^{corr}, increase as the cation size decreases. Discrepancies of the same type were found by Corrigan and Evans[9] during the reduction of 2-methyl-2-nitrobutane and by Kakutani *et. al.*[16] in studying the reduction of some nitrobenzenes. In both cases the effects were attributed to double layer effects.

Table 2. The effect of Bu_4NBF_4 concentration on the heterogeneous rate constant for the reduction of benzonitrile.

[Bu_4NBF_4] (M)	$-\phi_2$/ mV	k_s^{app}/ cm s^{-1}	k_s^{corr}/ cm s^{-1}	λ^a	$-\phi_r$/ mV
0.01	176.0	0.17	5.25	0.04	169.0
0.025	152.8	0.26	5.10	0.04	146.7
0.05	135.1	0.35	4.86	0.03	131.0
0.10	117.6	0.45	4.44	0	117.6

[a] See note [a] in Table 1.

Table 3. The effect of Pr_4NBF_4 concentration on the heterogeneous rate constant for the reduction of benzonitrile.

[Pr_4NBF_4] (M)	$-\phi_2$/ mV	k_s^{app}/ cm s^{-1}	k_s^{corr}/ cm s^{-1}	λ^a	$-\phi_r$/ mV
0.01	183.5	0.74	26.3	0.49	93.6
0.025	160.0	1.12	25.3	0.55	72.0
0.05	142.4	1.16	18.6	0.51	69.8
0.01	124.8	1.16	13.2	0.42	72.4

[a] See note [a] in Table 1.

Table 4. The effect of Et_4NBF_4 concentration on the heterogeneous rate constant for the reduction of benzonitrile.

[Et_4NBF_4] (M)	$-\phi_2$/ mV	k_s^{app}/ cm s^{-1}	k_s^{corr}/ cm s^{-1}	λ^a	$-\phi_r$/ mV
0.01	184.2	1.63	58.8	0.72	51.6
0.025	160.8	1.5	34.4	0.65	56.3
0.05	143.0	1.35	21.9	0.57	61.5
0.10	125.4	1.20	13.8	0.46	67.7

[a] See note [a] in Table 1.

A further test of the simple theory can be made by changing the electrolyte concentration during rate constant determinations. Results of experiments of this type are given in Tables 2, 3 and 4 using Bu_4NBF_4, Pr_4NBF_4 and Et_4NBF_4, respectively. In all cases, the values of k_s^{corr} increase substantially with decreasing electrolyte concentration. The conclusion we arrive at is, that if the data can be accounted for in terms of eqn. (3), the pre-electrode site cannot be at the OHP.

In order to find a model which is capable of explaining all of the results, the double layer can be divided into two distinct regions, according to the Stern treatment.[20] The two regions are the compact layer, encompassing the space from the electrode surface to the OHP, and the diffuse

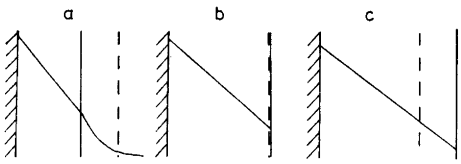

Fig. 4. A schematic picture of the double layer. – – – pre-electrode site; ———, outer Helmholtz plane. a. $1 \leqq \lambda$ and $\lambda \geqq 0$. b. $\lambda = 0$. c. $\lambda\phi_2 \leqq \phi_m$ and $\lambda < 0$.

layer, which is from the OHP out to the end of the diffuse layer.

If we assume that there exists a pre-electrode site at a certain distance from the electrode which is independent of the size of the electrolyte cation, OHP will be situated differently for the different electrolytes. It is commonly assumed that the potential falls linearly to the OHP in the compact layer and asymptotically to zero from the OHP in the diffuse layer.[20] The parameter λ is defined so that $|\phi_r|=|\phi_2|\ (1-\lambda)$ where ϕ_r is the potential at the pre-electrode site. Then for $\lambda \leq 1$ and $\lambda>0$, $\lambda=0$ and $\lambda\phi_m<\phi_2$ and $\lambda<0$ the pre-electrode site will be in the diffuse layer, at the OHP, and in the compact layer, respectively. Fig. 4 shows the potential drop in the double layer for the various cases.

From the capacitance data we can conclude that Me_4N^+ adsorbs specifically to the electrode. If the coverage is large the potential will drop to zero at the inner Helmholtz plane, IHP, and the apparent rate constant should equal the true value without need for correction. If we assume this to be the case, we can evaluate λ in other cases. In Table 1, we note that k_s^{corr}, where the correction was made assuming the pre-electrode site to be the OHP, with Bu_4N^+ as the electrolyte cation is nearly the same as the k_s^{app} with Me_4N^+. The model then calls for the pre-electrode site to be near the OHP when Bu_4N^+ is the supporting electrolyte cation. For the smaller cations the pre-electrode site will be in the diffuse layer while it will be situated in the compact layer for cations larger than Bu_4N^+. The values of λ listed in Table 1 are those that give k_s^{corr} equal to k_s^{app} for the case where the supporting electrolyte cation was Me_4N^+. In Table 1, ϕ_2 is the potential at the OHP and ϕ_r is that evaluated according to the model.

Table 5. The distance to the outer Helmholtz plane for the different electrolytes.

Electrolyte	Radii (Å)	x_2 (Å)
Me_4N^+	3.88	10.38
Et_4N^+	4.28[a]	10.78
Pr_4N^+	4.68	11.18
Bu_4N^+	5.12	11.62
Hx_4N^+	5.93[a]	12.43

[a] The value is taken from the linear plot of radii = f (number of carbon atoms).

The distance, x_2, from the electrode to the OHP can be estimated from the solvated radii of the tetraalkylammonium cations in DMF[17] if we assume that there is one layer of solvent molecules between the electrode and the OHP. In order to estimate the radii for cations for which data are not available, the radii were plotted as a function of the number of carbon atoms in the alkyl groups and a reasonably linear relationship was observed. Using these radii and a radius of 6.5 Å for DMF[16] resulted in the x_2 listed in Table 5. Since we have concluded that x_r, the distance from the electrode to the pre-electrode site, is independent of the electrolyte cation and that with Bu_4N^+ this corresponds to the OHP, x_r according to our model is 11.6 Å. We can estimate ϕ_r, the potential at x_r, for Hx_4N^+ since the potential is assumed to drop linearly to the OHP. In this case, $\phi_2=-125$ mV and $k_s^{app}=0.13$ cm/s (Table 1). A linear potential drop gives $\phi_r=-247$ mV and a corrected rate constant of 15.9 cm/s. This value is clearly too large, but the example does show that a small change in the pre-electrode site makes a large difference in the corrected rate constant.

An attempt was made to test the validity of the relation, $k_s^{app}=k_s^{corr}$ for Me_4N^+. The apparent

Table 6. The effect of the unsymmetrical electrolyte cations on the heterogeneous rate constant for the reduction of benzonitrile.

Electrolyte	k_s^{app}/cm s^{-1}
Bu_4NI	0.45
Bu_3MeNI	5±2
Bu_2Me_2NI	6±2
$BuMe_3NI$	3±2
Me_4NBF_4	4.5

Table 7. Determination of the heterogeneous rate constant for the reduction of benzonitrile as a function of temperature.

0.1M Hx_4N^+		0.1M Bu_4N^+		0.1M Pr_4N^+		0.1M Et_4N^+	
T(K)	$k_s{}^a$	T(K)	$k_s{}^a$	T(K)	$k_s{}^a$	T(K)	$k_s{}^a$
282	0.057	261	0.134	256	0.312	254	0.256
304	0.075	274	0.241	275	0.623	263	0.581
323	0.121	284	0.322	284	0.794	284	2.34
349	0.272	295	0.457	294	1.06	295	4.7
		318	0.790				

[a] Apparent k_s-values in cm s^{-1}.

rate constant for the reduction of benzonitrile in DMF in the presence of Me_4N^+ was determined at several electrolyte concentrations and found to be constant within experimental error. This is a rapid process and considerable error is associated with the measurement of the rate constants, *i.e.* of the order of ±50 %, which makes the independence of k_s^{app} on electrolyte concentration some what tenuous. In order to gain more confidence in this proposal, kinetic experiments were carried out using the unsymmetrical cations as well. The data are summarized in Table 6. In all cases, the error is relatively large but k_s^{app} are equal, within experimental error to the value obtained with Me_4N^+ as the cation.

Heterogeneous kinetic studies were carried out over a range of temperatures using some of the electrolytes. The data are shown in Table 7. The activation energies were evaluated by plotting ln k_s^{app} as a function of $1/T$ (Fig. 5). The plots are reasonably linear in all cases except when the electrolyte was Hx_4NBF_4. The activation parameters for the other electrolytes are given in Table 8. The values of E_a are nearly the same for Bu_4NBF_4 and Pr_4NBF_4 as electrolytes but nearly twice as great for Et_4NBF_4. The expected pre-exponential factor is given by $Z=RT/2\pi M$ where M is the molecular weight of the substrate.[18] This relationship gives $Z=6.8\times10^3$ which is close to that observed for Bu_4N^+ and Pr_4N^+. However, the value when Et_4N^+ was the electrolyte is 5 powers of 10 too great.

Since the heterogeneous charge transfer rate constants cannot be corrected in a simple way, it is expected that the transfer coefficients will also vary with the nature of the electrolyte.[19] How-

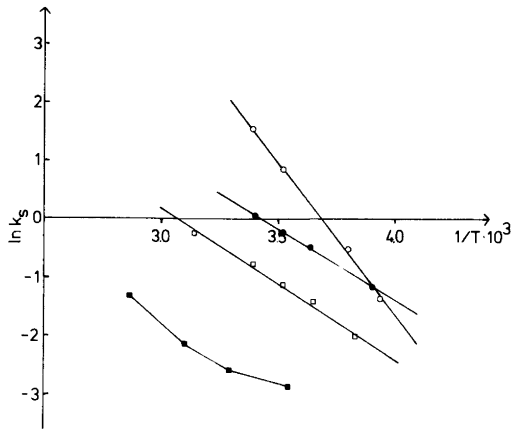

Fig. 5. Arrhenius plots for some tetraalkylammonium ions. ○, Et_4NBF_4; ●, Pr_4NBF_4; □, Bu_4NBF_4; ■, Hx_4NClO_4.

Table 8. The activation energy for the reduction of benzonitrile.

Electrolyte	E_a/kJ mol^{-1}	A
Bu_4NBF_4	21.2	$2.6\cdot10^3$
Pr_4NBF_4	20.1	$4.0\cdot10^3$
Et_4NBF_4	43.6	$2.4\cdot10^8$

Table 9. The reversible potential and the transfer coefficient for the reduction of benzonitrile.

Electrolyte	$-E_{rev}$/mV	α
Me_4NBF_4	2652.7	0.520
Et_4NBF_4	2652.5	0.534
Pr_4NBF_4	2649.4	0.535
Bu_4NBF_4	2650.4	0.530

Table 10. The effect of electrolyte cation on the heterogeneous rate constant for the reduction of dinitromesitylene.

Electrolyte	$-\phi_2$/mV	k_s^{app}/cm s^{-1}	k_s^{corr}/cm s^{-1}	λ^a	$-\phi_r$/mV
Me_4N^+	101.3	0.84	6.0	0	101.3
Et_4N^+	95.6	1.2	7.7	0	95.6
Pr_4N^+	102.9	1.08	8.0	0	102.9
Bu_4N^+	95.4	0.95	6.1	0	95.4
Hx_4N^+	103.0	0.42	3.1	–0.4	61.5

[a] See note [a] in Table 1.

ever, this was not observed to be the case. The transfer coefficients, listed in Table 9, were observed to be very nearly the same for all of the electrolytes. The reversible potential was also observed to be independent of the nature of the supporting electrolyte cation. The latter indicates that ion-pairing between the anion radical and the cations cannot be the source of the discrepancies.

The experimental results for the reduction of benzonitrile are difficult to explain within the realm of any model. The discrepancies could be due to the very negative charge on the electrode as previously discussed by Fawcett *et. al.*[6,19] In order to test for the dependence on the electrode potential, we chose to study the reduction of dinitromesitylene which takes place at -0.9 V *vs.* E_z. In this potential region none of the supporting electrolyte cations studied are specifically adsorbed to the electrode. The results are summarized in Table 10. Within experimental error, the corrected rate constants were observed to be constant with all electrolytes except Hx_4NBF_4. This indicates that the simple Frumkin correction is applicable to the data and that the electron transfer can be assumed to take place at the OHP. With Hx_4NBF_4 as the electrolyte cation there are still discrepancies. The λ value indicates that the pre-electrode site is in the compact layer in this case.

The data from the kinetic experiments on the reduction of dinitromesitylene can be incorporated in the description of the model if we neglect the unexplained results when Hx_4NBF_4 is the electrolyte. Since with the other electrolytes the Frumkin correction appears to be valid for this process we propose that, at potentials not too far removed from E_z, the OHP is the preferred pre-electrode site. At more negative potentials, the pre-electrode site will be located approximately at the same distance, x_r, from the electrode, independent of the size of the electrolyte cation. In the case of Bu_4N^+, x_r corresponds closely to x_2. Charge transfer in the presence of tetraalkylammonium ions smaller than Bu_4N^+ would be retarded at OHP and therefore takes place in the diffuse layer. In the case of cations with greater radii than Bu_4N^+, the potential at the OHP is sufficiently reduced due to the larger value of x_2 that the pre-electrode site can penetrate the OHP and charge transfer takes place in the compact layer. This model predicts that the Frumkin correction will be valid for all tetraalkylammonium ions with effective radii equal to or smaller than Bu_4N^+ up to some value of the electrode potential above which electron transfer is retarded at the OHP for the smaller cations and charge transfer begins to take place in the diffuse layer. The value of x_r then is predicted to be potential dependent and happens to coincide with x_2 during the reduction of benzonitrile. The model also predicts that x_r will always be less than x_2 for cations of greater radii than Bu_4N^+.

Although the model described above accounts for the double layer corrections necessary in the case of the two substrates used in this study, much further work would be necessary to twist the generality of the model.

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Short Communications

Sequence Homology between Tissue Polypeptide Antigen (TPA) and Intermediate Filament (IF) proteins

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Partial sequences of Tissue Polypeptide Antigen (TPA) a protein isolated from human carcinomas or human placenta [1] have been published.[2] Comparisons with other proteins did not show any homology or analogy.

Recently the total structure of desmin laid the basis for a comparison covering epidermal keratins, hard keratins, vimentin and neurofilament proteins, showing these to form a class of filamentous proteins (IF proteins) with similar architecture.[3–5] Homology reaches nearly 80 % in some conservative regions and the secondary structure is based on three helices, Helix Ia, Helix Ib and Helix II, separated by spacers, a 6K headpiece and a tail of varying length and structure. The partial sequences of TPA when aligned with those of the IF proteins show an extensive homology (Figs. 1–3). Between TPA fragment BrCN:B and human epidermal 50K keratin there is 71 % homology (Fig. 1). In this region, which covers most of helix Ia, an epitope is located since the synthetic peptides 64 and 118

```
B    —— MAVLNDRLAQYLDEVRALEAANG-LEVL ——————————
64        TDLDDRLAKYLDKVRALEAADGELGV
118       ALLNDELAEYLALVRALEAADGKLGV
7    ——————————— RFAAFIDKVR ———————————————————
8      59 MQFLNDRLASYLEKVRQLERENAELESRILER.....
E      56 MQNLNDRLASYLDKVRALEEANADLEVKIRDW.....
D     103 LQELNDRFANYIEKVRFLEQQNALMVAEVNRL.....
GFA ———— MLNEEFARYIERVVFLEEQKRARAALLDE————
V      89 LQELNDRFADYIDKVRFLEQQNKILLAELEQL.....
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Fig. 1. The amino acid sequence of TPA BrCN:B fragment and synthetic peptides 64 and 118 aligned with IF proteins. TPA BrCN:B fragment (B), Synthetic peptides 64 (64) and 118 (118), sheep wool α-keratin 7c (7), Sheep wool α-keratin 8c-1 (8), human epidermal 50K keratin (E), chicken gizzard desmin (D), porcine vimentin (V), bovine glial fibrillary acidic protein (GFA). Sequences of IF proteins are from Ref. 5, where further references are found. Solid lines denote unknown sequences.

```
E1   ———— MD-IIAE-VKAQYED-A-RM ————————————————
F    ——————————————————————————MLEE(F/V)-L(F/W)————
7    248 DLNMDCIVAEEIRAQYDDIASRSRAEAESWYRSK....
8    219 DLN---RVLNETRAQYEALVETNRRDVEEWYIRQ....
E    216 DLS---RILNEMRDQYEKMAEKNRKDAEEWFFIK....
D    260 DLT---AALRDVRQQYESVAAKNLQEAEEWYKSK....
V    246 DLT---AALRDVRQQYESVAAKNLQEAEEWYKSK....
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Fig. 2. The amino acid sequences of TPA BrCN fragments E_1 and F aligned with IF proteins. TPA BrCN:E_1 (E_1), TPA BrCN:F (F), sheep wool α-keratin 7c (7), sheep wool α-keratin 8c-1 (8), human epidermal 50K keratin (E), chicken gizzard desmin (D), porcine vimentin (V). Sequences for IF proteins are from Ref. 5, where further references are found.

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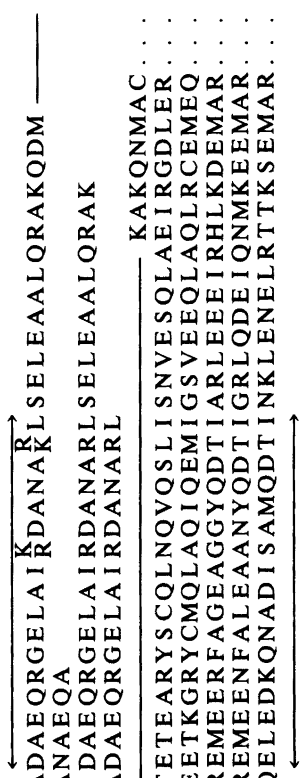

Fig. 3. The amino acid sequences of TPA BrCN:C and synthetic peptides 107, 255 and 269 aligned with IF proteins. TPA BrCN:C (C), peptides 107 (107), 255 (255), and 269 (269), sheep wool α-keratin 7c (7), sheep wool α-keratin 8c-1 (8), human epidermal 50K keratin (E), chicken gizzard desmin (D), porcine vimentin (V), porcine neurofilament 68 K protein (NF). Sequences of IF proteins are from Ref. 5, where further references are found. Double headed arrow denotes the approximate location of the epitope in TPA BrCN:C.

are recognized by the antibody to TPA. Arginine is essential for the binding[6] and since the only arginine located at the same place in the protein and the synthetic peptides is the one corresponding to E 71 the epitope should be centered there. The high homology in this area will also explain why TPA antibody stains various epithelia in addition to carcinomas. The homology in this area with non-epithelial proteins is lower (30–40 %).

Assuming further homology between TPA and the 50K epidermal keratin, the nearest methionine would give an 11K fragment, which is very nearly the weight observed for the BrCN:B fragment.

The fragment BrCN:E_1 can be aligned with the IF proteins around the start of Helix II (Fig. 2). The TPA sequence shows homology with different proteins in the group totalling 66 %. During sequencing BrCN:E_1 no amino acids are recorded after 17 steps. Assuming a methionine at step 18, fragment BrCN:F, which has been proven to contain the single tryptophan residue of TPA[6] can be inserted after the BrCN:E_1 fragment, giving the tryptophan residue the "right" position (Fig. 2). Further along Helix II the TPA fragment BrCN:C can be aligned (Fig. 3). After sequencing the fragment for 56 steps no further amino acids could be detected. Aligned with the IF proteins four of them have methionine at the next step, indicating that the TPA fragment also should be terminated here, although it was originally believed to be somewhat larger.

The pattern of homology between this fragment and the other IF proteins is different from that in the previous two examples (Fig. 3). The TPA sequence borrows features from all the other IF proteins and an overall homology with the group is 46 %. However, the homology is low, only 9 % in the central third of the fragment and approaches 70 % in the two terminal thirds.

Synthetic peptides matching the natural sequences were tested by hemagglutination and RIA methods in an attempt to reveal the position of the epitope in BrCN:C. The peptides 255 and 269 are recognized by the TPA antibody whereas peptide 107 is not. The epitope should therefore be situated in the area of overlap between peptides 255 and 269, which is also the region of low homology with other IF proteins. The epitope located in TPA fragment BrCN:C could thus be expected to be more specific for the protein than the epitopes in the BrCN:B fragment. Arginine is essential for the ability to bind to the antibody,[6] and around arginine 31 in BrCN:C there is an area of low probability for α-helix formation. However, the coiled coil

heptade structure is retained.

The earlier ideas about a triple stranded architecture of the IF proteins has been doubted by Weber[3] who found evidence for a double stranded coiled coil.[3] The sedimentation analysis of TPA[1] confirms this observation as TPA forms a rod like dimer at high and low pH (2.1*S*, f/f_o=2.4). Around pH 7 soluble 4*S* aggregates are formed, which however, easily precipitate, properties intermediate between those observed for the desmin rod and the complete desmin molecule.[3]

Based on sequence homology between various parts of the TPA molecule and a number of IF proteins TPA should belong to that group and immunological cross reactions can be expected. However chemical differences in at least one epitope may explain immunological differences between TPA and other IF proteins.

Experimental conditions for the preparation of TPA and its antibody, as well as the testing of antibody-antigen binding have been presented[1,2,6]. Peptides were synthesized by Merrifield technique.[7]

Acknowledgement. Thanks are due to Prof. Klaus Weber for valuable discussions and for giving access to Refs. 4 and 5 prior to their publication.

Note added in proof. A recently published Type II human epidermal cytoskeletal keratin [Hanukoglu, I. and Fuchs, E. *Cell 33* (1983) 915] shows a high homology (60 %) with the TPA BrCN:C fragment although TPA is a Type I cytokeratin.

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NMR Studies of Lignins. 6. Interpretation of the ^{1}H NMR Spectrum of Acetylated Spruce Lignin in a Deuterioacetone Solution

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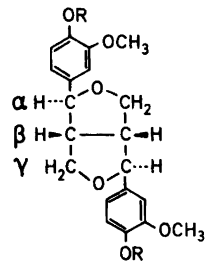

1 R=H or adjacent lignin unit

2 R=CH_3CO

3 R=CH_3

^{1}H NMR investigations of spruce and birch lignins have been reported in this series.[1] Attempts to detect $\beta-\beta$ structures of the pinoresinol type (*1*) in spruce lignin have failed,[1d,e] while clear evidence for the occurrence of this type of structure could be obtained in studies of birch lignin.[1b,e] It has now been found that signals from pinoresinol structures appear in spectra of acetylated spruce lignin in an acetone solution. Such spectra also exhibited signals which could be attributed to the presence of trace amounts of coniferyl alcohol end groups. Furthermore, the spectra provided a basis for a discussion of the occurrence of α-aryloxypropiophenone structures in spruce lignin. The exchange of chloroform for acetone as a solvent results in distinct alterations of the spectrum of acetylated spruce lignin (see Ref. 1d and Fig. 1). Since the spectral changes are those expected from model compound studies, the solvent dependence provides support for the interpretations presented in Table 2 and in Ref. 1d.

Model compound data are summarized in Table 1. The results from both the present examination of model compounds and previous studies of chloroform solutions of model compounds[1c] provided a basis for the interpretation of the spectrum for spruce lignin (Fig. 1) given in Table 2.

A small but clearly discernible peak at δ 3.12 in the lignin spectrum (Fig. 1) is attributed to H_β in $\beta-\beta$ structures of the pinoresinol type (the peak at δ 3.12 was found in spectra of Björkman lignins as well as in those of lignins purified by liquid–liquid extraction, *cf.* Ref. 1d). A compari-

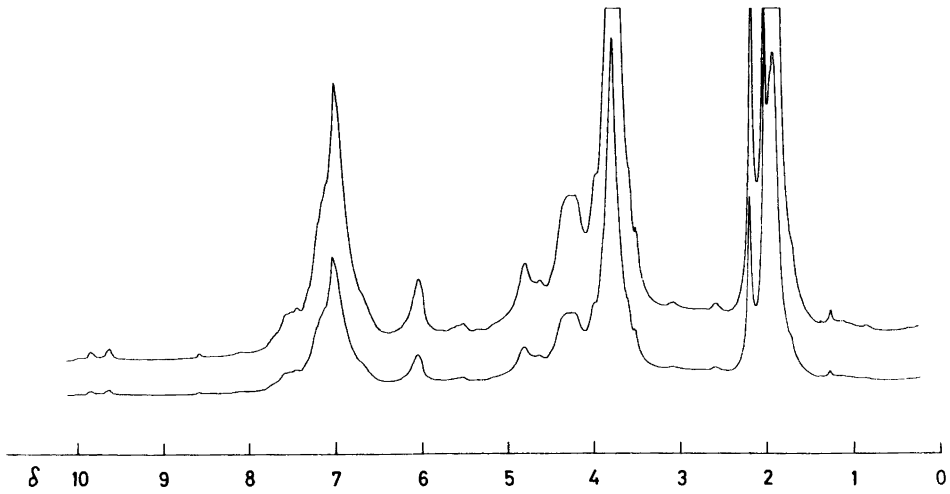

Fig. 1.

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Table 1. ^{1}H NMR data for lignin model compounds (solvent. CD_3COCD_3). δ Values; J values in Hz are given in parentheses.

Compound	H_α	H_β	H_γ	H_γ'	CH_3O	CH_3CO
2	4.79(4.2)	3.14(m)	3.92(3.7,9.1)	4.29(7.0,9.1)	3.82[a]	2.22[a]
3	4.71(4.3)	3.10(m)	4.22(m)[b]		3.80[a],3.81[a]	—
4	5.57(6.6)	3.76(m)	4.32(7.7,11.1)	4.44(5.5,11.1)	3.82,3.86	1.98,2.01,2.22
5	5.49(7.0)	—	4.31(7.5,11.0)	4.41(5.6,11.0)	3.79,3.80,3.85	1.98,2.01
6 (*erythro*)	6.07(5.1)	4.83(m)	4.22(4.2,11.8)	4.38(5.9,11.8)	3.81,3.83	1.93,2.07,2.22
7 (*erythro*)	6.01(5.0)	4.82(m)	4.19(4.0,11.9)	4.35(6.1,11.9)	3.79,3.80,3.81	1.93,2.03
7 (*threo*)	6.06(7.0)	4.78(m)	3.97(5.7,11.9)	4.23(3.8,11.9)	3.79,3.80,3.82	1.96,1.97
8 (*erythro*)	5.54(5.4)	4.84(m)	4.46(3.9,11.8)	4.55(5.8,11.8)	3.77,3.79,3.85	1.89,2.19
8 (*threo*)	5.58(4.8)	4.84(m)	4.13(6.5,11.6)	4.41(4.1,11.6)	3.78,3.79,3.81	1.92,2.21
9	—	5.75(4.0,6.4)	4.48(6.4,11.8)	4.60(4.0,11.8)	3.78,3.85,3.90	1.96
10	6.62(1.4,16)	6.22(6.4,16)	4.66(1.4,6.4)[b]	—	3.80,3.83	2.02
Diaryl-propanediol[c]	6.08(7.4)	3.41(m)	4.11(6.9,11.1)	4.26(6.3,11.1)	3.72,3.76,3.78[a]	1.91[a]

[a] 6H. [b] 2H. [c] The diacetate of *erythro*-1,2-bis(3,4-dimethoxyphenyl)-1,3-propanediol.

son with the peak due to formyl protons in cinnamaldehyde units (δ 9.62) suggests that 2–3 % of the units are involved in pinoresinol structures. The number of cinnamaldehyde units has previously been determined as 4 %,[1a] a result which is in agreement with those obtained by an independent method.[2] The baseline for the 3.12 peak is difficult to define due to the vicinity of the large methoxyl (δ 3.81) and acetate (δ 2.21 and 1.95) peaks; this makes the estimation uncertain. Assuming that the peak at β 3.12 is actually due to H_β in β–β structures, their number cannot be much less than 2–3 %. A substantially higher frequency (about 5 %) cannot be excluded, however, but seems unlikely judging from earlier experiments with non-derivatized spruce lignin.[1e]

Convincing evidence for the occurrence of pinoresinol structures in spruce lignin has previously been obtained in ^{13}C NMR investigations of acetylated spruce lignin.[3] Ogiyama and Kondo[4] concluded from the yield of "dilactone" in nitric acid oxidation that spruce lignin contained 5–10 % units in pinoresinol structures. Our results point to a figure which, at the most, is in the neighborhood of the lower limit of this interval.

In previous ^{1}H NMR spectral studies of spruce lignin it was concluded that the frequency of α-aryloxypropiophenone structures was low[1a] (presumably less than 2 % of the units). This was

Table 2. Assignment of signals in the ^1H NMR spectrum of acetylated spruce lignin (Fig. 1). Solvent, CD_3COCD_3. Several peaks are broad and have irregular shapes; δ values given always refer to the highest point of the peak.

δ Value/ppm	Assignment
1.29	Hydrocarbon contaminant
1.95	Aliphatic acetate
2.04	Acetone (solvent)
2.21	Aromatic acetate
2.62	$Ar-CH_2-$ (*cf.* Refs. 1d and 1e)
3.12	H_β in pinoresinol structures
3.81	CH_3-O-
4.24, 4.32	H_γ in several types of structures
4.65	Methylene protons in cinnamyl alcohol groups
4.81	H_β in $\beta-O-4$ structures
5.54	H_α in benzyl aryl ethers (cyclic as well as non-cyclic)
6.05	H_α in $\beta-O-4$ structures
7.03, 7.45	Aromatic protons
9.62, 9.84, 9.99	Formyl protons (*cf.* Ref. 1a)

derived from attempts to reveal the signal from H_β in such structures (δ 5.58 in $CDCl_3$). In acetone solution the signal from H_β in such units (δ 5.75) is distinctly separated from signals of other types of structures (Table 1). The absence of a peak at δ 5.75 in the lignin spectrum (Fig. 1) supports the conclusion drawn in previous ^1H NMR studies.

Experimental. *^1H NMR spectra* were recorded with a 270 MHz instrument, working in the pulse Fourier mode (Bruker WH 270). Acetone-d_6 was used as a solvent (internal reference TMS). Temperature: 308 K. Number of scans (lignin spectra): 1000–1200.

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Recirculation of Cellulolytic Enzymes in an Ultrafiltration Membrane Reactor *

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In recent years the enzymatic hydrolysis of lignocellulosic materials has become a subject of intensified interest because the hydrolysate can serve as a raw material for fermentatively produced chemicals.[1] However, available cellulolytic enzyme systems have a relatively low specific activity, about a hundred times lower than comparable amylolytic enzymes.[2] Therefore, high enzyme concentrations have to be used in order to achieve a reasonable rate and degree of hydrolysis, which in turn makes enzyme recycling an economic necessity in such a process.

Enzyme recycling has been reported to be possible by adsorption onto fresh substrate,[3] immobilization to a solid matrix, [4] as well as with the use of ultrafiltration methods.[5] Cellulolytic enzyme systems consist of at least three different activities which have been reported to adsorb with different strengths to cellulose.[6] It will therefore be difficult to obtain a complete recovery of the whole enzyme complex using adsorption alone. Immobilization of enzymes to solid matrices, plus the utilization of macromolecular or solid substrates, result in poor enzyme efficiency due to the introduction of diffusion limitation and steric hindrance. Ultrafiltration, on the other hand, allows complete recovery of the enzymes without the problems accompanying immobilization, provided that the right membrane is selected.

Cellulose hydrolysis in ultrafiltration membrane reactors has so far only been carried out with pure cellulose substrates.[5]

The present communication describes hydrolysis studied, with emphasis on enzyme recovery, in a membrane reactor using pretreated lignocellulosic material from a fast growing species of sallow (Salix Q082) at concentrations of 100 g/l dry weight. The fraction >1 mm from the lignocellulosic material, prepared by milling, was pretreated with 4 % NaOH (g/g) for 2 h at 160 °C.

The cellulolytic enzymes used were the following: 10 g/l cellulase from *Trichoderma reesei*, SP 122, with an *endo*-glucanase activity of 157 mg RS/ml min measured with CMC as substrate,[7] and 10 g/l cellobiase from *Aspergillus niger*, NOVOZYME 188, with a β-glucosidase activity of 45 μmol *p*-nitrophenol/g min using *p*-nitrophenyl-β-*D*-glucopyranoside as substrate.[8] Both enzymes were generously supplied by NOVO A/S, Copenhagen, Denmark.

A cylindrical ultrafiltration membrane reactor with a working volume of approximately 250 ml was equipped with a polyamide membrane, BM 100, molecular weight cut off 10 000 (Berghof GmbH, Tübingen, West Germany). The reactor was operated continuously by replacing the permeate stream with buffer solution from a 5l pressure feed container by means of an automatic level controll.

Semicontinuous hydrolyses were carried out in

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Table 1. Consecutive semicontinuous enzymatic hydrolyses experiments in an ultrafiltration membrane reactor utilising *one* initial addition of cellulolytic enzymes. 0.1 M acetate buffer, pH 4.8, 40 °C, dilution rate 1.0 h^{-1}, stirring 500 rpm.

Exp.	Substrate added (g)				ΣS	Σ *RS* in [b] permeate (g)	Yield $\frac{\Sigma RS}{\Sigma S}$	Productivity g RS/g enz.	Initial conversion rate g RS/g enz. h
I	25[a]	2.35	2.35	2.35	32.0	30.1	0.94	12.1	0.58
II	16.5[a]	2.3	2.3	2.3	23.4	18.7	0.80	7.5	0.44
III	16.5[a]	2.3	2.3	2.3	23.4	15.4	0.66	6.2	0.28
Total					78.8	64.2	0.81	25.8	

[a] Initial substrate addition in each experiment. Subsequent additions are intended to keep the dry weight in the reactor constant (see also text). [b] *RS*=reducing sugars measured by the DNS method (9).

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the ultrafiltration membrane reactor in order to study how much the productivity of the enzyme in terms of g sugar/g enzyme could be improved. In each experiment, three intermittent additions of fresh substrate were made, after which the hydrolysis was left to proceed until no more soluble sugars were produced. The amount of substrate to be added was estimated from the product concentration in the permeate, with the intention of keeping the dry weight in the reactor at a level of 100 g/l. The amount of reducing sugars in the permeate volume was derived from

$$\Sigma RS\,(\mathrm{g}) = \int_0^v (\mathrm{glucose} + \mathrm{cellobiose})\,\mathrm{d}v$$

Table 1 summarizes three consecutive experiments where a total of 78.8 g substrate were hydrolyzed using only the initially added enzymes. 81 % of the substrate was recovered as soluble sugars in the permeate, giving an enzyme productivity of 25.8 g sugar/g enzyme. In a parallel batch hydrolysis experiment under equivalent conditions, only 4.7 g sugar/g enzyme was obtained. Thus, the productivity of the enzymes could be increased more than 5 times by their recovery in an ultrafiltration membrane reactor.

However, the initial conversion rates, measured during the first hour of each experiment, declined 25 % with each experiment (Table 1), indicating that a fraction of the enzymes became successively inactivated. This could be due to operational conditions such as pH, temperature and mechanical stress.

In order to estimate the influence of these different factors, the enzyme activities were measured as a function of time under hydrolysis conditions, in the absence of substrate, with and without stirring. With stirring, 8 % of the *endo*-glucanase and 10 % of the β-glucosidase activity were lost over a 6 day period, compared to an unstirred reference where 6 and 3 %, respectively, were lost. It was therefore assumed that the adsorption of enzymes to the non-degradable fraction of the substrate could account for the decrease in hydrolysis rate.

Table 2. Soluble enzyme activity after completed hydrolysis, % of initial activity.

	I	II	III
endo-Glucanase	22	18	9
β-glucosidase	74	59	44

This was investigated by measuring soluble enzyme activity at the end of each of the three experiments (Table 2). After the first experiment, when digestion was complete, soluble *endo*-glucanase activity was reduced by 78 % and soluble β-glucosidase activity by 26 %. The fact that hydrolysis took place with the recycled enzyme during the second and third experiments indicates that the adsorbed enzymes were desorbed when fresh substrate was added – however, not completely. When undigested substrate accumulates in the reactor more enzyme seems to be adsorbed to it (Table 2). This points out the importance of relating the pretreatment of lignocellulosic materials not only to hydrolyzability, but also to its influence on a possible recovery of the enzymes.

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Determination of Amygdalin and Cyanide in Industrial Food Samples using Enzymic Methods *

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The use of enzymes in analysis promotes specific determination of a compound in complex mixtures. Especially in monitoring of biotechnical processes, for instance in food and pharmaceutical industry as well as in waste water treating plants, such methods appear to be of particular value.

Here are reported two such enzyme-based analytical procedures, tailor-made for the analysis of samples from a plant manufacturing almond pastes (from almond and apricot kernels) used in pastry and confectionery. Apricot and almond kernels contain the flavouring substance almond oil. Amygdalin, the major constituent of almond oil, is decomposed to glucose, benzaldehyde and hydrogen cyanide by the enzyme β-glucosidase which naturally occurs in the kernels,[1] Scheme 1.

In the manufacturing of almond paste the kernels are debittered by extraction first in 40–50 °C water and then in cold water during 2–4 days. This procedure is highly water and energy consuming and furthermore it delivers a rather toxic waste water that requires detoxification before discharge. Since the feedstocks contain varying amounts of amygdalin, the need for monitoring and control is apparent and should preferably be performed in a continuous and automatic fashion.

For the analysis of amygdalin we have chosen to measure the heat evolved by the enzymic reaction shown above with an enzyme thermistor,[2] which is a semi-adiabatic flow microcalorimeter. The instrument contains a small column (0.8 ml) filled with β-glucosidase (E.C. 3.2.123, from sweet almond, 50 U/mg; salicine, 25 °C; Boehringer-Mannheim, FRG.) immobilised on controlled pore glass (CPG).

To 1 ml of CPG 10–700 80/120 mesh (Corning Glasswork, USA) was added 40 mg of β-glucosidase. Coupling to the alkyl-amino-derivatised glass beads was performed using glutaraldehyde.[3] The β-glucosidase completely converts the amygdalin present in intermittently injected sample pulses, resulting in a corresponding temperature pulse that is recorded with a thermistor placed at the outlet of the column. Fig. 1 shows the heat released, expressed as a temperature change, as a result of injecting calibration solutions made up in 0.1 M sodium phosphate and 0.1 M sodium citrate buffers with pH ranging from 4 to 8. The optimum pH for the assay was found to lie between 5.0 and 7.0. The calibration curve then was linear up to 15 mM amygdalin using 0.5 ml sample at a flow rate of 0.8 ml/min. These samples were injected manually. In some experiments, however, we also used an automatic sample injection system with a sampling interval of 5 min.[4]

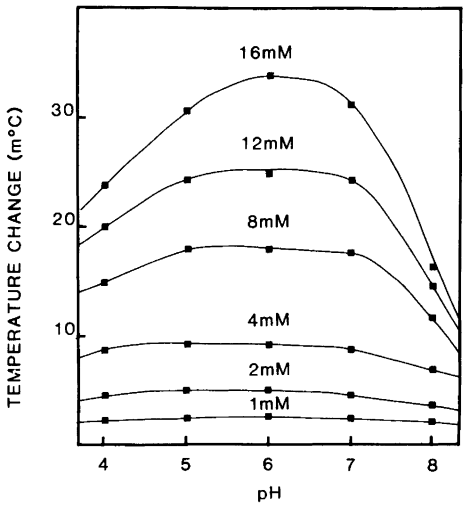

Fig. 1. Temperature responses of the enzyme thermistor versus pH at different concentrations of calibration solutions of amygdalin.

Table 1. Determination of amygdalin.

Sample of kernels	Enzyme thermistor (mg amygdalin per gram of kernels)	Spectrophotometer
Bitter almond	39.3	44.5
Apricot	11.8	12.2
Sweet almond	0	0

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0302-4369/83 $2.50

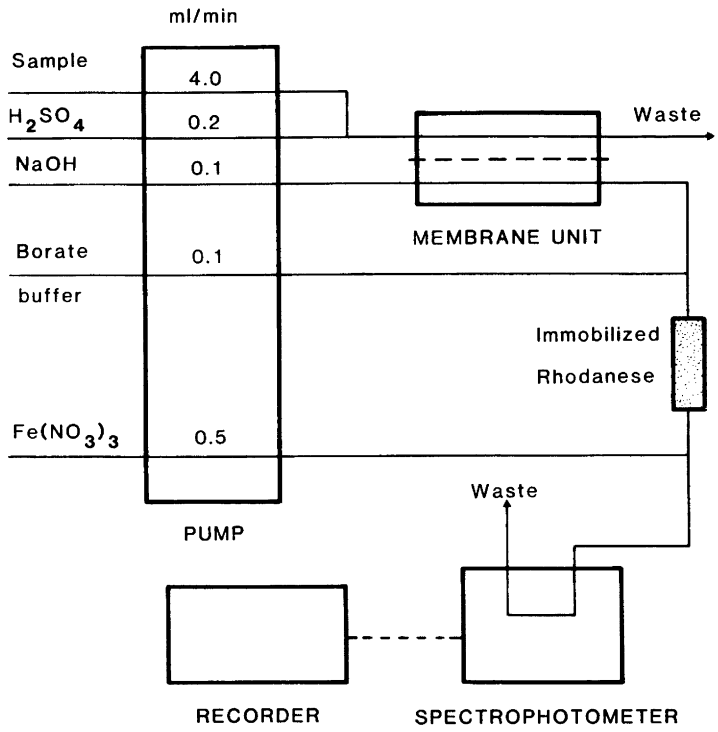

Fig. 2. Experimental set-up of the cyanide analyzing system with membrane enrichment, enzymic conversion and spectrophotometric monitoring. The $Fe(NO_3)_3$ contained 10 v/v % HNO_3.

The determination of amygdalin in crude samples is interfered with by naturally occurring β-glucosidase. This β-glucosidase activity could, however, be eliminated by denaturation of the enzyme by heating the sample or by acid treatment as the following experiment showed. Amygdalin was added to a concentration of 10 mM to an amygdalin-free extract of almond kernels (20 % dry material). The hydrolysis of amygdalin in the extract was followed by enzyme thermistor analysis of 0.5 ml samples withdrawn every 5 min.

In the untreated extract the amygdalin content was reduced by 50 % in 17 min, whereas initial treatment of the almonds in boiling water for 5 min prior to extraction, decreased the decomposition rate to 5–10 % in 30 min. Treatment with phosphoric acid at pH 2 had an even larger denaturing effect as only 5 % of the amygdalin was hydrolysed in 50 min. It should be noted that these treatments had no direct effect on the amygdalin concentration. A combination of these two methods virtually eliminated the unwanted decomposition of amygdalin in crude samples.

Different samples obtained from a food industry (Viktoria fabriken, Helsingborg) were analysed for amygdalin by the present method and by a spectrophotometric technique based on determination of cyanide with picric acid.[5] Data given in Table 1 show a good agreement between the two techniques. In conclusion, the enzyme thermistor method for determination of amygdalin described here turned out to work well with crude food samples. It is a rapid and direct method with sufficient sensitivity for such samples. Furthermore, it was capable of adequately monitoring the debittering process employed in the factory. The useful concentration range was 0.1–20 mM.

Since the highest permissible cyanide concentration in sewage water is set very low, enrichment of the cyanide present in the waste water samples had to be made prior to analysis. This was accomplished with an on-line filter unit containing a hydrophobic, porous teflon membrane. Such membranes are highly permeable to gases, including hydrogen cyanide. The membrane was mounted in a rectangular membrane holder exposing 400 mm^2 of each side of the membrane in 100 mm long channels with the

cross section 1×4 mm^2. A multichannel peristaltic pump (Gilson, Minipulse, France) was used for administration of the different flow streams according to Fig. 2. Cyanide present in the sample was protonated to hydrogen cyanide by lowering the pH to below 1. The pH at the other side of the membrane was kept high (pH>12). A low flow rate on this side of the membrane compared to that of the sample side resulted in considerably higher cyanide concentration than in the sample. Different hydrophobic membranes from Millipore (Bedford, Mass. USA.) were investigated using a spectrophotometric cyanide assay.[6] We found that a Fluoropore membrane with 0.2 μm porosity gave the highest enrichment factor (8 times), whereas 5-fold enrichment was seen with 1 μm Fluoropore and 5 μm Mitex membranes. Advantages of this membrane technique are that loss of cyanide is eliminated by the use of a closed system and that the sample is cleaned up, with minimised interference and increased specificity as consequences. Determination of cyanide was performed by converting the cyanide to thiocyanide (rhodanide) with use of immobilised rhodanese followed by reacting the thiocyanide with the Fe(III) ions to give the coloured complex, $FeSCN^{2+}$:

$$CN^- + S_2O_3^{2-} \xrightarrow{\text{Rhodanese}} SCN^- + SO_3^{2-} \quad (2)$$

$$SCN^- + Fe^{3+} \rightleftharpoons Fe(SCN)^{2-} \quad (3)$$

Rhodanese (E.C. 2.8.1.1; 10 units per mg; Sigma Chemicals, Mo, USA) was immobilised on CPG (5 mg rhodanese/ml CPG) using the same procedure as for β-glucosidase.[3] The $Fe(SCN)^{2+}$ complex was measured at 460 nm by a Zeiss PM6 spectrophotometer equipped with a 0.030 ml, 10 mm flow cell. Under the conditions given, the change in absorbance was linear to the cyanide concentration of aqueous standard solutions up to 15 mg CN^-/l (0.6 mM). The limit of detection was 0.2 mg/l (0.008 mM) and each analysis took 10–15 min.

Table 2. Waste water analyses.

Sample		Rhodanese method (mg CN/l waste water)	Official method
Untreated Waste water	I	3.3	2.9
	II	1.1	1.5
Treated Waste water	I	0.25	0.5
	II	<0.2	<0.1

Waste water samples from the factory (Viktoria fabriken) collected before and after detoxification were analysed for cyanide content by the present method and by a conventional, calorimetric technique (performed by an official control laboratory, Swelab, Sweden). Table 2 compares some of the results obtained in this study. We also found good agreement in results obtained with the present method, and with a Beckman cyanide electrode. The electrode was more sensitive, but its operation with crude samples was less reliable. Alternatively an enzyme thermistor can be used for cyanide determination,[7] although it is more susceptible to interferences (non-specific heats) in work with crude solutions.

In conclusion, two analytical procedures have been developed for specific, on-line analyses of crude process solutions. Both of them have adequate sensitivity and time response to be used for process control in the situations they were investigated for.

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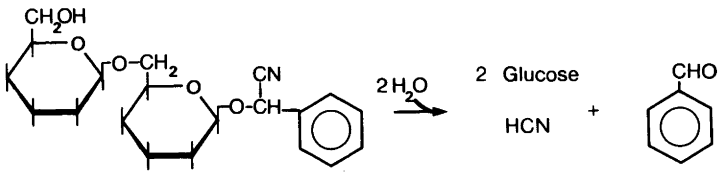

Scheme 1. Eqn. (1).

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Purification of Thymidine 2′-Hydroxylase from *Neurospora crassa* *

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Thymidine 2′-hydroxylase (EC 1.14.11.3) catalyzes the oxygenation of the deoxyribose moiety of thymidine and deoxyuridine. The enzyme is a 2-oxoglutarate-dependent oxygenase and high enzyme activities have been found in *Neurospora crassa*[1,2] and *Rhodutorula glutinis*.[3] Since low and variable concentrations of deoxypyrimidine nucleosidase have been found in Neurospora, thymidine 2′-hydroxylase seems to be the key enzyme for reutilization of thymidine in this organism.[4-6] After hydrolysis of ribosylthymine, thymine is oxidatively demethylated in another 2-oxoglutarate-dependent oxygenase reaction catalyzed by thymine 7-hydroxylase (EC 1.14.11.6).

These enzymes have been investigated by us in an attempt to improve the understanding of 2-oxoglutarate-dependent oxygenase reactions.[2,7] We have previously used partially purified enzyme preparations, but have now isolated thymidine 2′-hydroxylase from Neurospora in a 5-step procedure which gives a 8 000-fold purification from an intial 140 000 *g* supernatant (Table 1).

The molecular mass of the native enzyme is 47 kdalton as determined by gel filtration.[2] The enzyme consists of one polypeptide chain as judged from SDS polyacrylamide gel electrophoresis, where one major band is found with a molecular mass of 46 kdalton (Figs. 1 and 2).

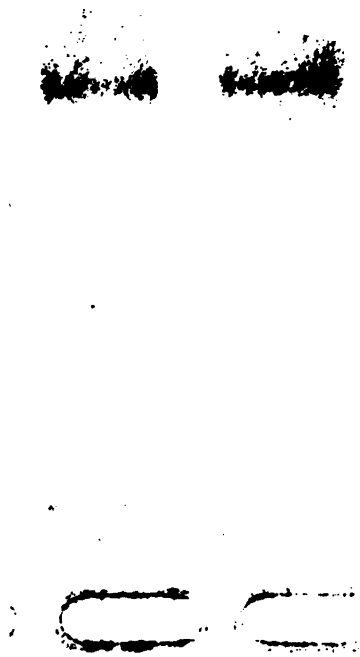

Fig. 1. SDS polyacrylamide gel electrophoresis of thymidine 2′-hydroxylase.

The specific activity of 765 μkat g^{-1} is the highest value reported for any 2-oxoglutarate-dependent oxygenase. The most active 2-oxoglutarate-dependent oxygenase reported hitherto is the bacterial γ-butyrobetaine hydroxylase (EC. 1.14.11.1) with a specific activity of 360 μkat g^{-1}.[9] If, however, the difference in molecular mass between these two enzymes is taken into account and molecular activity is calculated, the figures obtained are very similar *i.e.* 36 s^{-1} for thymidine

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Table 1. Purification of thymidine 2′-hydroxylase from *Neurospora crassa*.

Purification step	Protein g[a]	Total Activity μkat	Spec. Activity μkat g^{-1}
140 000 *g* supernatant	2.8	0.26	0.093
DEAE-cellulose chromatography	0.15	0.32	2.1
Hydroxylapatite chromatography	$8.6 \cdot 10^{-3}$	0.15	17
Sephadex G-100 filtration	$2.5 \cdot 10^{-3}$	0.082	33
Chromatofocusing on Mono P™	$0.35 \cdot 10^{-3}$	0.043	123
Anion exchange on Mono Q™	$28 \cdot 10^{-6}$	0.021	765

[a] Protein was determined according to Lowry *et al.*[11] except for the last step where $A_1^{1\%}{}_{cm} = 10$ at 280 nm was used.

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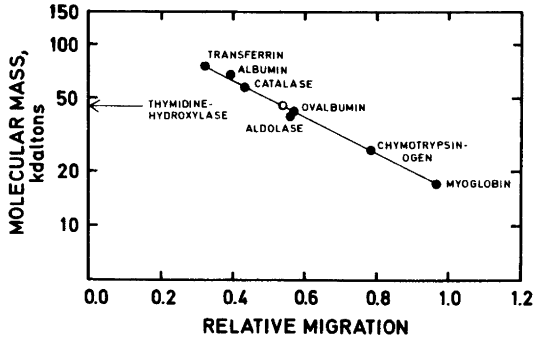

Fig. 2. Molecular mass determination of thymidine 2′-hydroxylase in SDS polyacrylamine gel electrophoresis.

2′-hydroxylase and 34 s^{-1} for γ-butyrobetaine hydroxylase. On high resolution chromatography on a Mono Q™ column (Fig. 3) coincidence of enzyme activity and a protein peak, which gives one dominating band on PAGE is found. Together with the high specific activity this indicates that the enzyme is essentially pure.

Experimental. Soluble extract. A soluble extract of *Neurospora crassa* strain STA 4 (FGSC 262 A) was prepared as described previously.[9]

DEAE Cellulose chromatography. After desalting on a Sephadex G-25 column the soluble extract (365 ml) was put onto a column (5×26 cm) of DE-cellulose (Whatman DE 52) equilibrated in 10 mM potassium phosphate buffer, pH 6.5, containing 25 mM KCl, 0.1 M glycine, and

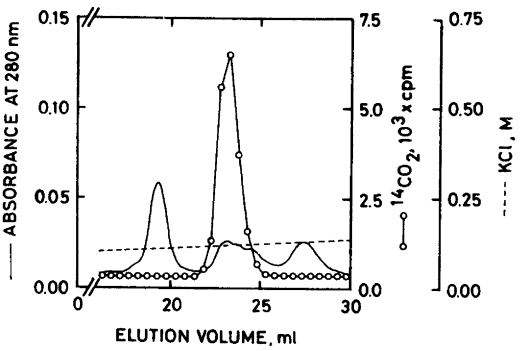

Fig. 3. Chromatography of thymidine 2′-hydroxylase in the final purification step on Mono Q™. The eluate was monitored for absorbance at 280 nm in a cell with 1 cm light path and collected in 0.5 ml fractions. The enzyme assay was performed as described in *Experimental;* 0.45 μl of eluate was used in the assay.

0.1 mM EDTA. The chromatogram was developed with a 7-1 linear gradient from 25 mM to 175 mM KCl. The enzyme activity was eluted around 150 mM KCl well separated from the preceding activity of thymine 7-hydroxylase.

Hydroxylapatite chromatography. The enzyme preparation from the DE cellulose column was concentrated by ultrafiltration on PM-10 Diaflo ultrafiltration membrane and the buffer was changed by passage through a Sephadex G-25 column. The enzyme preparation was then put onto a column (5×16 cm) of hydroxylapatite (Bio-Gel^R HTP from Bio-Rad Laboratories) equilibrated with 10 mM potassium phosphate buffer, pH 6.5, containing 0.1 M glycine. The chromatogram was developed with a linear gradient from 10 mM to 80 mM potassium phosphate buffer, pH 6.5, in a volume of 2.4 l. The enzyme activity was eluted around 60 mM.

Sephadex G-100 filtration. The hydroxylapatite eluate (350 ml) was concentrated by ultrafiltration on PM-10 Diaflo ultrafiltration membrane to 10 ml and further concentrated to 2 ml by vacuum dialysis in a Sartorius Membrane filter 13200 collodion bag. The enzyme preparation was then applied onto a Sephadex G-100 superfine column (1.5×90 cm) equilibrated with 50 mM potassium phosphate buffer, pH 6.5, containing 100 mM NaCl amd 0.03 % NaN_3.

Chromatofocusing. The buffer was changed to 25 mM BIS-TRIS, pH 6.0, by passing the previous eluate through a Sephadex G-25 column. The preparation was then chromatographed in a fast protein liquid chromatograph, FPLC, on a 3 ml Mono P™ column with an ampholyte, 7.5 % polybuffer in water (all components from Pharmacia fine chemicals, Uppsala, Sweden). The enzyme was eluted at pH 4.2.

QAE cellulose chromatography. The combined fractions (1 ml) from the preceding step were put through a Sephadex G-25 in order to change the buffer to 10 mM potassium phosphate pH 6.8. This solution was chromatographed in the FPLC-system on a 1 ml Mono Q™ column. The chromatogram was developed with a gradient that was 2 mM to 100 mM KCl in 10 ml and then 100 mM to 200 mM KCl in 40 ml (Fig. 3). The eluate was collected in 0.5 ml fractions and the enzyme activity was mainly found in three fractions which were combined and concentrated to 75 μl by vacuum dialysis in a collodion bag.

SDS polyacrylamine gel electrophoresis. Slab gels (12.5×26×0.3 cm) with a 7.5 % polyacrylamide gel were run with the continuous buffer system desbribed by Weber and Osborn.[10] Samples of protein were denaturated in 0.5 % SDS and 0.5 % mercaptoethanol for three minutes at 100 °C. Aproximately 3 μg of the purified thymi-

dine 2'-hydroxylase was applied to the gel. After staining in Coomassie brilliant blue the mobility was calculated relative to that of bromophenol blue.

Enzyme assay. The incubation system (0.2 ml) contained thymidine 2'-hydroxylase (about 10 pkat) in 50 mM potassium phosphate buffer, pH 7.5. The concentration of substrates and cofactors were: thymidine 0.5 mM, 2-oxo[1-^{14}C] glutarate 0.25 mM, 50 nCi, ascorbate 5 mM, dithiothreitol 5mM, Fe_2SO_4 1 mM, and catalase 2 g/l. The incubations were carried out for 10 min at 37 °C and stopped by the addition of 0.2 ml 0.3 M trichloroacetic acid. The incubations were carried out in 10-ml plastic test tubes. $^{14}CO_2$ was trapped on a piece of filter paper attached to a wire in the rubber stopper of the test tube. The filter papers had been soaked in 20 µl of a 1 M solution of hydroxide of Hyamine 10-X [*p*-(diisobutylcresoxyethoxyethyl)dimethylbenzylammonium hydroxide from Packard Instrument Company Inc.]. The diffusion was allowed to proceed for 1 h at 37 °C before the radioactivity was measured.

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Monitoring of Ethanol in Production of Baker's Yeast Using an Improved Membrane Gas Sensor *

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Previous work has shown the usefulness of membrane tubing methods for monitoring ethanol production in yeast fermentations.

The methods are based on the fact that the volatile ethanol can be separated from the fermentation broth by a gas permeable membrane and thereafter detected by a flame ionization detector [1] or a semiconductor gas detector.[2] The hydrophobic properties of porous Teflon and porous silicon have made these materials especially suitable, not only because of their permeability, but also due to their low degree of compatability with yeast cells.

Puhar *et al.*[2] have, among others, investigated the influence of factors such as aeration, impeller speed, carbon dioxide and oxygen concentration on the performance of the sensor. They found that only oxygen severely disturbs the detector response when nitrogen is used as carrier gas to the semiconductor.

Data presented here show how this drawback can be considerably reduced by using a dilution system incorporated into the sensor system. Now the ethanol samples can be continuously diluted between 2 to 1000 times before the detector analysis, thereby diluting oxygen far below harmful concentration levels. The complete detector system was from the prototype series manufactured by a local workshop. A comprehensive description of two dilution system designs is given elsewhere.[3]

A recent report[4] has described the use of a dilution system in combination with a semiconductor detector for measuring ethanol levels in an anaerobic fermentation using immobilized yeast. The fermentation broth was diluted before reaching the permeable membrane. We have chosen to use the gas dilution method to monitor ethanol production in an aerobic fermentation of baker's yeast, and to demonstrate the sensor's capability for continuously following the variation in ethanol concentrations.

The yeast used was *Saccharomyces cerevisiæ*, a gift from Svenska Jästbolaget, Rotebro, Sweden. The yeast was cultivated in YM Broth (Difco Laboratories, USA) supplemented with 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.4 g/l KH_2PO_4 and 0.6 g/l $Na_2HPO_4 \cdot 2H_2O$.

The glucose concentration was 10 g/l. The inoculum was cultivated in the above medium for 20 h on a rotary shaker. Batch cultivation was performed in a fermentor (Chemoferm AB, Hägersten, Sweden) with a working volume of 2 l. Temperature and pH were controlled at 30 °C and 5.5 respectively; 0.5 M NaOH was used to keep pH constant. Foaming was controlled by adding, when needed, the antifoam Adekanol

* Communication at the Meeting of the Swedish Biochemical Society in Lund, 9–10th June, 1983.

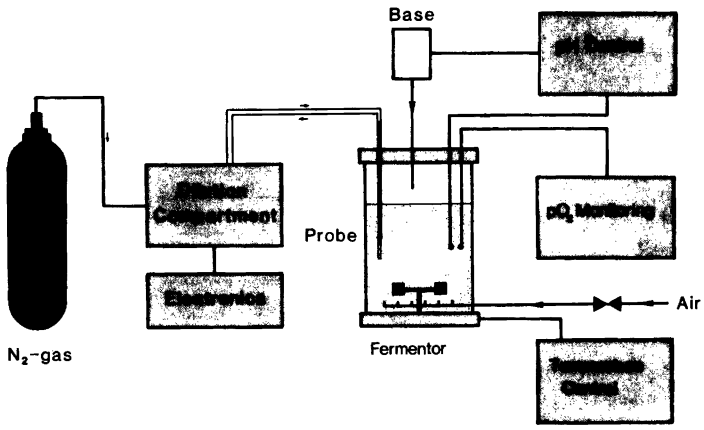

Fig. 1. Experimental set-up of the fermentor with the silicone tubing sensor and the equipment for temperature control, pH control and pO_2 monitoring.

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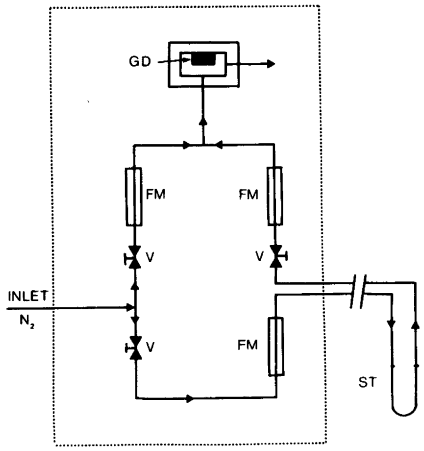

Fig. 2. Experimental set-up of the dilution compartment showing the gas detector in its insulated chamber (G.D.), precision valves (V), flow meters (F.M.) and the silicone tubing (S.T.).

LG-109 (Asahi Electro Chemical Co., Japan). The medium in the fermentor was inoculated with 5 % (v/v) inoculum. The level of dissolved oxygen was measured continuously with a galvanic oxygen electrode.[5] Aeration rate was 1.0 volume/volume min. Stirrer speed was set at 300 rpm.

The fermentor was also equipped with an ethanol probe (Fig. 1). This consisted of a stainless steel bar designed to fit the fermentor connections and suitable for autoclaving. The membrane tubing was mounted on this probe and was a 45 mm long and 0.2 mm thick silicone tubing connected to the continuous dilution system, which was connected to a SnO_2-semiconductor gas detector (Figaro 812, Figaro Eng. Inc. Japan) (Fig. 2).

Due to chemisorption of the gas onto its surface, the semiconductor underwent changes in its conductivity. Since the semiconductor is only able to detect small concentrations of ethanol (500–5000 ppm), above which it becomes saturated, dilution of the gas is quite favourable. By setting the dilution degree to 10 times, the detector gave a linear characteristic up to 20 g/l, whereby the signal response was 0–10 V. To investigate the performance and ability of the sensor for continuously and accurately reflecting the ethanol concentration, it was tested in a 26 h batch fermentation.

Fig. 3 shows the time course of the cultivation and the different parameters which were followed. The ethanol and glucose concentrations, as well as the optical cell density at 620 nm, were measured each hour. After sterile filtration (0.45 μm, Millipore), the glucose samples were analysed enzymatically, (glucose kit, Boehringer-Mannheim, FRG) and the ethanol samples were analysed in a GLC (Varian).

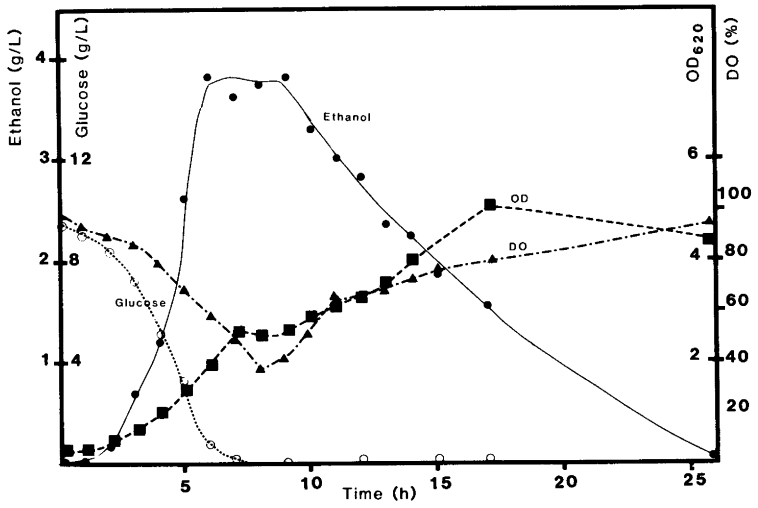

Fig. 3. Fermentation kinetics of ethanol production using baker's yeast, showing: glucose concentration (○); ethanol concentration continuously recorded with the sensor (−) or measured with GLC (●); optical density at 620 nm (■) and dissolved oxygen (▲).

The time course in Fig. 3 clearly shows the production of cell mass at the expense of glucose, but at the same time yielding ethanol concentrations up to 3.5 g/l. This ethanol can later serve as a second substrate when the glucose has been completely consumed after 9 h.

However, the ethanol consumption rate is lower, resulting in a lower growth. The diauxic effect also indicates the change of substrate. After 26 h the ethanol is completely consumed and the final cell mass reaches an optical density of 4.3. The dry weight was estimated to be 4.8 g/l, giving a total cell yield of 0.48 g dry weight/g glucose. This is in agreement with other reported results.[6]

The oxygen consumption during the first 8 h parallels that of glucose, and thereafter shows a slight increase as the ethanol is utilized more slowly.

The continuous ethanol signal agrees with the off-line performed GLC-analyses. The deviations are small and negligible. The changing oxygen concentration in the medium does not influence the sensor signal, nor does the cell density seem to cause fouling of the silicone membrane, which otherwise would have hampered the transfer of ethanol.

The results above show the advantages of using this type of ethanol sensor. Besides, being unaffected by several disturbances in the environment, this method of ethanol detection is also easy, inexpensive to operate, and has few maintenance requirements.

The ethanol sensor thus seems to be a suitable tool for continuous monitoring and later also control processes involving continuous production of ethanol, as is the case in ethanol fermentation[4] and production of baker's yeast.[7]

Acknowledgements. The project was supported in part by the National Swedish Board for Technical Development.

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Extractive Deacylation of Benzylpenicillin in Aqueous Two-Phase Systems *

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Penicillin acylase (PA) (EC 3.5.1.11) catalyzes the deacylation of benzylpenicillin (BP) to 6-aminopenicillanic acid (6-APA) and phenylacetic acid. The reaction has a pH dependent equlibrium, where high pH favours the deacylation reaction.

The interesting product from the deacylation step is 6-APA, key intermediate for the production of semisynthetic penicillins. PA immobilized to solid matrixes is used for industrial production of 6-APA. Several studies describe the binding of this enzyme to different matrix materials.[1-4]

Aqueous two-phase systems have lately become of increased interest in biotechnology. The purification of intracellular enzymes in large scale has been most actively studied and has been recently reviewed.[5-6] Aqueous two-phase systems have also been applied to biological analysis[7] and for the regeneration of coenzymes.[8] The utilization of aqueous two-phase systems in bioconversions has only recently been observed.[9] This communication investigates the use of aqueous two-phase systems for bioconversions using the deacylation of BP with PA as a model system.

PA was partitioned in different aqueous two-phase systems in order to obtain a very low partition coefficient where K=activity (top phase)/activity (bottom phase). For best results, the enzyme should be almost completely partitioned to the bottom phase. Phase systems consisting of poly(ethylene glycol) (PEG) and dextran only resulted in $K>0.03$, which was not considered satisfactory. However, phase systems consisting of PEG and salts gave K values <0.01.

The enzyme stability in these phase systems was determined from measurements of the activity[10] (Figure 1). The stability was very poor in the phase system containing magnesium sulfate. In the phase system composed of PEG-potassium phosphate, the enzyme even seems to be stabilized. This system was chosen for further studies. The partition coefficient in this phase system for substrate and products were: $K_{BP}\sim 8.0$, $K_{6\text{-APA}}=1.35$ and $K_{\text{phenylacetic acid}}=1.7$.

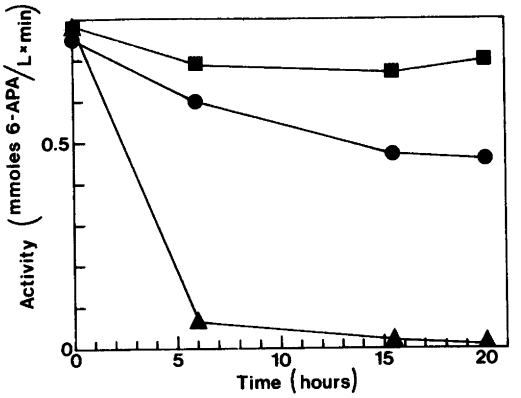

Fig. 1. The stability of PA. Aqueous two-phase systems: (■) PEG 20000, 8.9 % (w/w)/potassium phosphate, 7.6 % (w/w) and (▲) PEG 3350, 12 % (w/w)/magnesium sulfate, 10 % (w/w). (●) 0.05 M sodium phosphate buffer. pH was 7.8 and the temperature 37 °C.

The conversion of BP to 6-APA was then studied in a repeated batch conversion (Figure 2). The study was performed as follows: when the 6-APA formation in the stirred tank ceased, the stirring was stopped. When the phases had separated, the top phase was removed, followed by addition of new top phase with fresh BP. The stirring was than started again. pH control was not used. This was shown to affect the reaction velocity(decrease). Thus, before the fourth conversion, the pH was increased by titration, which increased the reaction velocity again. The specific productivity for the conversions measured during the first hour of each conversion was 0.89–3.16 μmol 6-APA/mg protein×min. The specific productivity for PA immobilized to solid matrices has been reported to be between 0.51–4.77 μmol 6-APA/mg protein×min.[11]

Advantages achieved by using aqueous two-phase systems for biotechnological conversions are the following: the enzyme can be reused; good mass transfer is achieved between the phases, which will prevent accumulation of inhibitory products; high enzyme concentrations can be used; the activity can easily be reestablished when it is falling; no activity is lost due to the immobilization procedure. One disadvantage is that the product stream is polluted with PEG. In conclusion, aqueous two-phase systems can be

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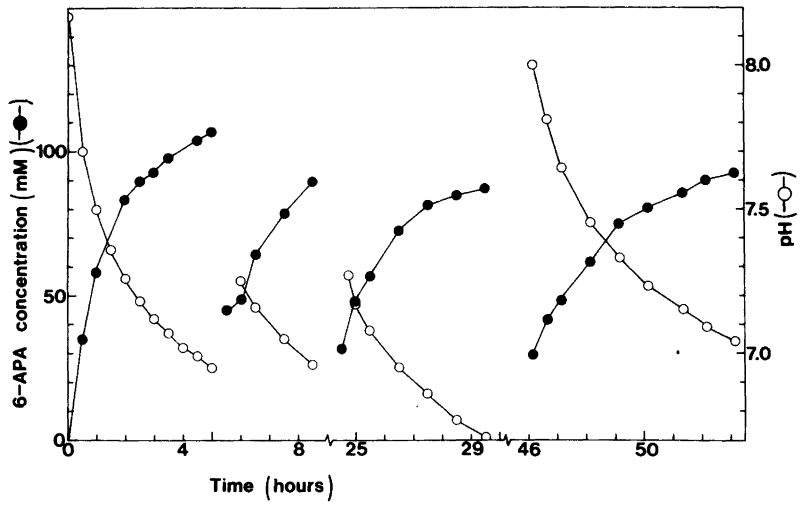

Fig. 2. Repeated batch conversion of BP (100 mg/ml phase system) to 6-APA with PA (0.3 mg protein/ml phase system, 105 U/mg protein) at 37 °C. The volume of the phase system was 50 ml. Aqueous two-phase system: PEG 20000, 8.9 % (w/w)/potassium phosphate, 7.6 % (w/w). Composition of added top phase: PEG 20000, 17 % (w/w)/potassium phosphate, 4.5 % (w/w). 6-APA concentration (●) and pH (○) are indicated.

an interesting alternative to immobilization to solid matrices.

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Levels of Metabolic Intermediates in *Streptococcus lactis* Grown on Different Carbon Sources and the Effect on Product Formation *

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The growing interest in using fermentation processes for producing both high value chemicals and chemical feed stocks, points out the necessity of understanding how the metabolism of the microbial cell is regulated.[1] With a deeper knowledge about these mechanisms it will be possible to direct fermentation processes towards higher yields of desired products, which is of utmost importance in situations where the price of the raw material often makes up much more than 50 % of the price of the product.

There are essentially two ways of gaining an understanding of the regulation of microbial metabolism: one is to identify the various enzymes along a route, show their specificity and regulatory function; the other is to quantify intermediary metabolites and from there identify which steps are regulatory. This communication describes the latter approach.

Methods for determining intermediary metabolite levels were developed by Lowry and Passonneau.[2] These methods preferably use the glycolytic and Krebs cycle enzymes to "pull" a certain metabolite towards a reduction of NAD or oxidation of NADH. This can then be measured fluorimetrically. These methods have so far only been used in a few applications. The metabolism of *E. coli* grown on various carbon and nitrogen sources has been studied.[3] Homo- and heterolactic fermentation in *S. lactis* has also been elucidated.[4]

This communication deals with the application of these fluorimetric methods to investigate the shift in product formation when *S. lactis* is grown on maltose as compared to glucose. A previous investigation had shown a pronounced heterofermentative product formation when *S. lactis* was grown in maltose, whereas glucose grown cells gave a homofermentative pattern.[5] In Fig. 1 the proposed metabolic routes for *S. lactis* grown on glucose and maltose, respectively, are shown. *S. lactis* 65.1 from the Swedish Dairies Association, Malmö, was grown on a complex media containing tryptone (5 g/l), yeast extract (5 g/l), caseamino acids (1 g/l), phosphate, magnesium sulfate and carbohydrates, (20 g/l glucose or 20 g/l maltose).

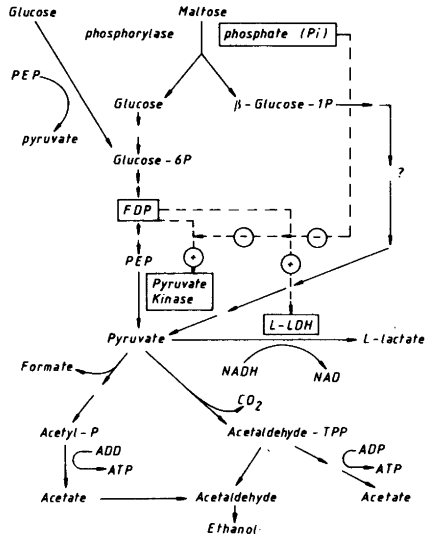

Fig. 1. Effects of metabolic regulation on product formation in *Streptococcus lactis* as suggested by Häggström (1981).

In a preliminary investigation it was found that the complex medium interfered with the measurements of pyruvate (PYR) and phosphoenolpyruvate (PEP), the disturbing components being tryptone and yeast extract. The concentration of these components was then decreased to 1/20 of the original values without decline in growth rate. At these concentrations they did not interfere with the measurements of metabolite levels.

The other problem involved when determining intracellular metabolite concentrations was to find a sampling technique which enabled the metabolism to be stopped in less than 2 s so that the pools of metabolites would not be changed due to starvation. Several sampling techniques were compared in the present study (Table 1). The reliability of these methods was tested by measuring intracellular levels of fructose – 1,6-

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Table 1. A comparison of the sampling techniques. All perchloric acid extracts were vortexed vigorously and were placed on ice for 5 min before centrifugation and neutralization to pH 7.0 with solid K_2CO_3. The clarified supernatants were stored at – 80 °C until analyzed, usually the next day. Values for FDP were calculated on the basis that 1 mg dry weight of cells contains 1.67 μl cytoplasmic fluid.[6]

Method	Glucose grown cells FDP (mM)	Maltose grown cells FDP (mM)	Advantages	Dis-advantages
Syringe[a]	30.1	20.5	Fastest immediate stop in metabolism	Cells dilute Medium present
Filter[b]	25.6	14.7	Fast Removes medium Concentrates cells	Vacuum may affect metabolism
Lyophilization[c]	26.7	13.6	Fast Immediate stop in metabolism Concentrates cells	Concentrates medium
Centrifuge[d]	7.3	4.5	Removes medium Concentrates cells	Slow

[a] With the syringe method samples are taken up in a syringe containing enough concentrated perchloric acid to give a 0.6 M final concentration. [b] The samples are filtered and the filter is transferred to liquid nitrogen in less than 2 seconds. Filters are subsequently extracted with 0.6 M perchloric acid. [c] The samples are dropped into liquid nitrogen and then lyophilized. The residue is then extracted as in b. [d] The samples are centrifuged for 5 min at 10 000 *g*, the supernatant decanted, and the pellet immediately extracted as in b.

Table 2. Effect of carbon source on metabolite levels and product patterns.

Carbon source	FDP(mM)	PEP(mM)	PYR(mM)	g/l EtOH	g/l HOAc	Lactate (mM)
Glucose	33	11.8	<3	N.D.[a]	N.D.[a]	0.246
Maltose	15	13.6	<3	0.199	0.133	0.206

[a] N.D.=not detectable.

diphosphate (FDP) in both glucose and maltose grown cells. Within experimental error the filter, syringe and lyophilization methods gave the same results (Table 1), whereas centrifugation appeared to interfere with metabolism, most likely in that it was the slowest method. Because of its simplicity and rapidity, the filter method was utilized in all further experiments.

Intracellular levels of fructose-1,6-diphosphate (FDP), phosphoenolpyruvate (PEP) and pyruvate (PYR) were then determined using batch fermentations of *S. lactis* grown on glucose or maltose, since these metabolites were considered key intermediates in understanding the shift from homo to heterolactic product formation. At least eight samples were taken during the exponential growth phase and analyzed in duplicate. The data given in Table 2 are the average values. The extracellular products lactate, ethanol and acetate were also determined.[5]

When *S. lactis* was grown on glucose, the intracellular concentration of FDP was about double compared to when the organism was grown on maltose (Table 2). The levels of PEP and PYR, however, were about the same for both carbon sources.

The FDP data support the hypothesis that the two sugars in maltose are metabolized along

different metabolic routes (Fig. 1) and that only half of the maltose molecule is processed along the Embden Meyerhof Pathway. As such, the decreased level of FDP in maltose grown cells may not be high enough to stimulate the L-lactate dehydrogenase to completely convert pyruvate to L-lactate, thus, opening the possibility for pyruvate to be metabolized to other end-products. Phosphate involved in the internal splitting of maltose has also been proposed as a regulatory factor.[5]

Although PEP in the maltose grown cells would also be expected to be one half of that found in glucose grown cells, the fact that PEP is used in the transport of glucose, but not necessarily of maltose,[5] may explain why this metabolite has a similar concentration in both cases (Table 2). Pyruvate concentrations are very low in the two situations, due to rapid funneling of this metabolite into the various pathways.

In conclusion, this study shows that it is possible to measure concentrations of intracellular intermediary metabolites even in fermentations based on complex media, and that these measurements can contribute to the understanding of how microbial metabolism is regulated.

Acknowledgements. This study was supported by the Swedish Board for Technical Development and the Swedish Natural Science Research Council.

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Temperature Dependence of Activation Parameters in the Neutral Ester Hydrolysis in Methanol–Water Solutions

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The kinetic behaviour of the uncatalysed reactions of methyl trifluoroacetate and chloromethyl dichloroacetate in MeOH-water solutions have been studied. In the water-rich region, the plots of $\Delta H^{\neq}$ *vs.* x_w for the hydrolysis of methyl trifluoroacetate have a maximum at lower and a minimum at higher temperatures. The plots of $T\Delta S^{\neq}$ *vs.* x_w have no extreme points in the temperature range studied. In the case of chloromethyl dichloroacetate, the values of activation parameters represent the simultaneous hydrolysis and methanolysis.

The heat capacity of activation has a minimum value -324 ± 6 J mol^{-1} K^{-1} at x_w 0.843 for methyl trifluoroacetate and -244 ± 13 J mol^{-1} K^{-1} at x_w 0.950 for chloromethyl dichloroacetate. For the same reactions in water the values of $\Delta C_p^{\neq}$ are -245 ± 5 and -182 ± 8 J mol^{-1} K^{-1}, respectively. The positions of the minima of $\Delta C_p^{\neq}$ are in accord with the anticipated effects of the substrate at the "magic" mole fraction 0.85 of water, but in the case of chloromethyl dichloroacetate the value of $\Delta C_p^{\neq}$ at the minimum may be too positive owing to the effects of the simultaneous methanolysis and hydrolysis.

Liquid water has a random, three-dimensional structure in which each water molecule attempts to form four tetrahedrally-disposed hydrogen bonds with its nearest neighbours.[1] If an organic co-solvent is added to water it is possible to change this hydrogen-bonded network continuously. Considerable information is available on the properties of dilute aqueous solutions of alcohols[2] which, according to their thermodynamic properties, belong to a class of typically aqueous (TA) solutions.[3–5] It is known that small amounts of a monohydric alcohol promote the formation of hydrogen bonds between the surrounding water molecules in a co-operative manner but larger amounts break the enhanced water structure. Thus, in the water-rich region, there is an alcohol concentration where a maximal water structure is reached. This "magic" mole fraction of water is determined by the nature of the added organic co-solvent. In alcohol–water solutions it has been found that the maximal water structure is reached when the mole fraction of water (x_w) is about 0.85, 0.91, 0.94, 0.96 and 0.98 in the case of methanol (MeOH), ethanol (EtOH), isopropyl alcohol (i-PrOH), *tert*-butyl alcohol (*t*-BuOH), and 2-butoxyethanol (2-BE), respectively.[3,6]

The thermodynamic activation parameters, activation enthalpy ($\Delta H^{\neq}$), activation entropy ($\Delta S^{\neq}$), and especially the heat capacity of activation ($\Delta C_p^{\neq} = \mathrm{d}\Delta H^{\neq}/\mathrm{d}T$)) have been widely employed as probes for the study of solvent effects in aqueous binaries.[3,5,7–12] The heat capacity of activation, found to differ from zero in most ionogenic solvolytic reactions, is usually assumed to be a genuine rate parameter of a single-step reaction.[7,13,14] Its value may depend on temperature and is mainly determined by the solvation differences between the initial and transition states. Another explanation for $\Delta C_p^{\neq}$ is based on a two-step mechanism with a tetrahedral intermediate, the first step of the reaction being reversible.[15–23] It has been, however, recently concluded that the heat capacity of activation and its derivatives are real rate parameters which may include a "spurious" contribution caused by the partitioning of a possible intermediate.[14]

The aim of the present work was to study the behaviour of the reactions of methyl trifluoro-

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acetate and chloromethyl dichloroacetate in different MeOH–water solutions over a wide temperature range. The reactions are assumed to take place as a general base catalysed ester hydrolysis, $B_{AC}3$.[9,10,24]

EXPERIMENTAL

Methyl trifluoroacetate, a commercial product (E. Merck AG, zur Synthese), was redistilled before use. Chloromethyl dichloroacetate was prepared by chlorinating methyl dichloroacetate with gaseous chlorine.[25] The solvent mixtures were prepared by diluting a known weight of distilled water with MeOH (E. Merck AG, getrocknet, *pro analysi*) to a known volume in a volumetric flask. The initial ester concentrations were about 10^{-4} M. The temperature was stable to about 0.01 K.

The reactions were followed conductometrically as described earlier.[11] Actually the concentrations of trifluoroacetic acid in the hydrolysis of methyl trifluoroacetate and that of hydrochloric and dichloroacetic acids in the reactions of chloromethyl dichloroacetate were measured.[9-11]

The rate constants were calculated by Guggenheim's method.[26] In some cases also final values were measured, and the obtained rate constants were essentially the same. The accuracy of the kinetic data was as described earlier.[11] The thermodynamic activation parameters were calculated by an extended Arrhenius equation, eqn. (1), employing the method of Clarke and Glew[27] after an orthogonalization procedure.

$$\ln k = A + B/T + C \ln T + DT + ET^2 + \ldots + \varepsilon \quad (1)$$

According to Student's *t*-test only the first three parameters of eqn. (1) were significant at the 95 % level. Exceptions to this are the reactions of methyl trifluoroacetate at x_w=0.740 and that of chloromethyl dichloroacetate at x_w=0.599 where also the fourth parameter was significant, probably because of, *e.g.*, systematic errors in rate measurements. In the following treatment, eqn. (1) has been used in its three-parametric form.

When studying the reaction of chloromethyl dichloroacetate in MeOH–water solutions, the possibility that both hydrolysis and methanolysis reactions take place concurrently cannot be ignored. In fact, a gas-chromatographic product analysis from the uncatalysed reaction of chloromethyl dichloroacetate revealed that the methanolysis product, methyl dichloroacetate, was formed during the reaction when x_w was about 0.9 or smaller. On the other hand, it was found that the esterification of the hydrolysis product, dichloroacetic acid, did not produce methyl dichloroacetate under the reaction conditions used. These results are in agreement with those from the uncatalysed reaction of chloromethyl chloroacetate in MeOH–water solutions when it has been possible to separate the simultaneous hydrolysis and methanolysis reactions.[31] Further, it has been found that when x_w is gradually lowered, MeOH decreases the hydrolysis rate of chloromethyl chloroacetate but water accelerates the rate of the alcoholysis so that at about x_w 0.72 the rate of the methanolysis exceeds that of the hydrolysis.

Table 1. Temperature range (K), number of kinetic runs, calculated values of k (s^{-1}), and activation parameters $\Delta H^{\neq}$ (J mol^{-1}), $\Delta S^{\neq}$ (J mol^{-1} K^{-1}), and $\Delta C_p^{\neq}$ (J mol^{-1} K^{-1}) at 298.15 K in the reactions of methyl trifluoroacetate in MeOH–water mixtures at the mole fraction x_w of water.

x_w	Temp. range	No. of runs	10^3k	$\Delta H^{\neq}$	$-\Delta S^{\neq}$	$-\Delta C_p^{\neq}$
1.000	273–313	24	8.459	39700(40)	151.4(1)	245(5)
0.994	273–313	26	8.196	39950(40)	150.9(1)	250(5)
0.991	273–313	20	7.987	39810(30)	151.6(1)	242(4)
0.972	273–316	23	7.138	39770(20)	152.6(1)	252(4)
0.930	273–320	24	5.474	39300(20)	156.4(1)	281(3)
0.922	273–318	11	5.168	39190(50)	157.3(2)	294(8)
0.875	273–318	11	3.747	38750(60)	161.4(2)	322(8)
0.843	273–318	11	3.016	38580(40)	163.8(1)	324(6)
0.777	273–320	12	1.827	37870(50)	170.3(2)	292(7)
0.740	273–320	11	1.372	37770(40)	173.0(1)	265(6)
0.690	273–320	18	0.949	37760(60)	176.2(2)	250(8)

Table 2. Temperature range (K), number of kinetic runs, calculated values of k (s^{-1}), and activation parameters $\Delta H^{\ddagger}$ (J mol^{-1}), $\Delta S^{\ddagger}$ (J mol^{-1} K^{-1}), and $\Delta C_p^{\ddagger}$ (J mol^{-1} K^{-1}) at 298.15 K in the reactions of chloromethyl dichloroacetate in MeOH–water mixtures at the mole fraction x_w of water.

x_w	Temp. range	No. of runs	10^2k	$\Delta H^{\ddagger}$	$-\Delta S^{\ddagger}$	$-\Delta C_p^{\ddagger}$
1.000	273–303	11	1.460	39180(90)	148.7(3)	182(8)
0.980	273–303	12	1.322	39040(100)	150.0(3)	189(8)
0.968	273–306	14	1.199	38080(140)	154.0(5)	230(15)
0.950	273–306	14	1.115	38130(130)	154.4(4)	244(13)
0.894	273–313	9	0.819	37900(80)	157.7(3)	266(10)
0.843	273–318	12	0.602	38340(60)	158.8(2)	197(9)
0.814	273–320	14	0.432	39140(40)	158.9(1)	166(6)
0.697	273–328	14	0.250	39940(60)	160.8(2)	133(8)
0.599	273–328	12	0.165	40000(100)	164.0(4)	174(12)
0.500	273–328	14	0.113	40130(80)	166.7(3)	162(11)

RESULTS AND DISCUSSION

Kinetic data at 298.15 K for the neutral reactions of methyl trifluoroacetate and chloromethyl dichloroacetate in the MeOH–water mixtures studied, are given in Tables 1 and 2, respectively. As can be seen from the Tables, MeOH decreases the rates of the hydrolyses. The retarding effect seems to be quite small when compared to the rate lowering effect caused by other organic co-solvents such as acetone,[28,29] 2-butanone,[30] 2-BE[9,10] and acetonitrile[28,29] in the hydrolyses of the same esters. This observation agrees with the fact that, of these co-solvents, MeOH is the most water-like solvent as it is a small molecule which can be hydrogen-bonded to other alcohol and water molecules.

In most neutral solvolytic reactions, organic co-solvents added to water, have been found to produce large and almost compensating changes upon $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$.[5,7–12,28–37] In solvent mixtures, rich in water, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ first decrease to a minimum, the occurrence and position of which in the solvent composition has been thought to be connected with the solvent structure. In the reactions of methyl trifluoroacetate and chloromethyl dichloroacetate in MeOH–water solutions there seems to be shallow minima in $\Delta H^{\ddagger}$ at x_w about 0.7 and 0.9 at 298.15 K (Tables 1 and 2; Figs. 1 and 2), respectively. However, when $\Delta H^{\ddagger}$ is plotted *vs.* x_w at different temperatures for the hydrolysis of methyl trifluoroacetate (Fig. 1), the plots show the unusual behaviour that $\Delta H^{\ddagger}$ has a maximum at x_w=0.875 at 273 K and a minimum at x_w about 0.8 at 313 K. Although it is not possible to draw any far-reaching conclusions from the reaction of chloro-

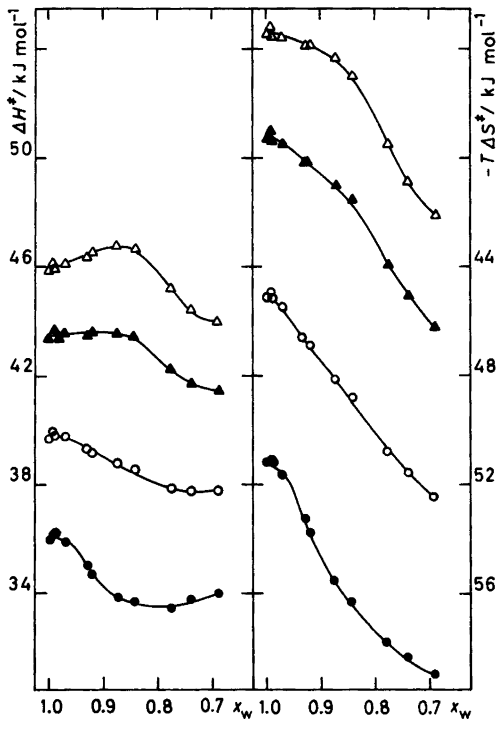

Fig. 1. Plots of $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ *vs.* x_w for the neutral hydrolysis of methyl trifluoroacetate in methanol-water mixtures at the temperatures 273 K (△); 283 K (▲); 298 K (○); 313 K (●).

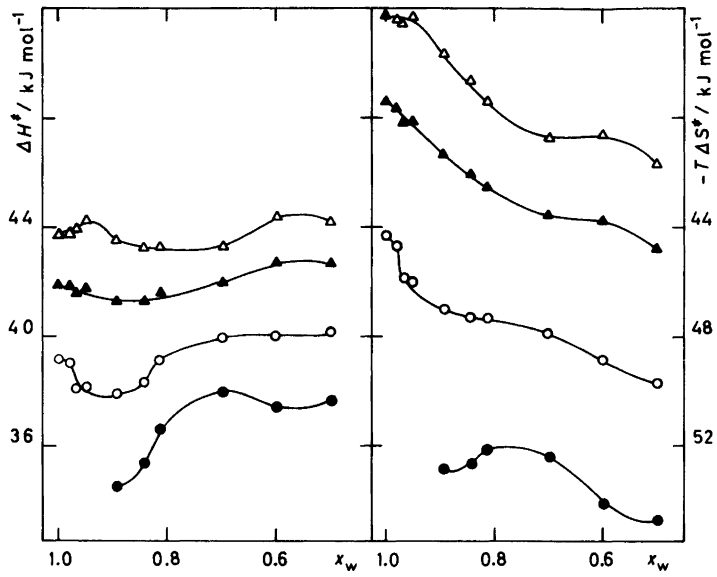

Fig. 2. Plots of $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ *vs.* x_w for the neutral reaction of chloromethyl dichloroacetate in methanol-water mixtures at the temperatures 273 K (△); 283 K (▲); 298 K (○); 313 K (●).

methyl dichloroacetate in MeOH–water mixtures because the activation parameters then include the simultaneous hydrolysis and methanolysis, it seems that also in this case $\Delta H^{\ddagger}$ first increases when $x_w \geq 0.95$ at 273 K (Fig. 2). These unusual concentration dependences of $\Delta H^{\ddagger}$ for the present reactions at different temperatures may be understood by the solvation variation of the initial state, in accord with the results of Arnett *et al.*,[38] if it is expected that carbon dioxide can serve as a model compound for the present esters. It has been found that the solubility of carbon dioxide in MeOH–water solutions increases when x_w goes from 1.00 to about 0.95 and then decreases to x_w about 0.9 leading to a minimum in the enthalpy of solution in the highly water-rich MeOH solutions.[39] These properties are especially important at lower temperatures and gradually vanish when the temperature is raised from 277.9 to 333.4 K.

Contrary to the hydrolysis of chloromethyl dichloroacetate, it has been found that for the hydrolysis of methyl trifluoroacetate in aqueous binaries there are no minima in $\Delta S^{\ddagger}$ but the plots of $\Delta S^{\ddagger}$ *vs.* x_w have only an inflexion point.[7,8,28,29,34] This is also true for the neutral hydrolysis of methyl trifluoroacetate in the MeOH–water solutions studied (Fig.1), in spite of the peculiar behaviour of the plots of $\Delta H^{\ddagger}$ *vs.* x_w at different temperatures.

Although in an aqueous binary the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of solvolytic reactions are certainly connected with the solvent structure, the heat capacity of activation is the most valuable rate parameter for studying the specific role of the solvent reorganization during the activation process. In the water-rich region of an aqueous solution which belongs to the class TA, the values of $\Delta C_p^{\ddagger}$ decrease until a mimimum value is reached. The place and the depth of the minimum depend on the organic co-solvent used and the hydrophobic character of the reactant. Thus Robertson and Sugamori[33] have found that for the solvolysis of *tert*-butyl chloride, $\Delta C_p^{\ddagger}$ goes from the value -347 ± 19 J mol^{-1} K^{-1} in water to the minimum values -569 ± 33, -565 ± 17, and -812 ± 33 J mol^{-1} K^{-1} in EtOH–, i-PrOH–, and *t*-BuOH–water solutions at x_w 0.925, 0.95, and 0.95, respectively. In these cases the positions of the minima occur at the "magic" mole fractions of the binary systems studied[3] and further, within the limits of the experimental accuracy, the changes in the values of $\Delta C_p^{\ddagger}$ from water to the minimum values, $\Delta\Delta C_p^{\ddagger} = \Delta C_p^{\ddagger}(H_2O) - \Delta C_p^{\ddagger}$ (minimum), become greater when the alcohol component of the binary is changed from EtOH

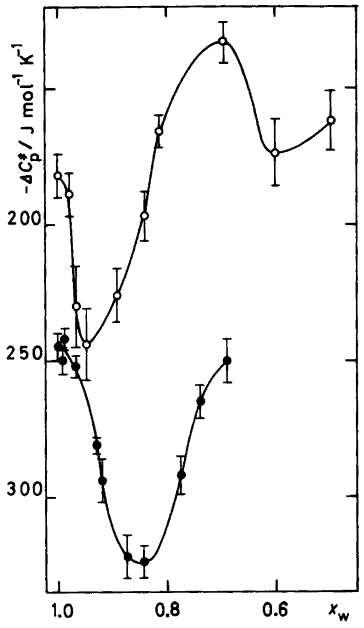

Fig. 3. Plots of $\Delta C_p^{\neq}$ *vs.* x_w for the neutral reactions of methyl trifluoroacetate (●) and chloromethyl dichloroacetate (○) in methanol-water mixtures at 298.15 K.

through i-PrOH to *t*-BuOH, $\Delta\Delta C_p^{\neq}$ being 222, 218 and 465 J mol^{-1} K^{-1}, respectively. In accordance with the above conclusions, $\Delta C_p^{\neq}$ is at a minimum at x_w=0.843 and 0.950 for the reactions of methyl trifluoroacetate and chloromethyl dichloroacetate, respectively, in the MeOH–water solutions studied (Tables 1 and 2; Fig. 3). It has been earlier found for the hydrolyses of the same esters that, if the organic component of an aqueous binary is the same, *e.g.*, acetone, $\Delta C_p^{\neq}$ reaches a minimum value at a lower x_w in the case of methyl trifluoroacetate than in that of chloromethyl dichloroacetate.[7,8,28,29] Thus when chloromethyl dichloroacetate reacts in aqueous methanol the effect of the substrate explains the fact that maximal water structure around the ester molecule is reached in a more water-rich region than could be expected on the basis of the "magic" mole fraction 0.85 of water. Further, according to the above-mentioned results for the solvolysis of *tert*-butyl chloride in alcohol–water solutions,[33] the depth of the minimum ($\Delta\Delta C_p^{\neq}$=79 J mol^{-1} K^{-1}) for the hydrolysis of methyl trifluoroacetate in MeOH–water solutions seems to be quite natural. The conclusion that the co-solvent in water primarily determines the value of $\Delta\Delta C_p^{\neq}$, is further supported by the results from the hydrolysis of the same ester in *t*-BuOH–[40] and 2-BE–water solutions[9,10] the values of $\Delta\Delta C_p^{\neq}$ at 298.15 K being 254 and 378 J mol^{-1} K^{-1}, respectively. In the last-mentioned hydrolyses the values of $\Delta C_p^{\neq}$ depend considerably on temperature. On the other hand, the simultaneous hydrolysis and methanolysis in the reaction of chloromethyl dichloroacetate in water-rich methanol solutions may cause a positive contribution to the values of $\Delta C_p^{\neq}$ and thus the depth of the minimum ($\Delta\Delta C_p^{\neq}$=62 J mol^{-1} K^{-1}) may be too small.

In disagreement with the solvent effects on $\Delta C_p^{\neq}$ described above are the results of Huq[35] for the hydrolysis of *tert*-butyl chloride in MeOH–water solutions. In this case the values of $\Delta C_p^{\neq}$ decrease from −111 J mol^{-1} K^{-1} in water to a minimum value of −3046 J mol^{-1} K^{-1} at x_w=0.935. However, these results, based on only four experimental temperatures (from 273 to 293 K), do not fulfil all of the demands which are generally expected for heat capacity measurements.[11]

The present results show that the solvent effects, caused by a water-structure-making co-solvent, MeOH, on the activation parameters, especially on $\Delta C_p^{\neq}$, are small when compared to the solvent effects of the higher analogues in an alcohol series. This is in accordance with the small effect of added MeOH on water structure. Further, contrary to the high temperature dependence of $\Delta C_p^{\neq}$ found in *t*-BuOH–[40] and 2-BE–water solutions[9,10] in the hydrolyses of methyl trifluoroacetate and chloromethyl dichloroacetate, the values of $\Delta C_p^{\neq}$ for the reactions of the same esters in MeOH–water solutions do not seem to depend on temperature.

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Metal Ion Oxidation. IX.* Oxidation of Aromatic Hydrocarbons and Arylacetic Acids by Heteropoly Anions Containing Ni(IV), Mn(IV) and Co(III) Ions as Central Atoms

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Heteropoly ions containing Ni(IV) and Mn(IV) as central atoms have been shown to oxidize aromatic hydrocarbons and arylacetic acids in acetic acid and acetic acid–water, yielding acetates and alcohols. The product pattern of these reactions supports an outer-sphere electron transfer mechanism. Substituted arylacetic acids are decarboxylated when treated with 12-tungstocobalt(III)ate ion and 9-molybdonickel(IV)ate ion. These decarboxylation reactions are proposed to be outer-sphere electron transfer processes.

Recent work on the oxidation of aromatic compounds with potassium 12-tungstocobalt(III)ate, $K_5[Co(III)W_{12}O_{40}]$, [hereafter denoted Co(III)W] has demonstrated that heteropoly ions with high valent metal ions as central atoms can act as outer-sphere electron transfer reagents.[1–3] Co(III)W consists of a cobalt(III) ion buried within a shell of WO_6 octahedra and has no possibility of binding to external species.[4] Other heteropoly ions with Ni(IV) or Mn(IV) contained within a shell of nine MoO_6 octahedra may also act as outer-sphere oxidants in organic redox processes.[4] I now report that ammonium 9-molybdonickel(IV)-ate,[5] $(NH_4)_6[Ni(IV)Mo_9O_{32}]\cdot 6\ H_2O$, and ammonium 9-molybdomangan(IV)-ate,[5] $(NH_4)_6[Mn(IV)Mo_9O_{32}]\cdot 6\ H_2O$ [denoted Ni(IV)Mo and Mn(IV)Mo] can oxidize aromatic hydrocarbons and arylacetic acids in non-aqueous or aqueous media with concomitant reduction to Ni(II) and Mn(II) species [eqns. (1)–(3].

$$ArCH_3 \xrightarrow[AcO^-]{\text{Ni(IV)Mo or Mn(IV)Mo}} ArCH_2OAc \quad (1)$$

$$ArH \xrightarrow[AcO^-]{\text{Ni(IV)Mo}} ArOAc \quad (2)$$

$$ArCH_2COOH \xrightarrow[AcO^-]{\text{Ni(IV)Mo or Co(III)W}} ArCH_2OAc + CO_2 \quad (3)$$

In a recent paper it was shown that Co(III)W also has the ability to oxidize arylacetic acids in acetic acid [eqn. (3)].[2] Hence it is possible to study the metal-ion-promoted decarboxylation of arylacetic acids by several outer-sphere oxidants. Other metal ion decarboxylations of arylacetic acids have been extensively studied, and two different mechanisms have been suggested,[6–8] one involving homolysis of a metal-carboxylate and the other electron transfer from the aromatic ring. These suggestions have mainly been based on studies of competitive decarboxylations of substituted arylacetic acids by different metal ion complexes, such as Co(III), Ce(IV) and Cu(III), which can form a ligand-to-metal bond to the acid (inner-sphere mechanism).

In order to compare outer-sphere decarboxylation with these inner-sphere decarboxylations, a

* Part VIII, see Jönsson, L. *Acta Chem. Scand. B 35* (1981) 683.

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series of competitive oxidations by Co(III)W and Ni(IV)Mo has been studied. The results indicate that decarboxylations by Ni(IV)Mo, Co(III)W, $Co(III)(OAc)_3$, Cu(III) and Ce(IV) follow an outer-sphere electron transfer mechanism.

RESULTS

Oxidation of aromatic hydrocarbons. The Ni(IV)Mo and Mn(IV)Mo complexes were soluble in water and acetic acid–water, but insoluble in glacial acetic acid. The Ni(IV)Mo complex was slowly reduced and hydrolyzed in water or acetic acid–water at room temperature, whereas the Mn(IV)Mo complex was stable against hydrolysis and redox processes induced by these media below 60 °C. In connection with these qualitative studies of the redox stability of the heteropoly anions, the potential of the Mn(IV)/Mn(III) or Mn(II) heteropoly ion couple has been determined to be 1.4 V *vs.* NHE by cyclic voltammetry. It was impossible to obtain a value for the Ni(IV)/Ni(II) couple by this method. Latimer gives[9] an E_o value of 1.8 V for $Ni(IV)O_4^{2-}$/Ni(II), and it is reasonable that Ni(IV)Mo also has this high oxidizing power.

In spite of the instability of Ni(IV)Mo in acetic acid–water it was possible to oxidize 4-methoxytoluene in this medium, resulting in a moderate yield (44 %) of the corresponding benzyl acetate and alcohol. With 0.5 M KOAc present in glacial acetic acid, the yields of acetate and aldehyde increased to 51 % and 13 %, respectively, and the proportion of alcohol decreased. The results of these and other oxidations of 4-methoxytoluene by Ni(IV)Mo and Mn(IV)Mo are presented in Table 1. Toluene gave 40 % benzyl acetate and 10 % benzaldehyde on oxidation by Ni(IV)Mo (in acetic acid-0.5 M KOAc), whereas 4-nitrotoluene completely reduced Ni(IV)Mo, but gave no detectable products. This remarkable behaviour was also noted for Co(III)W

Table 1. Acetoxylation of 4-methoxytoluene by Ni(IV)Mo and Mn(IV)Mo under different conditions.[a]

Reagent	Reaction conditions	Yield/ %[b] 4-Methoxybenzyl acetate	4-Methoxybenzaldehyde	4-Methoxybenzyl alcohol
Ni(IV)Mo	$HOAc/H_2O$ (4:1 v/v)	44	2	9
Ni(IV)Mo	$HOAc/H_2O$ (1:1 v/v)	21	4	28
Mn(IV)Mo	$HOAc/H_2O$ (1:1 v/v)	20	6	25
Ni(IV)Mo	HOAc	42	13	7
Ni(IV)Mo	HOAc, 0.5 M KOAc	51	13	<1
Mn(IV)Mo	HOAc, 0.5 M KOAc	7	1	<1

[a] Reaction conditions: 4-Methoxytoluene (4 mmol), reagent (0.2 mmol), solvent (10 ml), reflux temperature, reaction period 2 h under an argon atmosphere. [b] GLC based on Ni(IV) or Mn(IV).

Table 2. Nuclear acetoxylation of aromatic compounds by Ni(IV)Mo.[a]

Compound	Yield of nuclear acetates/%[b]	Isomer distribution This work			Co(III)W oxidation[c]			Anodic oxidation[d]		
		o	*m*	*p*	*o*	*m*	*p*	*o*	*m*	*p*
Anisole	13	53	5	42	54	4	42	60	2	38
Biphenyl	40	18	<0.1	82	16	<0.1	84	31	1	68

[a] Reaction conditions, unless otherwise noted: Aromatic compound (1 mmol), Ni(IV)Mo (0.2 mmol), glacial acetic acid containing 0.5 M KOAc (5 ml) acetic anhydride (1 ml) reflux temperature, reaction period 1 h. [b] GLC yield based on Ni(IV). [c] In acetic acid–water (4:1 v/v) containing 0.1 M KOAc, temperature 102 °C, Ref. 2. [d] In acetic acid–water – 1.0 M NaOAc (in anisole oxidation $[OAc^-]$=0.1 M) at 25 °C, Ref. 2.

Table 3. Relative rates of Co(III)W and Ni(IV)Mo oxidation of substituted arylacetic acids in acetic acid-KOAc.[a]

Substituent	Relative rate[b] Co(III)W	Ni(IV)Mo
p-OCH_3	2200±500	44.6±1
m-OCH_3	69.4±4	7.1±0.2
p-Ph	14.8±3	2.6±0.1
p-CH_3	2.00±0.08	1.95±0.02
m-CH_3	1.28±0.05	1.15±0.02
H	1.00	1.00
p-Cl	0.36±0.05	1.00±0.02
m-Cl	0.25±0.04	0.55±0.05

[a] Reaction conditions: Substituted arylacetic acid (2 mmol), phenylacetic acid (2 mmol), potassium 12-tungstocobalt(III)ate or ammonium 9-molybdonickel(IV)ate (0.1 mmol), glacial acetic acid–0.5 M KOAc (10 ml) (at Ni(IV) oxidation glacial acetic acid –1 M KOAc (10 ml) and acetic anhydride (2 ml)), reflux temperature, reaction period 2 h under an argon atmosphere. [b] Values given are the average of two or four independent experiments, each analyzed twice by GLC.

oxidation.[2]

Under non-aqueous conditions with 0.5 M KOAc present, substrates without α hydrogen gave nuclear acetoxylation (see Table 2). The isomer distribution is analogous to that observed for Co(III)W and anodic acetoxylation.[2] In all reactions above, and in the decarboxylation reactions, Ni(IV)Mo was reduced to a weak blue-green coloured 6-heteropoly anion, containing Ni(II). The Mn(IV)Mo was reduced to Mn^{2+} during decomposition of the heteropoly anion.

Isotope effects. The kinetic isotope effect was determined for the Ni(IV)Mo α acetoxylation of toluene and 4-methoxytoluene by allowing Ni(IV)Mo to react with a large excess of an equimolar amount of protiated and deuterated compound. By determining the deuterium contents (MS) of the benzyl acetates, a k_H/k_D value of 4.2±0.1 and 2.7±0.1 was obtained for toluene and 4-methoxytoluene, respectively.

Decarboxylation of arylacetic acids. The oxidative decarboxylation of arylacetic acids by Co(III)W and Ni(IV)Mo in refluxing acetic acid, containing 0.5 M KOAc and acetic anhydride, resulted in the formation of benzyl acetates, which accounted for more than 80 % of all products observed. An attempt to decarboxylate 4-nitrophenylacetic acid by Co(III)W and Ni(IV) gave no detectable products, but the reagents were completely reduced within 2 h.

Competitive experiments. Competitive decarboxylations of a series of substituted arylacetic acids by Co(III)W and Ni(IV)Mo were carried out. The relative rates were determined from the relative amounts of the corresponding benzyl acetates produced. (For results and reaction conditions, see Table 3.) Excluding the rate constants for the methoxy and phenyl compounds, which appear to be abnormally high, the logarithms of the relative rates were plotted against the substituent constants[10] σ and σ^+ and gave a ρ value of −1.71 (r=0.997) and a ρ^+ value of −0.77 (r=0.985) for Co(III)W and Ni(IV)Mo decarboxylation, respectively. If the data for the methoxy and phenyl compounds are included, the correlation is not significant. Correlation of the logarithms of the relative rates for Ni(IV)Mo decarboxylation against σ gave no significant correlation. The logarithms of the relative rates of Ni(IV)Mo decarboxylation were also plotted against the logarithms of the relative rates of Co(III)W, $Co(III)(OAc)_3$, Cu(III) and Ce(IV) decarboxylation. Data from this work, and Refs. 7, 8 and 6b, respectively. See Figs. 1–4.

DISCUSSION

The results of the Ni(IV)Mo and Mn(IV)Mo oxidation of aromatic hydrocarbons show that the product and isomer distributions are in conformity with those obtained in the acetoxylation of the same compounds by established electron transfer reagents such as anodic,[11]

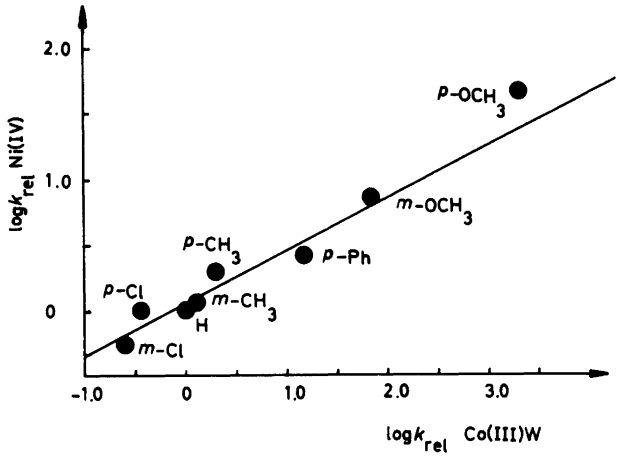

Fig. 1. Plot of log k_{rel} of decarboxylation by Ni(IV)Mo *vs.* log k_{rel} of decarboxylation by Co(III)W. Slope=0.40 (r=0.97).

Ce(IV),[12] Ag(II)[13] and 12-tungstocobalt(III)ate ion[2] (Co(III)W). (See Tables 1 and 2.) The relatively large amount of aldehydes produced in acetic acid–acetate ion media is due to geminal bis-acetoxylation of methyl arenes yielding $ArCH(OAc)_2$, followed by decomposition in GLC to aldehydes. This reacion is well-known from other electron transfer acetoxylations of alkyl-aromatic compounds.[11]

The observed primary kinetic isotope effects (toluene, k_H/k_D=4.2, 4-methoxytoluene, k_H/k_D=2.7) are in accord with a mechanism including an electron transfer step, followed by a rate-limiting proton transfer step. (For a discussion of the isotope effect in an electron transfer initiated reaction followed by proton transfer, see Refs. 2a and 14.)

Since Ni(IV)Mo and Mn(IV)Mo are inert to substitution they are able to oxidize aromatic compounds *via* an electron transfer initiated reaction with no bridging ligand between the oxidant and reductant in the transition state.

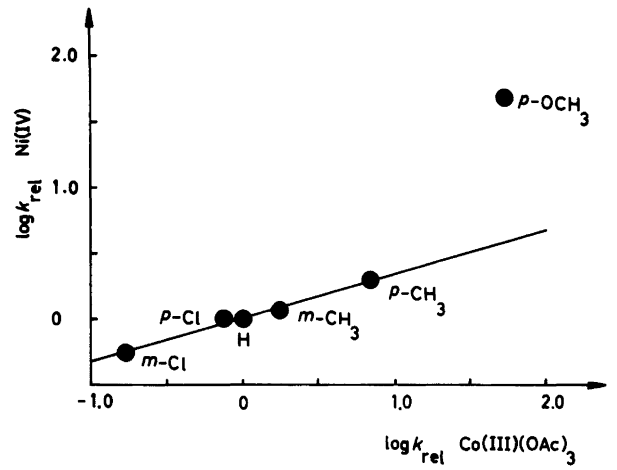

Fig. 2. Plot of log k_{rel} of decarboxylation by Ni(IV)Mo *vs.* log k_{rel} of decarboxylation by $Co(III)(OAc)_3$. Slope=0.33 (r=0.99).

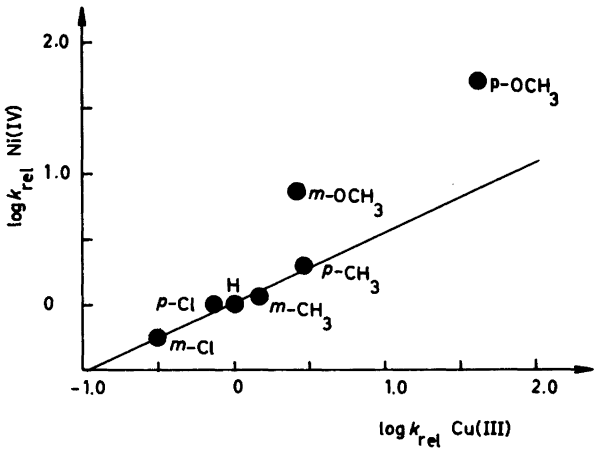

Fig. 3. Plot of log k_{rel} of decarboxylation by Ni(IV)Mo *vs.* log k_{rel} of decarboxylation by Cu(III). Slope=0.54 (r=0.98).

In the light of these results and the properties of the reagents, a mechanism, analogous to that for Co(III)W acetoxylation, involving a reversible electron transfer step followed by a rate-limiting proton transfer step, is most probable (eqns. (4)–(7)).

$$ArCH_3 + M(IV)Mo \rightleftarrows ArCH_3^{\cdot +} + M(III)Mo \quad (4)$$

$$ArCH_3^{\cdot +} + B \rightarrow ArCH_2^{\cdot} + HB^+ \quad (5)$$

$$ArCH_2^{\cdot} + M(III)Mo \rightarrow ArCH_2^+ + M(II)Mo \quad (6)$$

$$ArCH_2^+ + Nu^- \rightarrow ArCH_2Nu \quad (7)$$

However, this mechanism is more complicated than Co(III)W acetoxylation, since Co(III)W is a one-electron reagent. Ni(IV)Mo and Mn(IV)Mo are two-electron reagents, and an intermediate M(III)Mo species can act as oxidant ion in both steps 1 and 3, in competition with M(IV)Mo.

We therefore need more quantitative information about these reactions, such as kinetic data, the effect of additives *etc.*, to establish a definitive mechanism. We have now also begun to

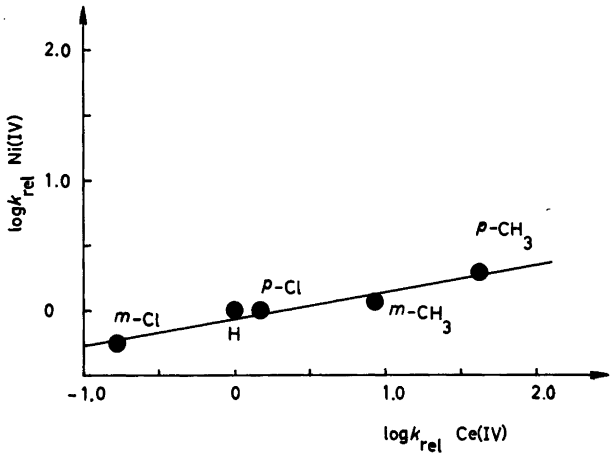

Fig. 4. Plot of log k_{rel} of decarboxylation by Ni(IV)Mo *vs.* log k_{rel} of decarboxylation by Ce(IV). Slope=0.21 (r=0.97).

investigate the remarkable fact that Co(III)W and Ni(IV)Mo are completely reduced by media containing 4-nitrotoluene or 4-nitrophenylacetic acid, with no detectable products so far.

The relative rates calculated in Co(III)W and Ni(IV)Mo initiated decarboxylation of arylacetic acids containing electron-releasing substituents are very high, based on expectations derived from the Hammett correlations (see Table 3). These results are in agreement with work on Ce(IV) and Cu(III) promoted decarboxylation of arylacetic acids.[6b,8]

To explain these observations Trahanovsky *et al.* suggested two mechanistic pathways for Ce(IV) decarboxylation of arylacetic acids.[6b] Acids which give a good Hammett correlation (see Table 4) are suggested to follow a pathway involving homolysis of a metal-carboxylate complex (inner-sphere complex). The unusually rapid rates of oxidation of both 3- and 4-methoxyphenylacetic acids suggest that these acids are oxidized *via* a mechanism involving initial formation of the radical cation of the aromatic ring. The analogous results obtained in Cu(III) initiated decarboxylation were explained in the same manner.[8]

Since it is impossible to form an inner-sphere complex between Co(III)W or Ni(IV)Mo and the substrate, one of these explanations is not relevant for the decarboxylations described in this paper. The results instead show that two different outer-sphere electron transfer mechanisms must exist. One mechanism is assumed to involve an electron transfer from the aromatic ring in arylacetic acids containing electron-rich substituents (Scheme 1).

Table 4. ρ-Values for decarboxylation of arylacetic acids by different oxidants.

Oxidant	ρ	ρ^+	Ref.
$SO_4^{\cdot -}$	−0.60[a]	−0.44[a]	17
Ce(IV)		−2.9	6b
Co(III)		−2.9[a]	7
Cu(III)		−1.4	8
Co(III)W	−1.7		This work
Ni(IV)Mo		−0.77	This work

[a] The correlation includes results from arylacetic acids containing electron-releasing substituents.

$$ArCH_2COOH \xrightarrow{M^{n+}} Ar^{\cdot}\overset{+}{C}H_2COOH \xrightarrow{fast} ArCH_2^{\cdot} + CO_2 + H^+$$

$$ArCH_2^{\cdot} \xrightarrow{M^{n+}} ArCH_2^+ \xrightarrow{Nu^-} ArCH_2Nu$$

Scheme 1.

Another pathway involves a Kolbe-like (anodic oxidation of carboxylic acids) mechanism *via* an electron transfer from the carboxylic group, possibly with concerted loss of carbon dioxide (Scheme 2).

$$ArCH_2COOH \xrightarrow[-H^+]{M^{n+}} ArCH_2COO^{\cdot} \longrightarrow ArCH_2^{\cdot} + CO_2$$

$$ArCH_2^{\cdot} \xrightarrow{M^{n+}} ArCH_2^+ \xrightarrow{Nu^-} ArCH_2Nu$$

Scheme 2.

Compounds following this pathway contain less electron-releasing substituents and follow a good Hammett correlation. The ρ-values in these correlations are −1.71 and −0.77 for Co(III)W and Ni(IV)Mo decarboxylation, respectively, indicating a process leading to a benzyl radical in the rate-determining step. It is known that ρ-values for most such processes are in the range of −0.3 to −1.5.[6b,15,16]

A comparison between the values in Table 4 shows that the ρ-values for decarboxylation of more electron-poor acids by Co(III)W, Ni(IV)-Mo, Cu(III)[8] and $SO_4^{\cdot -}$ [17] support an electron transfer mechanism without a metal-carboxylate intermediate. The plots of the logarithms of the relative rates for Ni(IV)Mo, Co(III)W, $Co(III)(OAc)_3$, Cu(III) and Ce(IV) decarboxylations against each other (see Figs. 1–4) give good linear correlations except for the *para*- and *meta*-methoxy compounds. This is good support for a Kolbe-like mechanism, involving outer-sphere electron transfer for all these decarboxylation reactions.[18] The deviation of methoxy compounds from the linear plots indicate, even if the deviation in some cases is not clearly significant, that these acids follow a mechanism with electron transfer from the aromatic ring. In the light of this, it is probable that Ce(IV) decarboxylation

Table 5. Rate constants for decarboxylation of 4-methoxyphenylacetic acid together with different parameters in eqn. 8.[a]

Oxidant	$k_{obs}/M^{-1}\ s^{-1}$	$k_{calc}/M^{-1}\ s^{-1}$	λ/kcal mol^{-1}	W/kcal mol^{-1}	$\Delta G^{o'}$/kcal mol^{-1}
Co(III)W	~0.5	0.08	31.5	3.3	16.3
Ni(IV)Mo	fast	$8 \cdot 10^4$	32.5	4.0	−2.8

[a] All parameters are based on data and methods given in Ref. 14. $\Delta G^{o'}$ values are based on E^o for Co(III)/Co(II)=1.0 V (Ref. 14), E^o for Ni(IV)/Ni(II)=1.8 V (Ref. 9) and E_p for 4-methoxyphenylacetic acid=1.85 V (Ref. 19), all values *vs.* NHE.

proceeds *via* electron transfer instead of a process involving a metal-carboxylate intermediate, which was suggested by Trahanovsky *et al.*[6b]

A comparison between crude estimated rate constants (k_{obs}) based on observations in connection with competition experiments for Co(III)W and Ni(IV)Mo decarboxylation shows good agreement with calculated rate constants (k_{calc}) from eqn. (8), which is based on the Marcus theory for the outer-sphere electron transfer processes.* [See Table 5 and eqn. (8)].In eqn. (8), k_d is assumed to be $2 \cdot 10^{10}$ M^{-1} s^{-1}, A=constant with a value of 0.2, W is the electrostatic work expended to bring the two reactants together, corrected for ionic strength (μ=0.5). λ is the reorganization energy of the reaction and $\Delta G^{o'}$ is the free energy change of the reaction corrected by a term involving the electrostatic energy (W).

$$k_{calc}=\frac{k_d}{1+A\exp\left\{\left[W+\frac{\lambda}{4}\left(1+\frac{\Delta G^{o'}}{\lambda}\right)^2\right]/RT\right\}} \quad (8)$$

The relatively good agreement between observed and calculated rate constants lends additional support for an outer-sphere electron transfer mechanism for Co(III)W and Ni(IV)Mo decarboxylation of arylacetic acids.

EXPERIMENTAL

Materials. Ammonium 9-molybdonickel(IV)-ate and ammonium 9-molybdomangan(IV)ate were prepared according to a literature method,[5] and were recrystallized twice from hot water (70 °C). Potassium 12-tungstocobalt(III)ate was prepared according to the procedure given by Simmons,[20] and was recrystallized three times from hot water. All other chemicals used in this investigation were either purchased of the highest quality available or prepared according to known methods described in earlier work.[11,21,22] 4-Biphenylacetic acid, 4-nitrophenylacetic acid, and 3-chlorophenylacetic acid were recrystallized twice from ethanol–water.

Oxidation procedure. A mixture of the aromatic compound, heteropoly ion and the solvent was stirred at reflux temperature under an argon atmosphere. The mixture was worked up by addition to saturated sodium hydrogen carbonate solution, followed by ether extraction and GLC analysis. The competition experiments were carried out in the following way: 2 mmol of each of two arylacetic acids were heated at reflux temperature in a solvent–reagent mixture for 2 h under an argon atmosphere. The work-up procedure was the same as above.

Analysis. Yields and isomer distributions were determined using a Varian 1400 gas chromatograph equipped with an electronic integrator (Hewlett-Packard 3380 A), on a 2 m×3 mm 5 % NPGS on Chromosorb W and a 2 m×3 mm 3 % OV 101 on Chromosorb Q column. The yield was determined using an internal standard (hexamethyl-benzene and bimesitylene) calibrated against authentic samples. The identification of the products was based on GLC-MS comparison (Finnigan 4021 instrument) with authentic samples. The isotope effect was determined by mass spectroscopy.

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The Synthesis and Crystal Structure of 2-(3-Methyl-1-oxoisoindoline-2-yl)-butyramide

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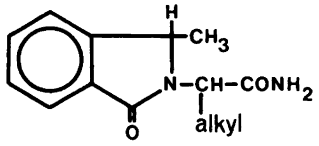
The title compound was obtained by *N*-alkylation of racemic 3-methyl-1-oxoisoindoline with a racemic ethyl-2-bromobutyrate followed by ammonolysis. The two pairs of racemates thus formed could be separated because of their different solubility. The high melting racemate which was obtained in pure form was subsequently recrystallized for the single crystal X-ray study.

$C_{13}H_{16}N_2O_2$, M_r=232.3, monoclinic, $P2_1/c$, a=10.230(2), b=8.017(2), c=16.192(3) Å, β=110.31(2)°, V=1245(1) Å^3, Z=4, D_x=1.24 Mg m^{-3}, μ(Mo$K\alpha$)=0.079 mm^{-1}, F(000)=496. Out of 2910 independent reflexions measured on an automatic diffractometer 1821 had significant intensities. The structure was solved by direct methods and refinement by full-matrix least-squares calculations gave a final R=0.050 for 203 parameters. The structure consists of centrosymmetrically related molecules forming infinite chains by N–H···O hydrogen bonds (2.91 and 2.94 Å). The isoindoline group is nearly planar although the benzene part is slightly distorted.

For studies on structure-activity relationships in potentially teratogenic phthalimides and phthalimidines [1,2] we also prepared some amides of 2-(3-methyl-1-oxoisoindoline-2-yl)alkanoic amides.



0302-4369/83 $2.50

These compounds were obtained by *N*-alkylation of racemic 3-methyl-1-oxoisoindoline either with a racemic α-bromoalkanoic amide or with a racemic α-bromoalkanoic ester, in this case followed by ammonolysis of the intermediate ester. The amides contain two asymmetric carbon atoms (α and 3) and can accordingly form two pairs of racemates which were obtained as mixtures.

In the case of the butyramide (Alkyl=C_2H_5) differences in solubility permitted easy separation of the mixture into a pure, high melting racemate and a low melting one containing about 10 % of the high melting form. The separation could be monitored by ^{1}H NMR spectroscopy as the 3-methyl groups give distinctly separated doublets (δ 1.57 for high melting, 1.51 for low melting form.) Generally corresponding peaks are shifted to a slightly lower field in the high melting form. This is especially notable for the amide hydrogen atoms, δ 6.05 and *ca.* 7.5 in the high melting form, 6.0 and 6.8 in the low melting form, suggesting a higher degree of hydrogen bonding in the former. The high melting racemate has now been studied by X-ray crystallography and it is the purpose of the present paper to report this study.

EXPERIMENTAL

Synthesis. 3-Methyl-1-oxoisoindoline[3] (25.5 g; 0.173 mol) in anhydrous dimethylformamide (60 ml) was added below 70 °C as rapidly as foaming

permitted to a suspension of sodium hydride (4.8 g; 0.20 mol) in dimethylformamide (60 ml). After stirring for an additional hour ethyl-2-bromobutyrate (40 g; 0.205 mol) was added dropwise at 70–80 °C during 1 h and the mixture was heated for a further hour on a boiling water bath. Most of the solvent was removed *in vacuo* and the remaining oil was treated with water and extracted into ether, washed and dried (Na_2SO_4). Distillation afforded 38 g of an oil boiling at 205–208 °C/12 mm Hg. The ester (38 g; 0.145 mol) was added to methanol (500 ml) saturated at 0 °C with ammonia. The solution was kept in a sealed, thick-walled bottle for 4 days and then evaporated to dryness. The solid residue was repeatedly crystallized from ethanol to yield 15.6 g of amide, m.p. 164–165 °C, which according to NMR was a pure racemate. Evaporation of the mother liquid from the first crystallization afforded an oil which solidified after several weeks. Repeated recrystallization gave 9.1 g of low melting racemate, m.p. 138–140 °C, which according to NMR contained about 10 % of the high melting isomer.

^1H NMR (Perkin-Elmer R12, 60 MHz, $CDCl_3$, tetramethylsilane standard). High melting racemate: δ 7.3–7.8 (m, 5H, ArH and NH), 6.05 (broad signal, 1H, NH), 4.65 (quartet, J=6 Hz, 1H, CH_3CH 1), 4.27 (t, J=8 Hz, 1H, C_2H_5CH), 2.25 (quintet, J=7 Hz, 2H, CH_3CH_2), 1.57 (d, J=6.6 Hz, 3H, CH_3CH), 0.98 (t, J=7 Hz, 3H, CH_3CH_2).

Low melting racemate: δ 7.3–7.9 (m, 4H, ArH), 6.8 and 6.0 (broad signal, 1H, NH), 4.44–4.80 (m, 2H, CH_3CH and C_2H_5CH), 2.10 (quintet, J=7 Hz, C_2H_5CH), 1.51 (d, J=6.6 Hz, 3H, CH_3CH), 0.95 (t, J=7 Hz, CH_3CH_2).

X-Ray study. The high melting form of the title compound was recrystallized from ethanol. The space group and approximate cell dimensions were determined from Weissenberg photographs. One optically perfect crystal trimmed to an almost perfect sphere with a diameter of 0.35 mm was mounted about b in a single crystal diffractometer (Philips PAILRED). Graphite monochromatized Mo$K\alpha$ radiation was used and accurate cell dimensions (see abstract) were determined. The intensities were collected up to 2θ=55°, scan range ±1.8–±2.4°, scan speed 2.5° min^{-1}. Fainter reflexions were scanned up to three times and the background was measured for 1 min at each limit of the scan. Out of the 2910 independent reflexions recorded, 1821 were considered observed [$I>3\sigma(I)$]. There was no decrease in intensity as a function of time in periodically measured standard reflexions. For the data reduction including corrections for Lorentz and polarization effects our own data program[4] was used. Absorption corrections were not applied due to the low μ-value.

Structure determination and refinement. The structure was determined with the aid of MULTAN 80.[5] An E-map was obtained where all but one of the non-hydrogen atoms could easily be

Table 1. Final positional and thermal parameters for non-hydrogen atoms (×10^4). The e.s.d.'s are given in parentheses. The temperature factors are defined by: $\exp[-2\pi^2(h^2a^{*2}U_{11}+\cdots+2klb^*c^*U_{23}\cdots)]$.

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	5511(1)	1693(2)	4807(1)	275(8)	585(8)	430(10)	–20(7)	190(7)	10(7)
O(2)	265(2)	1724(2)	4336(1)	248(8)	617(8)	575(11)	134(8)	151(7)	76(7)
N(1)	3514(2)	2539(2)	5036(1)	216(9)	455(8)	335(10)	–29(7)	117(8)	–13(7)
N(2)	1636(2)	–548(2)	4780(1)	297(10)	488(8)	512(12)	83(8)	182(9)	23(8)
C(1)	4918(2)	2279(2)	5280(1)	256(11)	381(8)	362(12)	47(8)	108(9)	3(8)
C(2)	5529(2)	2837(2)	6205(1)	296(11)	405(8)	361(12)	20(8)	99(9)	–55(8)
C(3)	6911(2)	2772(3)	6769(2)	328(12)	531(10)	434(14)	36(10)	69(11)	–46(10)
C(4)	7199(3)	3344(3)	7626(2)	427(14)	658(13)	426(16)	59(12)	–1(12)	–126(12)
C(5)	6146(3)	3979(4)	7886(2)	672(19)	780(15)	338(14)	–93(12)	129(13)	–230(15)
C(6)	4775(3)	4046(3)	7322(2)	505(16)	720(14)	417(15)	–142(12)	182(12)	–73(13)
C(7)	4484(2)	3434(3)	6473(1))	360(12)	480(10)	373(13)	–14(9)	138(10)	–70(9)
C(8)	3091(2)	3283(2)	5738(1)	319(11)	482(9)	385(13)	–47(9)	162(10)	–14(9)
C(9)	2315(3)	4932(3)	5475(2)	497(17)	564(11)	665(18)	–85(12)	223(14)	101(11)
C(10)	2546(2)	1816(2)	4217(1)	241(10)	488(9)	293(12)	23(9)	92(9)	–31(9)
C(11)	2023(3)	3057(3)	3466(2)	420(14)	711(13)	450(15)	185(12)	143(12)	22(11)
C(12)	1187(3)	2230(5)	2600(2)	511(17)	1248(23)	377(16)	134(16)	90(13)	81(17)
C(13)	1364(2)	986(2)	4444(1)	215(10)	483(9)	295(11)	15(9)	62(8)	–29(8)

Table 2. Final positional parameters for the hydrogen atoms ($\times 10^3$) which all were given U=0.06 Å^2. Standard deviations in parentheses.

	x/a	*y/b*	*z/c*
HN(2)A	249(3)	-95(3)	489(2)
HN(2)B	104(3)	-96(3)	505(2)
HC(3)	756(3)	228(3)	657(2)
HC(4)	823(3)	324(3)	797(2)
HC(5)	635(3)	440(3)	848(2)
HC(6)	403(3)	462(3)	752(2)
HC(8)	247(3)	249(3)	591(2)
HC(9)A	139(3)	475(3)	499(2)
HC(9)B	216(3)	546(3)	598(2)
HC(9)C	289(3)	561(3)	525(2)
HC(10)	309(3)	96(3)	407(2)
HC(11)A	289(3)	354(3)	340(2)
HC(11)B	146(3)	378(3)	361(2)
HC(12)A	34(3)	167(3)	257(2)
HC(12)B	92(3)	316(3)	214(2)
HC(12)C	172(3)	137(3)	240(2)

located. Switching to the SHELX program system[6] the missing atom was found in a difference map. The positional coordinates of the non-hydrogen atoms were refined with isotropic thermal parameters to an R-value of 0.10. A subsequent difference synthesis gave the positions of all hydrogen atoms. Refinement of positional and anisotropic thermal parameters for the non-hydrogen atoms only and the introduction of a weighting scheme w=0.80/$[\sigma^2(F_o)+0.0005F_o^2]$ brought the R-value to 0.052. The three strongest reflexions $|F_o|>65$ were now excluded from further calculations due to indications of extinction. In the final refinement cycle (R=0.050) the positional parameters of the hydrogen atoms were refined with fixed isotropic thermal parameters (U=0.06 Å^2). A difference map based on the final parameters of all atoms showed no peak higher or lower than 0.2 eÅ^{-3}.

Final positional and thermal parameters for the non-hydrogen atoms are given in Table 1 and the positional parameters for the hydrogen atoms are given in Table 2. Atomic scattering factors were those of International Tables for X-Ray Crystallography.[7] A list of the final structure factors are available on request from the authors.

RESULTS AND DISCUSSION

The atomic numbering is given in the perspective drawing of the molecule (Fig. 1). The bond distances and angles uncorrected for the effects

Table 3. Intramolecular bond distances (Å) and angles (°) with estimated standard deviations in parentheses.

C(1)-O(1)	1.222(2)	C(7)-C(8)	1.509(3)
C(1)-N(1)	1.367(2)	C(8)-C(9)	1.525(3)
C(1)-C(2)	1.472(3)	C(8)-N(1)	1.472(2)
C(2)-C(7)	1.370(2)	N(1)-C(10)	1.467(3)
C(2)-C(3)	1.393(3)	C(10)-C(13)	1.532(2)
C(3)-C(4)	1.387(3)	C(10)-C(11)	1.514(3)
C(4)-C(5)	1.381(3)	C(11)-C(12)	1.516(4)
C(5)-C(6)	1.382(3)	C(13)-O(2)	1.228(2)
C(6)-C(7)	1.388(3)	C(13)-N(2)	1.334(3)
O(1)-C(1)-N(1)	125.3(2)	C(7)-C(8)-C(9)	114.1(2)
O(1)-C(1)-C(2)	128.4(2)	C(7)-C(8)-N(1)	100.8(2)
N(1)-C(1)-C(2)	106.3(2)	C(9)-C(8)-N(1)	113.6(2)
C(1)-C(2)-C(3)	128.9(2)	C(1)-N(1)-C(8)	113.5(2)
C(1)-C(2)-C(7)	108.8(2)	C(1)-N(1)-C(10)	120.8(2)
C(7)-C(2)-C(3)	122.2(2)	C(8)-N(1)-C(10)	124.5(2)
C(2)-C(3)-C(4)	117.1(2)	N(1)-C(10)-C(11)	113.6(2)
C(3)-C(4)-C(5)	120.4(2)	N(1)-C(10)-C(13)	107.3(2)
C(4)-C(5)-C(6)	122.3(3)	C(11)-C(10)-C(13)	112.8(2)
C(5)-C(6)-C(7)	117.2(2)	C(10)-C(11)-C(12)	112.3(2)
C(6)-C(7)-C(2)	120.8(2)	C(10)-C(13)-N(2)	115.3(2)
C(6)-C(7)-C(8)	128.6(2)	C(10)-C(13)-O(2)	121.1(2)
C(2)-C(7)-C(8)	110.6(2)	N(2)-C(13)-O(2)	123.6(2)

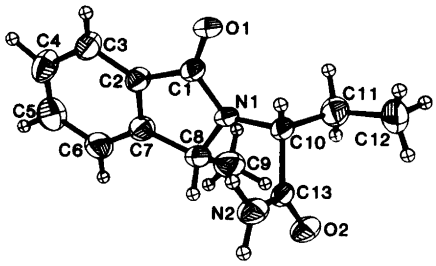


Fig. 1. ORTEP [11] drawing of the molecule showing atomic numbering and vibrational ellipsoids for the non-hydrogen atoms drawn at the 50 % probability level.

of thermal motion are given in Table 3. The C–H distances lie in the range of 0.90–1.02 Å and the two N–H distances are 0.87 Å and 0.97 Å. The bond lengths and angles are much as expected but a few angles deviate from the standard values. Thus, in the benzene ring fused to the five-membered heterocycle two opposite angles, C(2)–C(3)–C(4) and C(5)–C(6)–C(7) show a decrease from 120° by 2.9° (13σ). This seems to be a common feature for indole rings where these two angles always are found to be less than 120° (mean value for 9 structures=117.9°). The same situation is true for isoindoles where one finds a mean value of 118.1° in the three structures reported (Gieren *et al.*,[8] Brisse and Sygusch[9] and Fukuyama *et al.*[10]).

The isoindoline nucleus is essentially planar with a maximum deviation for a ring atom of 0.028(3) Å. Looking at the two fused rings individually the planarity is more pronounced. The maximum deviation of the pyrroline ring is 0.006 (2) Å and that of the benzene ring is 0.012 (3) Å. The angle between these two planes is 2.1 (4)°. As could be expected the atoms of the amide group C(10), C(13), O(2) and N(2) are likewise nearly coplanar, the maximum deviation of an atom being 0.008 (2) Å.

The carbon chain C(10)–C(12) attached to N(1) is approximately perpendicular to the plane of the rings and almost maximally extended, the torsion angles C(8)–N(1)–C(10)–C(11) and N(1)–C(10)–C(11)–C(12) being 90.4(3)° and 172.2(3)°, respectively.

A view of the packing along the *b* axis is presented in Fig. 2. The crystal structure consists of infinite hydrogen bonded chains of molecules related by centres of symmetry. The hydrogen bonds run essentially in the *a* direction. Each molecule is linked to two others by the following hydrogen bonds: N(2)–H(N2)A···O(1)^{I} and N(2)–H(N2)B···O(2)^{II} where the symmetry codes are (i) 1–x, –y, 1–z and (ii) –x, –y, 1–z. The geometry of these bonds are characterized by N···O distances of 2.907(2) and 2.937(2) Å, respectively. Corresponding N–H···O angles are 176(4) and 176(4)°.

Acknowledgements. This work has been supported by the Swedish Medical Research Council (project No. 144) and funds from *Karolinska Institutet.*

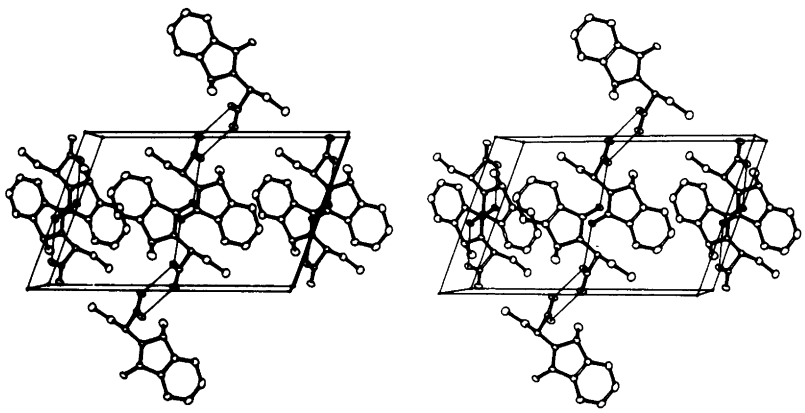


Fig. 2. Stereoview (ORTEP [11]) of the crystal structure. The drawing is essentially seen along *b*. Hydrogen atoms are omitted for clarity. The hydrogen bonds are represented by thin lines.

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Mercury Iodide as a Catalyst in Oligosaccharide Synthesis

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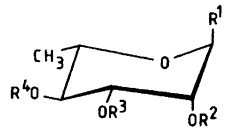
The disaccharide 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranose and the trisaccharide 2-*O*-α-D-galactopyranosyl-4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranose determinants, which are analogs of the repeating unit of *Salmonella* serogroup A, B and D, have been synthesized using mercury(II) iodide as a catalyst in the glycosylation reaction. The reducing end of the di- and the trisaccharide was substituted with a linking arm for covalent attachment to a protein carrier. Reaction of 8-ethoxycarbonyloct-1-yl 2,3-di-*O*-benzoyl-α-L-rhamnopyranoside with acetobromomannose in the presence of mercury(II) iodide gave, after deprotection, the disaccharide in 49 % yield. The trisaccharide was prepared by a block synthesis in which 6-*O*-acetyl-4-*O*-allyl-2-*O*-(6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-D-galactopyranosyl)-3-*O*-benzyl-α-D-mannopyranosyl bromide (*21*) and 8-methoxycarbonyloct-1-yl 2,3-*O*-cyclohexylidene-α-L-rhamnopyranoside were condensed in the presence of mercury(II) iodide. These conditions gave the trisaccharide (*26*) in 26 % yield. The disaccharide *21* was prepared by mercury(II) iodide catalyzed condensation of the protected galactopyranosyl bromide (*15*) and 4-*O*-allyl-1,6-anhydro-3-*O*-benzyl-β-D-mannopyranose followed by acetolysis and reaction with titanium tetrabromide.

Mercury(II) salts have found wide application as catalysts for promotion of glycosidic bond formation.[1] Thus mercury(II) cyanide and mercury(II) bromide are generally used in glycosylation with glycosyl halides of medium reactivity, mainly producing α-linked glycosides.[1] In reaction of less reactive glycosyl halides silver salts have been advantageously used as catalysts. The silver salts are very active catalysts and decomposition products are often observed on prolonged reaction times.[1] In the present paper mercury(II) iodide is introduced as a new catalyst of medium activity for glycosidation reactions. Based on the ion pair equilibration theory by Lemieux *et al.*[2] mercury(II) iodide may be an alternative catalyst to mercury(II) bromide in the formation of α-linked glycosides, the latter extensively used by Paulsen and Kolár.[3] The nucleophilicity of the iodide ion, the soft character and the solubility of mercury(II) iodide in unpolar solvents like chloroform and dichloromethane as well as the reactivity of the intermediate β-glycosyl iodide-mercury(II) iodide ion-pair complex all seem to favour selective α-glycosylation.

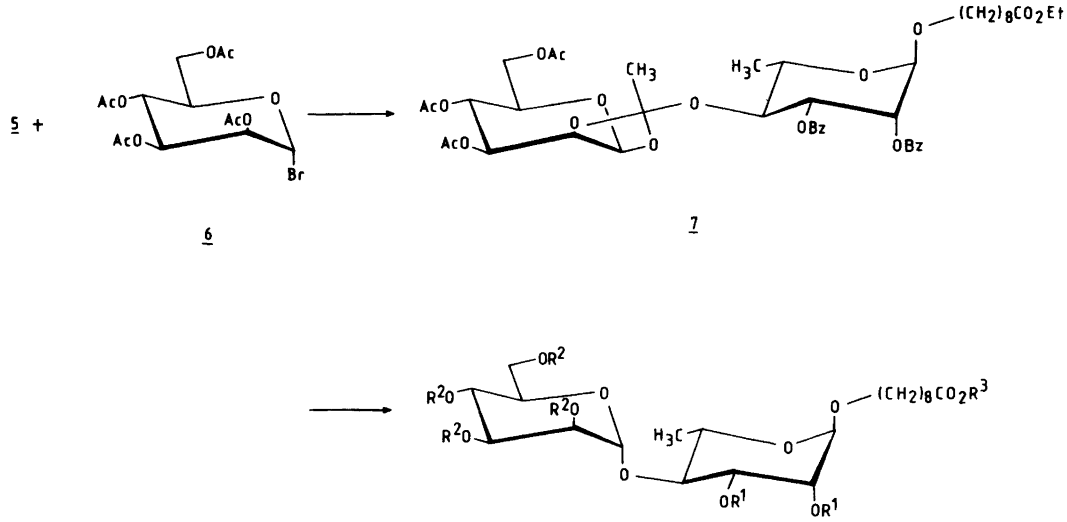
RESULTS AND DISCUSSION

In this investigation attempts were made to synthesize oligosaccharides related to *Salmonella* antigens. The selectively protected α-L-rhamnopyranoside (*5*) was prepared from 1,2,3 tri-*O*-



1 R^1=OBz; R^2=R^3=Bz; R^4=H
2 R^1=OBz; R^2=R^3=Bz; R^4=ClH_2CCO
3 R^1=Br; R^2=R^3=Bz; R^4=ClH_2CCO
4 R^1=$-O-(CH_2)_8CO_2Et$; R^2=R^3=Bz; R^4=ClH_2CCO
5 R^1=$-O-(CH_2)_8CO_2Et$; R^2=R^3=Bz; R^4=H
22 R^1=Br; R^2=R^3=R^4=Bz
23 R^1=$-O-(CH_2)_8CO_2Et$; R^2=R^3=R^4=Bz
24 R^1=$-O-(CH_2)_8CO_2Me$; R^2=R^3=R^4=H
25 R^1=$-O-(CH_2)_8CO_2Me$; R^2, R^3=cyclohexylidene; R^4=H

0302-4369/83 $2.50

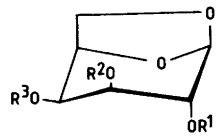


8 R^1=Bz; R^2=Ac; R^3=Et
9 R^1=R^2=H; R^3=Me

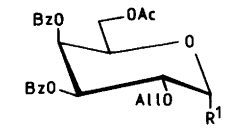
benzoyl-α-L-rhamnopyranose (*1*), by the method developed by Garegg and Norberg.[4] The rhamnose derivative (*1*) was chloroacetylated and transformed into the rhamnopyranosyl bromide (*3*) which, in turn, was reacted with 8-ethoxycarbonyloctan-1-ol in the presence of mercury(II) cyanide to give *4*. Selective removal of the chloroacetyl group with thiourea gave *5* in an overall yield of 52 % from *1*. Reaction of *5* with acetobromomannose (*6*) in the presence of mercury(II) iodide led almost exclusively to the formation of the orthoester (*7*) which was isolated in 86 % yield. Rearrangement of the orthoester with boron trifluoride etherate gave the α-linked disaccharide (*8*) in 49 % yield overall from *5*. Finally, deacylation of *8* afforded the disaccharide (*9*). From these results it appears that mercury(II) iodide was not a strong enough Lewis acid to rearrange the orthoester to the glycoside at room temperature.

Synthesis of the trisaccharide 8-methoxycarbonyloct-1-yl 2-*O*-α-D-galactopyranosyl-4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranoside, a moiety of the repeating unit of *Salmonella* lipopolysaccharides, was attempted in order to investigate the use of mercury(II) iodide as a catalyst in glycosylation with halides having inactive 2-*O*-substituents. 1,6-Anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose (*10*) was reacted with allyl bromide and sodium hydride to give 82 % of *11*. Removal of the isopropylidene group gave 92 % of *12* which was quantitatively benzoylated. Acetolysis of *13* gave *14* (90 % yield), transformed into the crystalline bromide (*15*) in 83 % yield. This bromide was used for mercury(II) iodide promoted condensation with 4-*O*-allyl-1,6-anhydro-3-*O*-benzyl-β-D-mannopyranose (*18*), obtained in 72 % yield from 1,6-anhydro-2,3-*O*-endo-benzylidene-β-D-mannopyranose[5] (*16*) by allylation and selective cleavage of the benzylidene group with lithium aluminium hydride–aluminium trichloride according to Liptak *et al.*[6]

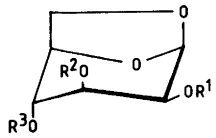
Condensation of *15* and *18* gave 60 % yield of the disaccharide (*19*); only one anomer was observed in the crude product. The 1,6-anhydro ring of *19* was opened by acetolysis in the presence of trifluoroacetic acid and the product (*20*) was transformed to the unstable bromide (*21*) on treatment with titanium tetrabromide according to Paulsen and Jansen.[7] In this reaction some removal of the benzyl group was observed when traces of hydrogen bromide were present. Condensation of *21* with 8-methoxycarbonyloct-1-yl 2,3-*O*-cyclohexylidene-α-L-rhamnopyranoside (*25*) was carried out in the presence of mercury(II) iodide. The cyclohexylidene derivative was considered more suitable than the benzoyl derivative (*5*) for this reaction due to the higher reactivity. Hence, *25* was prepared in four



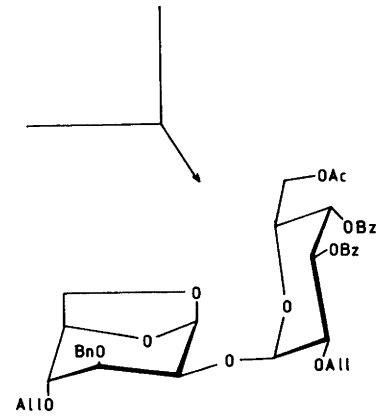
10 R^1=H; R^2, R^3=isopropylidene
11 R^1=All; R^2, R^3=isopropylidene
12 R^1=All; R^2=R^3=H
13 R^1=All; R^2=R^3=Bz



14 R^1=OAc
15 R^1=Br



16 R^1, R^2=*endo*-benzylidene; R^3=H
17 R^1, R^2=*endo*-benzylidene; R^3=All
18 R^1=H; R^2=Bn; R^3=All

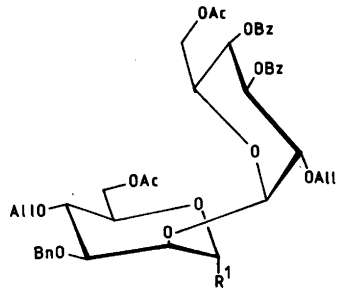


19

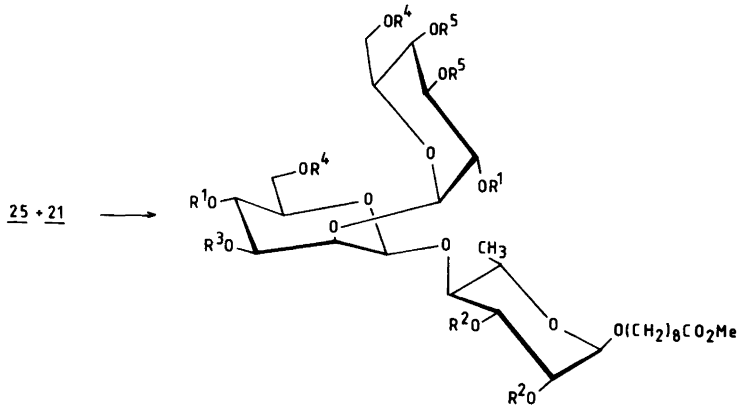
steps from tri-*O*-benzoyl-α-L-rhamnopyranosyl-bromide (*22*) in an overall yield of 92 %. The condensation of *21* with *25* gave unexpectedly the trisaccharide (*26*) in 26 % yield overall from *19*. The exclusive formation in this case of a β-linkage was ascribed to sterically conditioned suppression of the intermediate β-ion pair concentration through the proximity of the galactopyranosyl residue to the β-site of the anomeric center of the mannose unit. Such proximity problems could be interpreted on the basis of the well documented orientational effect of the exo-anomeric energy. In fact, this might be a general way to obtain oligosaccharides containing 2-*O*-α-D-glycosylated β-D-mannopyranoside or 2-*O*-α-L-glycosylated β-L-rhamnopyranoside moieties.

The allyl group of *26* was removed by the method of Gigg and Gent [8] and the cyclohexylidene group subsequently hydrolyzed. Acetylation gave *27* in 56 % overall yield, followed by

19 →



20 R^1=Ac
21 R^1=Br



26 R^1=All, R^2=cyclohexylidene; R^3=Bn; R^4=Ac; R^5=Bz
27 R^1=R^2=R^4=Ac; R^3=Bn; R^5=Bz
28 R^1=R^2=R^4=Ac; R^3=H; R^5=Bz
29 R^1=R^2=R^3=R^4=R^5=H

debenzylation to *28* which was deacylated to give overall 71 % of *29*. This trisaccharide constitutes an isomer of the repeating main-chain trisaccharide common to *Salmonella* antigens of serogroups A, B and D.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. NMR spectra were obtained on Bruker WH-90 and HX-270 NMR instruments. The spectra of protected compound were measured in $CDCl_3$ and non-protected products in D_2O. Acetone (δ 2.12) was used as internal reference for ^1H NMR spectra and dioxane (67.4 ppm) for ^{13}C NMR spectra in D_2O. Microanalyses were performed by NOVO microanalytical laboratory.

1,2,3-Tri-O-benzoyl-4-O-chloroacetyl-α-L-rhamnopyranose (2). 1,2,3-Tri-*O*-benzoyl-α-L-rhamnopyranose [9] (*1*) (10 g, 21 mmol) was dissolved in acetonitrile (75 ml) and pyridine (3.34 ml, 43 mmol) and cooled to 0 °C. Chloroacetyl chloride (3.34 ml, 42 mmol) dissolved in acetonitrile (10 ml) was added dropwise over a period of 15 min. The mixture was stirred for 2 h at 20 °C and evaporated three times with toluene (30 ml). The residue was dissolved in dichloromethane (40 ml) and washed with saturated sodium hydrogencarbonate (50 ml) and water (50 ml). The organic phase was dried, filtered through carbon and evaporated. It was redissolved in ethyl acetate (30 ml) and filtered through silica gel. Evaporation at 1 mm Hg and 40 °C left 11.3 g (97 %) of rather unstable *2*. ^1H NMR: δ 6.48 (H-1); 5.78 (H-2); 5.85 (H-3); 5.54 (H-4); 4.24 (H-5); 1.38 (H-6). J_{12} 1.5 Hz; J_{34} 9.8; J_{45} 9.8; J_{56} 6.4.

8-Ethoxycarbonyloct-1-yl 2,3-di-O-benzoyl-α-L-rhamnopyranoside (5). Compound (*2*) (11.3 g, 20.5 mmol) was dissolved in dichloromethane (20 ml) and cooled to 0 °C. Hydrogen bromide in acetic acid (35 %, 20 ml) was added and the mixture was stirred at 0 °C for 2 h. The solution was evaporated and dissolved in dichloromethane (50 ml). Successive washing with ice water (100 ml), cold saturated aqueous sodium hydrogencarbonate (50 ml) and ice water (50 ml), followed by drying for 40 min gave chromatographically pure 2,3-di-*O*-benzoyl-4-*O*-chloroacetyl-α-L-rhamnopyranosyl bromide (*3*). ^1H NMR: δ 6.45 (H-1); 5.79 (H-2); 6.01 (H-3); 5.51 (H-4); 4.30 (H-5); 1.39 (H-6). J_{12} 2.0 Hz; J_{23} 3.5; J_{34} 10.3; J_{45} 10.3; J_{56} 6.0.

The solution was dried over molecular sieves (4 Å, 3 g) for 2 h under nitrogen and added to a solution of 8-ethoxycarbonyloctan-1-ol (4.2 g, 20.8 mmol) in dichloromethane (20 ml) which had been stirred under nitrogen with mercury(II) cyanide (6 g, 23.8 mmol) and molecular sieves (4 Å, 5 g) for 2 h. The mixture was stirred for 18 h and filtered. The residue was washed with dichloromethane (20 ml) and the filtrate was washed with water (50 ml), saturated sodium hydrogencarbonate solution (50 ml) and water

(50 ml). Drying, filtration through carbon and evaporation gave *4* (12.1 g) as a syrup. ^{1}H NMR showed 90 % conversion to the glycoside. The product was dissolved in acetonitrile (50 ml) and water (9 ml) followed by addition of thiourea (2.58 g, 34 mmol). The mixture was stirred for 2 d and evaporated. The residue was extracted with chloroform (75 ml) and filtered. The filtrate was washed twice with saturated sodium hydrogencarbonate solution (50 ml) and water. The organic phase was dried, filtered through carbon and evaporated. Separation on a silica gel column (ethyl acetate–pentane: 1:2.8) gave 6.03 g (52 % overall from *1*) of *5*. $[\alpha]_D^{20}$ +24° (c 1.9, $CHCl_3$) Analysis for $C_{31}H_{40}O_9$: C,H. ^{1}H NMR: δ 4.89 (H-1); 5.56 (H-2); 5.49 (H-3); 3.88 (H-4); 3.88 (H-5); 1.44 (H-6). J_{12} 1.4 Hz; J_{56} 6.0.

*8-Ethoxycarbonyloct-1-yl 2,3-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-α-*L*-rhamnopyranoside (6).* Compound (*5*) (1.6 g, 2.9 mmol) was dissolved in chloroform (5 ml) and stirred overnight with red mercury(II) iodide (2.3 g, 5 mmol) and molecular sieves (4Å, 6 g) under nitrogen. 2,3,4,6-Tetra-*O*-acetyl-α-*D*-mannopyranosyl bromide (*6*) (1.8 g, 4.4 mmol), dissolved in chloroform (10 ml) and dried overnight over molecular sieves (4Å, 2g), was added and the mixture was stirred under nitrogen at 20 °C for 7 d. The mixture was filtered and the residue was washed with chloroform. The filtrate was washed 3 times with aqueous potassium iodide (75 ml), saturated sodium hydrogencarbonate solution (50 ml) and water (50 ml). The organic phase was dried, filtered and evaporated followed by separation on a silica gel column (ethyl acetate–pentane: 4:5). This gave the intermediate orthoester (*7*) (2.2 g, 86 %) containing 10 % of the disaccharide (*8*) according to a ^{1}H NMR spectrum. The orthoester (7) (530 mg) was dissolved in dichloromethane–chloroform: 1:1 and stirred with powdered 4Å molecular sieves for 4 h and boron trifluoride etherate (2 drops) was added. The mixture was stirred for 4 d. Filtration and evaporation gave a syrup, which was purified on a silica gel column to give 300 mg of *8* (49 % yield overall). $[\alpha]_D^{20}$+65.5° (c 2.8, $CHCl_3$). Analysis for $C_{45}H_{58}O_{18}$: C,H. ^{1}H NMR: δ 5.03 (H-1.1); 5.17–5.27 (H-2.1, H-3.1, H-4.1; 3.82 (H-5.1) 3.68 (H-6.1); 3.49 (H-6′.1); 4.87 (H-1.2); 5.55 (H-2.2); 5.70 (H-3.2); 3.94 (H-4.2); 4.03 (H-5.2); 1.46 (H-6.2). $J_{12.1}$ 1.1 Hz; $J_{56.1}$ 3.4; $J_{66'.1}$ 10.0; $J_{12.2}$ 1.7; $J_{23.2}$ 3.3; $J_{34.2}$ 9.0; $J_{45.2}$ 9.0; $J_{56.2}$ 5.9.

*8-Methoxycarbonyloct-1-yl 4-*O*-(α-*D*-mannopyranosyl)-α-*L*-rhamnopyranoside (9).* The disaccharide (*8*) (500 mg, 0.564 mmol) was dissolved in sodium methoxide (0.05 M, 50 ml) and the mixture was stirred for 16 h at room temperature. Sodium ions were removed by stirring for 2 h with ion exchange resin (Amberlite IRC 50). Evaporation gave a syrup which was separated on a silica gel column (methanol–ethyl acetate: 1:3). Fractions containing the title compound were evaporated giving 213 mg (76 %) of *9*. $[\alpha]_D^{20}$ −1.3° (c 0.4, MeOH). ^{1}H NMR: δ 4.87 (H-1.1); 3.87 (H-2.1); 3.66 (H-3.1); 3.62 (H-4.1); 3.82 (H-5.1); 3.75 (H-6.1); 3.68 (H-6′.1); 4.66 (H-1.2); 3.81 (H-2.2); 3.80 (H-3.2); 3.43 (H-4.2); 3.60 (H-5.2); 1.20 (H-6.2). $J_{12.1}$ 1.5 Hz; $J_{23.1}$ 2.8; $J_{34.1}$ 9.4; $J_{45.1}$ 9.4; $J_{56.1}$ 2.6; $J_{56'.1}$ 5.2; $J_{66'.1}$ 12.2; $J_{12.2}$ 1.1; $J_{23.2}$ 2.8; $J_{34.2}$ 9.4; $J_{45.2}$ 9.4; $J_{56.2}$ 6.0 ^{13}C NMR: 102.4 ppm (C-1.1); 71.3 (C-2.1); 71.5 (C-3.1); 68.4 (C-4.1); 74.0 (C-5.1); 61.7 (C-6.1); 100.5 (C-1.2); 71.5 (C-2.2); 70.3 (C-3.2); 82.4 (C-4.2); 67.7 (C-5.2); 17.8 (C-6.2). $J_{C1H1.1}$ 167 Hz; $J_{C1H1.2}$ 166.

*2-O-Allyl-1.6-anhydro-3,4-*O*-isopropylidene-β-*D*-galactopyranose (11).* 1,6-Anhydro-3,4-*O*-isopropylidene-β-*D*-galactopyranose (*10*) (25 g, 124 mmol) was dissolved in dry *N,N′*-dimethyl formamide (185 ml) and allyl bromide (15 ml, 175 mmol) was added. Sodium hydride (8.9 g, 185 mmol, 50 % oil-suspension) was washed several times with light petroleum and added in small portions over a period of 45 min at 20 °C with stirring and cooling. The mixture was stirred at 0 °C for 70 min and methanol (10 ml) was added dropwise. After 10 min the mixture was partitioned between dichloromethane (500 ml) and water (500 ml) and filtered through celite, the organic phase was separated and washed twice with water (500 ml). Drying, filtration and evaporation at 1 mmHg and 60 °C gave a syrup which crystallized on cooling. The material was recrystallized from ethanol (150 ml) and water (125 ml). Filtration and washing with water (25 ml) gave, upon drying *in vacuo* over potassium hydroxide, 19.5 g of *11* $[\alpha]_D^{22}$ −60° (c 0.8, $CHCl_3$), mp 56.5–57.5 °C, ^{1}H NMR: δ 5.38 (H-1); 3.50 (H-2); 4.36 (H-3); 4.51 (H-4); 4.17 (H-5); 4.05 (H-6); 3.54 (H-6′). J_{15} 1.0 Hz; J_{23} 1.5; J_{34} 6.3; $J_{66'}$ 7,5. Analysis for $C_{12}H_{18}O_5$: C,H. Processing of the mother liquors gave an additional 5.1 g of *11* bringing the total yield to 82 %.

*2-O-Allyl-1,6-anhydro-β-*D*-galactopyranose (12).* The anhydro compound (*11*) (4.06 g, 20 mmol) was dissolved in 75 % aqueous acetic acid (80 ml) and stirred for 4 d at 40 °C. The mixture was evaporated, dissolved in water (40 ml) and extracted twice with chloroform (20 ml). The aqueous phase was evaporated at 1 mmHg, 50 °C and the product crystallized from diethyl ether (20 ml)–pentane (20 ml). Filtration at 0 °C gave, after drying *in vacuo*, 3.11 g (92 %) of 12, $[\alpha]_D^{22}$ −34° (c 0.3, $CHCl_3$), mp. 55.0–57.0 °C. Analysis for $C_7H_{14}O_5$: C, H. ^{1}H NMR: δ 5.42 (H-1); 3.54

(H-2); 3.94 (H-3); 3.96 (H-4); 4.41 (H-5); 4.16 (H-6); 3.60 (H-6'); J_{12} 1.3 Hz; J_{15} 1.3; J_{23} 0.7; J_{34} 0.5; J_{45} 4.5; J_{56} 0; $J_{56'}$ 4.7; $J_{66'}$ 7.0.

2-O-Allyl-1,6-anhydro-3,4-di-O-benzoyl-β-D-galactopyranose (13). Compound (*12*) (3.59 g, 17.8 mmol) was stirred in pyridine (25 ml) and cooled to 0 °C. Benzoyl chloride (6.19 ml, 53.4 mmol) was added over a period of 45 min at this temperature. The suspension was stirred overnight at 5 °C and water (3 ml) was added. The mixture was diluted with dichloromethane (75 ml) and extracted successively with water (50 ml), cold hydrochloric acid (4 M, 150 ml and 50 ml), saturated sodium hydrogencarbonate solution (50 ml), and water (50 ml). The organic phase was dried, filtered through carbon and evaporated at 1 mm Hg and 50 °C to give 7.39 g (100 %) of (13). $[\alpha]_D^{23}$ −70° (c 1.5, $CHCl_3$). ^1H NMR: δ 5.50 (H-1); 3.61 (H-2); 5.60 (H-3); 5.63 (H-4); 4.68 (H-5); 4.55 (H-6); 3.83 (H-6'). J_{12} 1.3 Hz; J_{15} 1.5; J_{23} 1.3; J_{45} 4.8; J_{56} 0; $J_{56'}$ 4.8; $J_{66'}$ 12.3.

1,6-Di-O-acetyl-2-O-allyl-3,4-di-O-benzoyl-α-D-galactopyranose (14). Compound (*13*) (30.2 g, 32 mmol) was dissolved in acetic anhydride (80 ml, 800 mmol) and cooled to 0 °C with stirring. Sulfuric acid (20 drops) was added and the mixture was stirred for 20 min at 0 °C, and then poured onto crushed ice (200 ml) and ethanol (100 ml). After stirring for 2 h the crystalline product was isolated by filtration followed by washing three times with water (30 ml). Drying over potassium hydroxide *in vacuo* gave 34.03 g (90 %) of *14*. $[\alpha]_D^{22}$ +142° (c 0.3, $CHCl_3$) m.p. 141−146 °C. Analysis for $C_{27}H_{28}O_{10}$: C, H. ^1H NMR: δ 6.50 (H-1); 4.13 (H-2); 5.60 (H-3); 5.85 (H-4); 4.46 (H-5); 4.14 (H-6); 4.14 (H-6'). J_{12} 3.9 Hz; J_{23} 10.5; J_{34} 3.3; J_{45} 0.9; J_{56} 6.2; $J_{56'}$ 6.2.

6-O-Acetyl-2-O-allyl-3,4-di-O-benzoyl-α-D-galactopyranosyl bromide (15). The acetate (*14*) (18.5 g, 33 mmol) was dissolved in dichloromethane (50 ml) and cooled to 0 °C. Hydrogen bromide in acetic acid (35 %, 50 ml) was added and the mixture was stirred for 50 min and then concentrated at 35 °C. The residue was crystallized from diethyl ether (150 ml) and light petroleum (250 ml) giving 16.5 g (83 %) of *15* as a fine powder m.p. 90−95 °C. ^1H NMR: δ 6.67 (H-1); 4.03 (H-2); 5.67 (H-3); 5.89 (H-4); 4.69 (H-5); 4.31 (H-6); 4.29 (H-6'). J_{12} 4.1 Hz; J_{23} 10.5; J_{34} 3.1; J_{45} 1.3; J_{56} 6.0; $J_{56'}$ 6.0.

4-O-Allyl-1,6-anhydro-2,3-O-endo-benzylidene-β-D-mannopyranose (17). 1,6-Anhydro-2,3-*O-endo*-bennzylidene-β-D-mannopyranose (16) (2.5 g 10 mmol) was dissolved in dry *N,N'*-dimethyl formamide (15 ml) and allyl bromide (1.20 ml, 14 mmol) was added with stirring. Sodium hydride (720 mg, 15 mmol, 50 % oil suspension) was washed several times with pentane and added in portions at 20 °C to the stirred solution over a period of 40 min. The mixture was stirred for 1 h at 20 °C and excess of reactants were decomposed by addition of methanol (1 ml). The reaction mixture was diluted with dichloromethane (100 ml) and washed three times with water (100 ml). Drying, filtration and evaporation gave 2.91 g (100 %) of *17*. Crystallization from pentane gave m.p. 48−50 °C, $[\alpha]_D^{22}$ −15° (c 0.2, $CHCl_3$). ^1H NMR: δ 5.47 (H-1) 3.73 (H-2); 4.23 (H-3); 4.23 (H-4); 4.64 (H-5); 3.82 (H-6); 3.96 (H-6'). J_{12} 1.0 Hz; J_{15} 1.5, J_{56} 1.2; $J_{56'}$ 5.6; $J_{66'}$ 5.8. Analysis for $C_{16}H_{18}O_5$: C, H.

4-O-Allyl-1,6-anhydro-3-O-benzyl-β-D-mannopyranose (18). Compound (*17*) (3.00 g, 10 mmol) was dissolved in dry dichloromethane (30 ml) and cooled to 0 °C with stirring under a nitrogen atmosphere. Lithium aluminium hydride (433 mg, 11.4 mmol) was added and a solution of aluminium chloride (1.53 g, 11.4 mmol) in dry diethyl ether (30 ml) was added dropwise over a period of 10 min. The mixture was stirred for 30 min and excess of reagent was destroyed by addition of ethyl acetate (12 ml) at 0 °C. After 40 min at 0 °C water (4 ml) was added and stirring was continued for 20 min. The organic phase was separated and the solid residue was extracted twice with diethyl ether (50 ml). The combined organic extracts were washed twice with water (25 ml) and evaporated. The residue was redissolved in dichloromethane (30 ml), dried, filtered and evaporated leaving 2.86 g of a clear syrup. Purification on a silica gel column (ethyl acetate−pentane: 23:17) gave 2.17 g (72 %) of *18*, $[\alpha]_D^{23}$ −66° (c 0.6, $CHCl_3$). ^1H NMR: δ 5.31 (H-1); 3.67 (H-2); 3.74 (H-3); 3.45 (H-4); 4.50 (H-5); 4.10 (H-6); 3.70 (H-6'). J_{12} 1.5 Hz; J_{15} 1.0; J_{34} 1.0; J_{45} 0.5; J_{56} 0.7; $J_{56'}$ 6.0; $J_{66'}$ 6.9. Analysis for $C_{16}H_{20}O_5$: C, H.

4-O-Allyl-2-O-(6-O-acetyl-2-O-allyl-3,4-di-O-benzoyl-α-D-galactopyranosyl)-1,6-anhydro-3-O-benzyl-β-D-mannopyranose (19). Compound (*18*) (5.95 g, 20 mmol), red mercury(II) iodide (13.6 g, 30 mmol), molecular sieves (4Å, 15 g) and dichloromethane (40 ml) were stirred for 18 h under a nitrogen atmosphere and *15* (16.5 g, 30 mmol) in dry dichloromethane (20 ml) was added dropwise at 20 °C. The mixture was stirred for 48 h and filtered through celite. The residue was washed with dichloromethane (100 ml) and the filtrate was washed three times with aqueous potassium iodide (75 ml, 10 %) and with water (75 ml). After drying, filtration through carbon and evaporation 18.2 g of syrup was isolated. Purification on a silica gel column (ethyl acetate-pentane: 3:4) gave 8.9 g (60 %) of *19* $[\alpha]_D^{22}$ +73.3° (c 0.2, $CHCl_3$). ^1H NMR: δ 5.29 (H-1.1); 4.11

(H-2.1); 5.77 (H-3.1); 5.85 (H-4.1); 4.74 ((H-5.1); 4.18 (H-6.1); 4.25 (H-6′.1); 5.14 (H-1.2); 3.76 (H-2.2); 3.89 (H-3.2); 3.47 (H-4.2); 4.50 (H-5.2); 4.36 (H-6.2); 3.78 (H-6′.2). $J_{12.1}$ 3.5 Hz; $J_{23.1}$ 12.0; $J_{34.1}$ 2.8; $J_{45.1}$ 0.0; $J_{56.1}$ 7.6; $J_{56'.1}$ 5.0; $J_{66'.1}$ 11.2; $J_{12.2}$ 0.0; $J_{23.2}$ 4.8; $J_{34.2}$ 0.0; $J_{45.2}$ 0.0; $J_{56.2}$ 0.0; $J_{56'.2}$ 5.0; $J_{66'.2}$ 7.0.

*1,6-Di-*O*-acetyl-4-*O*-allyl-2-*O*-(6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-*D*-galactopyranosyl)-3-*O*-benzyl-α-*D*-mannopyranose (20).* Compound (*19*) (6.2 g, 8.33 mmol) was dissolved in acetic anhydride (75 ml) and trifluoroacetic acid (10 ml) was added. The mixture was stirred for 2 h at 20 °C and evaporated three times with toluene (25 ml) at 40 °C, 10 mm Hg and then at 40 °C, 1 mm Hg. This gave 7.09 g of a syrup which according to a ^{1}H NMR spectrum contained mainly the title compound. A small amount (115 mg) was purified on a silica gel plate (ethyl acetate–pentane: 1:1) to give 91 mg of *19*, $[\alpha]_D^{20}$ +135° (c 2.2, $CHCl_3$). Analysis for $C_{45}H_{50}O_{16}$: C, H. ^{1}H NMR: δ 5.47 (H-1.1); 4.05 (H-2.1); 5.64 (H-3.1); 5.84 (H-4.1); 4.54 (H-5.1); 4.22 (H-6.1); 4.14 (H-6′.1); 6.23 (H-1.2); 4.33 (H-2.2); 3.88 (H-3.2); 3.99 (H-4.2); 3.84 (H-5.2); 4.0–4.1 (H-6.2–H-6′.2). $J_{12.1}$ 4.8 Hz; $J_{23.1}$ 10.8; $J_{34.1}$ 3.2; $J_{45.1}$ 1.0; $J_{56.1}$ 5.8; $J_{56'.1}$ 6.8; $J_{66'.1}$ 11.0; $J_{12.2}$ 2.4; $J_{23.2}$ 2.8; $J_{34.2}$ 9.3; $J_{45.2}$ 9.6; $J_{56.2}$ 3; $J_{56'.2}$ 3.

*6-*O*-Acetyl-4-*O*-allyl-2-*O*-(6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-*D*-galactopyranosyl)-3-*O*-benzyl-α-*D*-mannopyranosyl bromide (21).* Compound (*20*) (6.98 g, 8.0 mmol) was stirred with titanium tetrabromide (5 g, 14 mmol), dry dichloromethane (50 ml) and dry ethyl acetate (7.5 ml) for 5 h at 20 °C. Acetonitrile (65 ml) and anhydrous sodium acetate (10.4 g) were added and the mixture was stirred until the color had disappeared (5–10 min). Toluene (75 ml) was added and the mixture was stirred for 10 min at 10 °C and filtered. The filtrate was evaporated and the residue extracted with toluene (30 ml). Filtration through celite and evaporation at 1 mm Hg gave 8.0 g of a syrup containing *21* as the main component. ^{1}H NMR: δ 6.63 (H-1.1); 5.80 (H-4.2); 5.55 (H-3.2); 5.00–5.36 (H-1.1 and terminal allyl protons), 4.70 (benzyl-CH_2-protons); 3.75–4.54 (H-2.1–H-6′.1, H-2.2, H-5.2–H6′.2 and aliphatic allyl protons); 2.06 (acetyl protons). The unstable bromide was used directly in the next step.

*8-Ethoxycarbonyloct-1-yl 2,3,4-tri-*O*-benzoyl-α-*L*-rhamnopyranoside (23),* 8-Ethoxycarbonyloctan-1-ol (40.5 g, 200 mmol) was dissolved in dry dichloromethane (250 ml) and stirred with drierite (15 g), molecular sieves (15 g, 4Å) and mercury(II) cyanide (50.4 g, 200 mmol) for 8 h under a nitrogen atmosphere. A suspension of 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (*22*)[9] (108 g, 200 mmol) in dichloromethane (200 ml), dried over molecular sieves (4Å), was added in portions with stirring over a period of 1 h at 20 °C. The mixture was stirred for 3 d. The solid material was removed by filtration through celite and the filtrate was washed twice with aqueous potassium iodide (200 ml), twice with saturated sodium hydrogen carbonate solution (200 ml), and with water (200 ml). Drying, filtration and evaporation at 1 mmHg, 50 °C left 131.7 g (99.5 %) of a syrup. ^{1}H NMR: δ 4.96 (H-1); 5.54 (H-2); 5.83 (H-3); 5.54 (H-4); 4.17 (H-5); 1.38 (H-6). J_{12} 1.3 Hz; J_{23} 3.3; J_{34} 9.8; J_{45} 9.8; J_{56} 6.0.

*8-Methoxycarbonyloct-1-yl α-*L*-rhamnopyranoside (24).* The glycoside (*23*) (131.5 g, 199 mmol) was dissolved in methanolic sodium methoxide (500 ml, 0.2 M) and stirred at 20 °C for 2 h. Sodium ions were removed by stirring with ion exchange resin (Amberlite IRC-50, 10 g) for 1.5 h. The filtrate was concentrated *in vacuo* and dissolved in water (150 ml). The aqueous phase was extracted twice with pentane–toluene (5:1, 100 ml) and then twice with toluene (100 and 50 ml). Repeated evaporation at 1 mmHg of the toluene phase left 73.3 g of chromatographically pure *24* containing a small amount of toluene. ^{1}H NMR data were identical to those reported [10] and showed no other impurities.

*8-Methoxycarbonyloct-1-yl 2,3-*O*-cyclohexylidene-α-*L*-rhamnopyranoside (25).* The glycoside (*24*) (72.3 g) was dissolved in dry acetonitrile (350 ml) and cooled to 0 °C with stirring. *p*-Toluenesulfonic acid (700 mg) and 1-ethoxycyclohexane (52.5 ml, 2 eqv) were added over 2 min and the stirring was continued for 10 min at 0–5 °C with cooling. The mixture was neutralized with pyridine (0.80 ml) and extracted twice with pentane (400 ml). The acetonitrile phase was filtered through charcoal and evaporated *in vacuo* giving 78.7 g of *25* (total yield of 92 % from *22*). An analytical sample gave $[\alpha]_D^{23}$ −19° (c 1.2, $CHCl_3$). Analysis for $C_{22}H_{38}O_7$: C, H. ^{1}H NMR: δ 4.87 (H-1); 4.05 (H-2); 4.01 (H-3); 3.33 (H-4); 3.61 (H-5); 1.27 (H-6). J_{12} 0 Hz; J_{45} 9.0; J_{56} 6.4.

*8-Methoxycarbonyloct-1-yl 4-*O*-(6-*O*-acetyl-4-*O*-allyl-2-*O*-(6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-*D*-galactopyranosyl)-3-*O*-benzyl-β-*D*-mannopyranosyl)-2,3-*O*-cyclohexylidene-α-*L*-rhamnopyranoside (26).* The glycoside (*25*) (3.1 g, 7.3 mmol) was dissolved in chloroform (75 ml) and stirred with mercury(II) iodide (4 g, 8.8 mmol) and molecular sieves (4Å, 20 g) under an atmosphere of nitrogen for 6 h. The bromide (*21*) (prepared from *20* (6.97 g, 8 mmol)) dissolved in dry chloroform was added dropwise over a period of 2 h at 20 °C. The mixture was stirred for 4 d at

20 °C and then filtered through celite. The residue was washed with chloroform (75 ml) and the filtrate was washed twice with aqueous potassium iodide solution (40 ml, 10 %), with saturated sodium hydrogencarbonate solution (50 ml) and with water (50 ml). The chloroform phase was dried, filtered and evaporated. The residue was acetylated in toluene (25 ml) with acetic anhydride (2 ml) and pyridine (4 ml) for 20 h at 20 °C. Filtration and evaporation left 9.58 g of syrup which was separated on a silica gel column (ethyl acetate–pentane 3:7) giving 2.29 g of *26* (26 %). $[\alpha]_D^{25}$ +70° (c 1.0, $CHCl_3$). Analysis for $C_{65}H_{84}O_{21}$: C, H. ^{1}H NMR: δ 5.64 (H-1.1); 3.99 (H-2.1); 5.66 (H-3.1); 5.81 (H-4.1); 4.93 (H-5.1) 4.1–4.2 (H-6.1–H6'.1); 4.98 (H-1.2); 3.42 (H-2.2); 3.58 (H-3.2); 3.83 (H-4.2); 3.40 (H-5.2); 4.49 (H-6.2); 5.02 (H-1.3); 4.08 (H-2.3); 4.10 (H-3.3); 3.66 (H-4.3); 3.70 (H-5.3); 1.38 (H-6.3). $J_{12.1}$ 3.5 Hz; $J_{23.1}$ 9.9; $J_{34.1}$ 3.5; $J_{45.1}$ 1.0; $J_{56.1}$ 5.9; $J_{56'.1}$ 6.8; $J_{12.2}$ 0.0; $J_{23.2}$ 1.9; $J_{34.2}$ 9.6; $J_{45.2}$ 9.6; $J_{56.2}$ 2.7; $J_{56'.2}$ 5.9; $J_{66'.2}$ 11.7; $J_{12.3}$ 0.0; $J_{34.3}$ 9.4; $J_{45.3}$ 9.6; $J_{56.3}$ 6.0.

*8-Methoxycarbonyloct-1-yl 2,3-di-*O*-acetyl-4-*O*-(3-*O*-benzyl-4,6-di-*O*-acetyl-2-*O*-(2,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-α-*D*-galactopyranosyl)-β-*D*-mannopyranosyl)-α-*L*-rhamnopyranoside (27).* The compound (*26*) (260 mg, 0.214 mmol) was dissolved in toluene (7 ml) and ethanol (3 ml), water (1 ml) and 1.8-diaza-[2.2.2]-biscyclooctane (25 mg) was added. The mixture was heated to reflux temperature and tris (triphenyl phosphine) rhodium chloride (100 mg, 0.054 mmol) was added with stirring. The mixture was stirred for 12 h at reflux temperature, filtered through celite and evaporated. The residue was dissolved in aqueous acetone (90 %, 6 ml) and red mercury-(II) oxide (120 mg, 0.55 mmol) was added. A solution of mercury(II) chloride (120 mg, 0.44 mmol) in aqueous acetone (90 %, 2.5 ml) was added over a period of 3 min and the mixture was stirred for 7 min. The mixture was evaporated and the residue was partitioned between diethyl ether (30 ml) and aqueous potassium iodide (10 %, 15 ml). The organic phase was washed twice with aqueous potassium iodide (10 ml), dried, filtered and evaporated to give 233 mg of a syrup containing one major product according to TLC (ethyl acetate–pentane 1:2). The product was dissolved in aqueous acetic acid (80 %, 13 ml) and heated to 50 °C for 2 d. Filtration through celite and evaporation with toluene gave a clear syrup (260 mg) which was dissolved in pyridine (5 ml) and acetylated by stirring with acetic anhydride (2 ml) for 20 h at 20 °C. The mixture was evaporated twice with toluene and the residue was purified on a silica gel plate (ethyl acetate–pentane: 1:1) to give 140 mg (56 %) of *27*. $[\alpha]_D^{23}$ +61° (c 0.5 $CHCl_3$). Analysis for $C_{61}H_{76}O_{25}$: C, H. ^{1}H NMR: δ 5.60 (H-1.1); 5.42 (H-2.1); 5.78 (H-3.1); 5.88 (H-4.1); 4.86 (H-5.1); 4.20 (H-6.1); 4.13 (H-6'.1); 4.56 (H-1.2); 3.98 (H-2.2); 3.42 (H-3.2); 5.33 (H-4.2); 3.54 (H-5.2); 4.29 (H-6.2); 4.14 (H-6'.2); 4.68 (H-1.3); 5.22 (H-2.3); 5.23 (H-3.3); 3.71 (H-4.3); 3.82 (H-5.3); 1.45 (H-6.3). $J_{12.1}$ 3.7 Hz; $J_{23.1}$ 10.8; $J_{34.1}$ 3.5; $J_{45.1}$ 1.0; $J_{56.1}$ 6.8; $J_{56'.1}$ 5.9; $J_{66'.1}$ 11.8; $J_{12.2}$ 0; $J_{23.2}$ 2.2; $J_{34.2}$ 9.4; $J_{45.2}$ 9.6; $J_{56.2}$ 3.0; $J_{56'.2}$ 5.9; $J_{66'.2}$ 12.2; $J_{12.3}$ 0; $J_{34.3}$ 9.6; $J_{45.3}$ 9.6; $J_{56.3}$ 6.0.

*8-Methoxycarbonyloct-1-yl 4-*O*-(2-*O*-α-*D*-galactopyranosyl-β-*D*-mannopyranosyl)-α-*L*-rhamnopyranoside (29).* Compound (*27*) (125 mg, 0.10 mmol) was hydrogenated in methanol (50 ml) and acetic acid (10 ml) with palladium on charcoal (5 %, 200 mg) under hydrogen pressure (1400 psi) for 20 h at 20 °C. The mixture was filtered through celite and evaporated twice with chloroform giving 110 mg of *28*. This product (95 mg) was dissolved in methanolic sodium methoxide (0.1 M, 15 ml) and the mixture was stirred for 12 h. After neutralization with ion exchange resin (Amberlite IRC-50) and filtration the mixture was evaporated and the residue was purified on silicagel (ethyl acetate–methanol–water: 6:3:1) to give 38 mg (71 %) of *29*. $[\alpha]_D^{23}$ +0.2° (c 3.0, H_2O). ^{1}H NMR: δ 5.18 (H-1.1); 3.74 (H-2.1); 3.82 (H-3.1); 3.88 (H-4.1); 4.16 (H-5.1); 3.55–3.61 (H-6.1–H-6'.1); 4.81 (H-1.2); 4.12 (H-2.2); 3.61 (H-3.2); 3.53 (H-4.2); 3.26 (H-5.2); 3.82 (H-6.2); 3.65 (H-6'.2); 4.65 (H-1.3); 3.77 (H-2.3); 3.81 (H-3.3); 3.53 (H-4.3); 3.59 (H-5.3); 1.55 (H-6.3). $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 9.8; $J_{34.1}$ 3.0; $J_{45.1}$ 1.2; $J_{56.1}$ 6.3; $J_{56'.1}$ 6.3; $J_{12.2}$ 0; $J_{23.2}$ 3.0; $J_{34.2}$ 9.5; $J_{45.2}$ 9.8; $J_{56.2}$ 2.5; $J_{56'.2}$ 6.3; $J_{66'.2}$ 12.2; $J_{12.3}$ 0; $J_{34.3}$ 9.8; $J_{45.3}$ 9.8; $J_{56.3}$ 6.0. ^{13}C NMR: 101.1 ppm (C-1.1); 69.1 (C-2.1); 70.4 (C-3.1); 70.2 (C-4.1); 71.5 (C-5.1); 62.2 (C-6.1); 101.9 (C-1.2); 78.1 (C-2.2); 77.7 (C-3.2); 71.9 (C-4.2); 75.4 (C-5.2); 61.8 (C-6.2); 100.9 (C-1.3); 71.7 (C-2.3); 70.6 (C-3.3); 81.2 (C-4.3); 68.5 (C-5.3); 18.2 (C-6.3). $J_{C1H1.1}$ 171 Hz; $J_{C1H1.2}$ 158; $J_{C1H1.3}$ 169.

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On the Formation of Nitrosation Reagents

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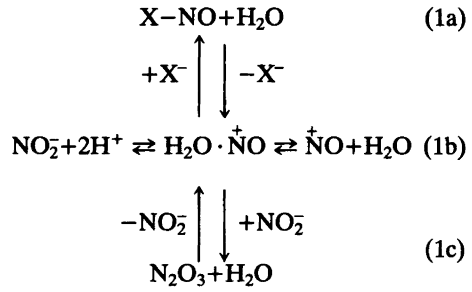
By use of quantum mechanical perturbation theory developed by Klopman and semi-empirical calculations, the change in perturbation energy for reactions of the nitrosyl ion with anions has been calculated. It is found that the calculated change in perturbation energy correlates with the experimental equilibrium constants for these reactions. The atomic net charges on the nitrogen of some nitrosation reagents have been calculated and found to be inversely proportional to the Pearson's nucleophilicity parameter of the nucleophiles (anions) to which the nitrosyl group is bound. The results are discussed in terms of the Hard and Soft Acids and Bases (HSAB) principle.

The kinetics of the reaction between nucleophiles and nitrous acid [1–4] or nitrosation reagents [1–5] are complex. Investigations have shown that the nitrosation reagent probably is neither the nitrite ion nor nitrous acid but a species in which the nitrosyl group ($^+N{=}O$) is free,[6] or bound to a carrier.[1–4] The carrier can be an anion or a neutral species (*e.g.* halide-, nitrite- or thiocyanate ions and alcohols, sulfides or water).

The first step in the formation of nitrosation reagents is protonation of nitrous acid (Scheme 1, 1b); from 1b different routes are possible depending on the reaction conditions.

Anions as chloride, bromide and thiocyanate (1a) are important as catalysts in nitrosation of nucleophiles, and the reaction rate for nitrosation of nucleophiles with anions (X^-) present is given by eqn. (2):[1]



Scheme 1.

$$\text{Rate}=k_2[\text{Nu}][\text{H}^+][\text{HNO}_2][\text{X}^-] \quad (2)$$

which is equivalent to the concentration product eqn. (3):[1]

$$\text{Rate}=k_3[\text{Nu}][\text{X–NO}] \quad (3)$$

In eqn. (2) k_2 contains implicity the equilibrium constants for the formation of X–NO which are known for some nitrosation reagents. For nitrosation with nitrous anhydride (1c) the reaction rate is given by eqn. (4),

$$\text{Rate}=k_4[\text{Nu}][\text{HNO}_2]^2 \quad (4)$$

where k_4 contains the equilibrium constant for the formation of N_2O_3.

By using perturbation theory, Klopman [7] has derived an expression for the energy (ΔE) which is gained or lost when orbitals of one reactant overlap with those of another:

$$\Delta E=-\sum_{ab}(q_a+q_b)\beta_{ab}S_{ab}-\sum_{k<l}\frac{Q_k\cdot O_l}{\varepsilon}\Gamma + \sum_{r}^{\text{occ.}}\sum_{s}^{\text{unocc.}}-\sum_{s}^{\text{occ.}}\sum_{r}^{\text{unocc.}}\frac{2(\Sigma_{ab}c_{ra}c_{sb}\cdot\beta_{ab})^2}{E_r-E_s} \quad (5)$$

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In eqn. (5) q_a and q_b are the electron population in the atomic orbitals a and b, β_{ab} is the resonance integral. S_{ab} is the overlap integral. Q_k and Q_l are the total charges on atoms k and l. Γ is $1/R_{kl}$; R_{kl} is the distance between the atoms k and l; c_{ra} is the coefficient of atomic orbital a in molecular orbital r (b,s respectively). E_r is the energy of molecular orbital r, and E_s is the energy of molecular orbital s.

The first term in eqn. (5) expresses the first order closed shell repulsion and is derived from the interaction of filled orbitals of one molecule with the filled orbitals of another. This term is ignored here, because the main idea of the frontier orbital theory is to explain features of differential reactivity. The second term in (5) is the Coulomb repulsion or attraction, and the third one is the interaction of filled orbitals of one molecule with the unfilled ones of another. When $E_r - E_s$ is large, the contribution from the third term is neglected and the reaction is said to be charge controlled, whereas if $E_r \simeq E_s$ the third term becomes the important one, and the reaction is said to be frontier orbital controlled.

Eqn. (5) can be approximated to eqn. (6) by using only the HOMO of the nucleophile and LUMO of the electrophile:

$$\Delta E = -\frac{Q_{\text{nucl}} Q_{\text{elec}}}{\varepsilon} \Gamma + \frac{2(c_{\text{nucl}} c_{\text{elec}} \beta)^2}{E_{\text{HOMO}} - E_{\text{LUMO}}} \quad (6)$$

Eqn. (6) is a good approximation of eqn. (5) because the interactions of the other orbitals have all much larger $E_r - E_s$ values and thus make a small contribution to third term in eqn. (6)

The present approach is based on a form of molecular orbital theory, termed energy weighted maximum overlap (EWMO) by Linderberg and Öhrn,[8] and within an electron propagator framework. The model has the attractive features that only atomic orbitals, orbital energy parameters and the molecular geometry enter. Extensive applications of the method show very satisfactory correlation between orbital energies and the ionization potentials.[10,11] Recently the basic ideas behind the model and the applications made so far have been reviewed.[12]

This paper presents an investigation of the formation of nitrosation reagents from protonated nitrous acid and anions, and comparison with experimental results is made. The atomic net charge of some nitrosation reagents has been calculated and discussed in relation to Pearson's nucleophilicity parameter[9] and the hard and soft acids and bases principle.

RESULTS AND DISCUSSION

A new version of an e.w.m.o. program has recently been developed,[13,14] in which atomic parameters, described elsewhere,[14] are stored in the program. The only essential input is the molecular geometry and atomic numbers, while the output gives the atomic net charges, molecular orbitals, molecular energies, *etc.* As input parameters known structures and mean bond lengths of molecules from the literature are used.

In Fig. 1 the calculated HOMO orbital energies of the anions are used to classify the ions as hard or soft according to Pearson,[8] a hard base having a low value of the occupied frontier orbital, whereas a soft base has a higher value. In other words, softness increases with increased energy of the occupied frontier orbital. A hard acid has a high value of the empty frontier orbital and the hardness will decrease with decreased energy.

Fig. 1 shows the HOMO energy levels for some anions, H_2O, and the LUMO energy levels for the nitrosyl group, free or bound to H_2O. It is

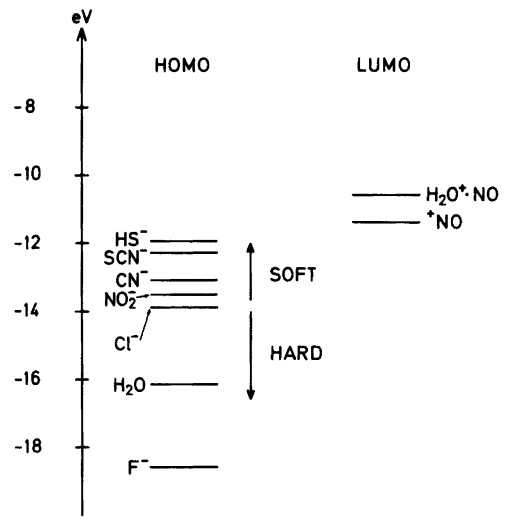

Fig. 1. Schematic representation of the HOMO energy levels in anions and H_2O and LUMO energy levels for the nitrosyl ion free or bound to H_2O.

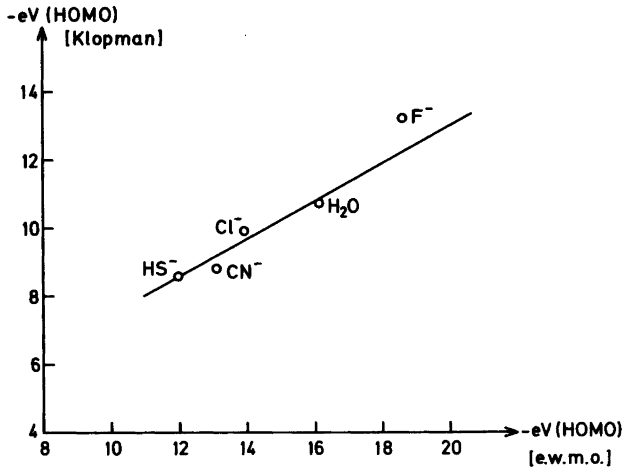

Fig. 2. The correlation between E_{HOMO} calculated by the e.w.m.o. model and E^{*}_{HOMO} calculated by Klopman.

also seen that the order of the energy of the nucleophiles is in accordance with that calculated by Klopman,[7] using eqn. (7)

$$E^{*}_{HOMO} = -\frac{IP+3EA}{4} + \frac{14.388(q+0.5)}{R_{ion}}\left(1-\frac{1}{\varepsilon}\right) \quad (7)$$

where IP is the ionization potential, EA the electron affinity, R_{ion} the effective ionic radii (in Å), q is the initial charge of the ion and ε is the dielectric constant of the solvent.

The correlation between E^{*}_{HOMO} from eqn. (7) and E_{HOMO} from e.w.m.o. calculations is linear (Fig. 2) (α=0.54±0.10 and corr.coef.=0.95). The E_{HOMO} values calculated by EWMO are generally found to be lower than those calculated by Klopman.[7]

As the LUMO-energy of $H_2O \cdot \overset{+}{N}O$ is larger than that of $\overset{+}{N}O$ (Fig. 2), $\overset{+}{N}O$ bound to water is the harder species of the two. Our value for E_{LUMO} for $\overset{+}{N}O$ (−11.42 eV) also correlates well with that of Yonezawa *et al.*,[15] (−11.51 eV), calculated by a semi-empirical SCF molecular treatment.

Using eqn. (6) the total perturbation (neglecting the contribution from the change in solvent energy ΔE for the reaction of $H_2O \cdot \overset{+}{N}O$ with X^-) can be calculated using the following constant values: $Q_{elec.}$=+1, ε=80 and $c_{elec.}$=1 and β obtained from Ref.7. The results are summarized in Table 1.

It is seen from Table 1 that ΔE increases with increased softness of the anions which shows that NO^+ prefers to coordinate with the soft anions rather than with the hard ones.

The effect of X on the atomic net charges of nitrogen (Q_N) of nitrosation reagents has been calculated (Fig. 3).

A good correlation between Q_N and Pearson's nucleophilicity parameter, n,[19] is found. It is observed that Q_N is largest when $\overset{+}{N}O$ is bound to hard species (H_2O), but tends to zero or negative, when $\overset{+}{N}O$ is bound to soft species (SCN^-, HS^-).

When $H_2O \cdot \overset{+}{N}O$ reacts with different nucleophiles either a frontier-controlled or a charge-controlled reaction can occur. The protonated nitrous acidium ion ($H_2O \cdot \overset{+}{N}O$) reacts with the fluoride ion to give nitrosyl fluoride in a charge-controlled reaction because the atomic net charge on the nitrogen changes from +0.80 in the protonated acidium ion to +0.92 in nitrosyl fluoride whereas in the reaction of thiocyanate ion complete charge transfer occurs from +0.80 in the nitrous acidium ion to −0.04 on the nitrosyl nitrogen in nitrosyl thiocyanate. It is also seen from Table 1 that $H_2O \cdot \overset{+}{N}O$ prefers to undergo a frontier-controlled reaction when n increases.

ΔE-values correlate well with the known ex-

Table 1. Calculations of ΔE from eqn. 6. [a,b]

	F^-	Cl^-	Br^-	NO_2^-	CN^-	SCN^- [b]	HS^-
R_{X-NO}(Å)	1.52	1.95	2.14	1.23	1.47	1.70	1.70
$-2\beta_X$(eV)	4.48	4.10	3.60	4.20	3.8	3.9	4.0
$-E_{HOMO}$(eV)	18.60	13.90	13.20 [c]	13.54	13.10	12.31	11.97
ΔE(eV)	0.70	1.33	1.46	1.57	1.63	1.85	2.90

[a] The value of E_{LUMO} ($H_2O^+ \cdot NO$) is −10.59 eV. [b] c_{nucl}=1, except for SCN^-, where c_{nucl} is calculated from $\psi_{HOMO}=0.876\,\phi_S+0.142\,\phi_C-0.456\,\phi_N$. The thiocyanate ion is ambident and if the energy gap between the HOMO orbital of thiocyanate and the LUMO orbital of the electrophile is large, then the reaction is charge-controlled. The thiocyanate ion will then prefer to coordinate with a cation through its nucleophilic nitrogen, which carries the highest negative charge. On the other hand, if there is only a minor energy gap between the HOMO and LUMO orbitals, the reaction is orbital-controlled and the cation will seak the largest electronic density pertaining to the highest occupied orbital of SCN^-. Assuming all other things to be equal, $H_2O \cdot NO^+$ will then prefer to attack sulfur which carries the highest electronic charge in this particular orbital: $c_S^2=(0.878)^2=0.771$; $c_N^2=(-0.456)^2=0.208$. The value for $c_s^1=0.771$ is then used in the calculation of ΔE for X=SCN. [c] E_{HOMO} for Br^- is obtained from Fig. 2 as the program has not been developed for atoms with atomic numbers higher than 17.

Table 2. ΔE compared with the experimental equilibrium connstants for the same reaction.

	Cl−NO	Br−NO	O_2N−NO	NCS−NO
ΔE(eV)	1.33	1.46	1.57	1.85
K	5.6×10^{-4} [a,16]	2.2×10^{-2} [b,17]	0.2 [c,18]	46 [d,19]

[a, b, d] at 0 °C; [c] At 20 °C.

perimental equilibrium constants and illustrate the semiquantitative agreement between the calculated and observed reactions of various nucleophiles with $H_2O \cdot \overset{+}{N}O$ (Tables 1 and 2).

The reaction between a nucleophile and an electrophile can be either ionic or frontier orbital controlled. If E_{HOMO} has a large negative value and E_{LUMO} a large positive value, the stable

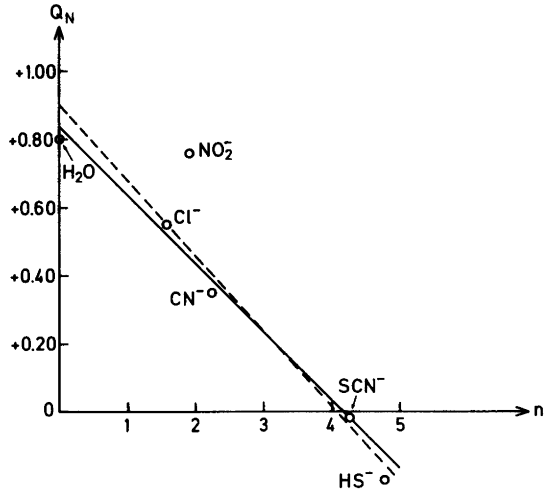

Fig. 3. Plot of Q_N (the atomic netto charge at the nitrosation nitrogen) as a function of n (Pearson's nucleophilicity parameter). *Full line:* without Q_N of NO_2^-: $Q_N=0.84-n \cdot 0.20$ [$s(Q_N^\circ)=0.048$ and $s(n)=0.016$]. *Dotted line:* with Q_N of NO_2^-: $Q'_N=0.91-n \cdot 0.22$ [$s(Q_N^\circ)=0.091$ and $s(n)=0.032$].

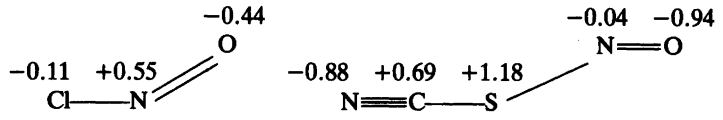

Fig. 4. Calculated atomic net charges on the atoms in nitrosyl chloride and nitrosyl thiocyanate.

hard–hard interaction can take place, whereas if E_{HOMO} has a small negative value and E_{LUMO} a large negative value, a soft–soft interaction can occur. Hard–hard interactions take place because of favourable entropy and soft–soft combinations due to favourable enthalpy. The interaction between the nitrosyl group and thiocyanate ion is then a typical soft–soft combination ($\Delta H = -2.9$ kcal/mol)[19] and the interaction of the nitrosyl group with the chloride ion is more of a hard–soft (borderline) interaction (ΔH=5,4 kcal/mol). The hard–hard bonding should then be electrostatic and the soft–soft one covalent. Fig. 4 shows the atomic net charges of Cl–NO and NCS–NO calculated by the EWMO model (observe that the values calculated "too high" which is due to an exaggerated polarization in the calculation by EWMO).

The values in Fig. 4 also indicate that a charge transfer takes place from the thiocyanate ion to the nitrosyl ion, which is typical for soft–soft interactions, whereas in nitrosyl chloride the bonding is more electrostatic.

CONCLUSION

The change in perturbation energy for the reaction of the protonated nitrous acidium ion with anions has been calculated. Correlation between the stabilization energy and the equilibrium constant is found. The atomic net charge of the nitrogen in the nitrosation reagent is found to be inversely proportional to the Pearson's nucleophilicity parameter of the anions.

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Addendum. After this manuscript was accepted for publication a quite recent paper was published: *Ab Initio Studies of a Proposed Mechanism for* N-*Nitrosamine Formation.*[22]

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The Crystal and Molecular Structure of 8-Hydroxy-1-methylquinolinium Chloride Hydrate and 1-Methylquinolinium-8-olate Dihydrate

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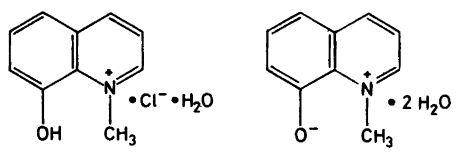
The structures of the title compounds, $C_{10}H_{10}ClNO.H_2O$ (*1*) and $C_{10}H_9NO.2H_2O$ (*2*) have been determined by X-ray methods. Full-matrix least-squares refinements led to final conventional *R*-values of 0.042 for *1* (3443 reflections) and 0.060 for *2* (2214 reflections). Crystals of *1* are triclinic, space group $P\bar{1}$, with (at −150 °C) a=7.765(3) Å; b=7.932(3) Å; c=8.913(3) Å; α=81.95(3)°; β=72.89(3)°; γ=70.45(3)°, Z=2. Crystals of *2* are monoclinic, space group $P2_1/c$, with (at −150 °C) a=7.282(2) Å; b=13.643(3) Å, c=10.049(2) Å; β=98.69(2)°, Z=4.

The structural changes upon *N*-methylation of 8-hydroxyquinoline comprise a lengthening of the C–N ring bonds by 0.02 Å and also a weakening of more distant bonds in the molecule. *2* is the conjugate base of the acid *1*. The deprotonation of the oxygen atom leads to a shortening of the C–O bond by 0.06 Å and an increase of the adjacent C–C bond lengths by 0.02 Å.

8-Hydroxyquinoline and its derivatives have been extensively studied, mainly because of their ability to coordinate with a wide range of metal ions and because of their desinfectant effect.[1]



1 *2*

Scheme 1.

Although the X-ray structure of the mother compound has been determined in its complex with 1,3,5-trinitrobenzene,[2] no account of the structure of the *N*-methylated derivative (*1*) has been found in the literature. There has been some interest in the betaine of the *N*-methyl derivative (*2*) because of certain peculiarities in its absorption spectrum.[3] For comparison we therefore decided to determine this structure as well.

EXPERIMENTAL

Materials. 8-Hydroxy-1-methylquinolinium iodide was prepared according to the method of Saxena *et al.*[3] with the exception that the reaction was carried out at room temperature overnight. Compound *2* was obtained from the salt using an anion exchanger (Amberlite®-IRA-400). Treatment of *2* with hydrochloric acid gave *1*.

X-Ray data. The data collection procedure for the two compounds were similar. A SYNTEX $P\bar{1}$ automatic diffractometer was used with graphite crystal monochromated Mo$K\alpha$ radiation. The temperature at the crystal site was −150 °C. Cell parameters were refined using the diffractometer settings of 15 general reflections with $2\theta > 30°$. Intensities were measured using the θ–2θ scan technique; the scan speed (2θ) was 2–4° min^{-1} depending on the peak intensity, scan width ±1°. Background counts were taken for 0.35 times the scan time at each of the scan limits. Data were collected up to a sin θ/λ value of 0.8 Å^{-1}. The three standard reflections monitored for every 100 data showed no systematic variation. Out of the 3520 unique reflections collected from

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compound *1* 3443 with $I \geq 2.5\sigma(I)$ were retained for the structure analysis; the corresponding numbers for *2* were 3229 and 2214. The standard deviation of the intensities was calculated as $\sigma(I)=|C_T+(0.02C_N^2|^{\frac{1}{2}}$, where C_T is the total number of counts and C_N is the scan count minus background count. Corrections were made for Lorentz and polarization effects but not for absorption.

CRYSTAL DATA

1. 8-Hydroxy-1-methylquinolinium chloride hydrate, $C_{10}H_{10}ClNO.H_2O$, m.p. 250 °C. (dec.) Triclinic, a=7.765(3) Å; b=7.932(3) Å; c=8.913 (3) Å; α=81.95(3)°; β=72.89(3)°; λ=70.45(3)°; V=493.9(3) Å^3; (t=−150 °C); M=213.66; Z=2; F (000)=224; μ(Mo$K\alpha$)=3.7 cm^{-1}; D_x= 1.436 gcm^{-3}. Space group $P\bar{1}$(No. 2).

2. 1-Methylquinolinium-8-olate dihydrate, $C_{10}H_9NO.2H_2O$, m.p. 130 °C. (dec.) Monoclinic, a=7.282(2) Å; b=13.643(3) Å; c=10.049(2) Å; β=98.69(2)°; V=986.9(3) Å^3; (t=−150 °C); M=195.22; Z=4; F(000)=416; μ(Mo$K\alpha$)=1.1 cm^{-1}; D_x=1.314 gcm^{-3}. Absent reflections: (h 0 l) for l odd, (0k0) for k odd. Space group $P2_1/c$ (No. 14).

STRUCTURE DETERMINATIONS

The computer programs used for the structure analyses are described in Ref. 4. The atomic scattering factors for Cl^-, O, N, and C are given by Doyle and Turner[5] and for H by Stewart, Davidson and Simpson.[6]

Both structures were solved using the program assembly MULTAN.[7] The usual sequence of isotropic and anisotropic refinements was followed; hydrogen positions were calculated and refined, isotropic thermal parameters were applied. In order to avoid the influence of bonding electrons on the atomic positions only reflections with sin θ/λ larger than 0.45 Å^{-1} were used during the last refinement cycles.

The refinements converged to conventional R factors of 0.042 (*1*) and 0.060 (*2*), the R_w values were 0.042 (*1*) and 0.052 (*2*) and goodness of fit, $[\Sigma w\Delta F^2/(m-n)]^{\frac{1}{2}}$, 2.03 (*1*) and 1.45 (*2*). Difference Fourier syntheses were calculated, giving only minor peaks mainly attributable to valence electrons.

Final atomic coordinates are given in Table 1. Lists of structure factors and thermal parameters are available from the authors.

Table 1. Fractional atomic coordinates.

Atom.	x	y	z
Compound *1*			
C1	0.1641(1)	0.3601(1)	−0.3767(1)
C2	0.1744(1)	0.5343(1)	−0.4176(1)
C3	0.0447(1)	0.6713(1)	−0.3274(1)
C4	−0.0906(1)	0.6349(1)	−0.1916(1)
C5	−0.2186(1)	0.7756(1)	−0.0950(1)
C6	−0.3472(1)	0.7393(1)	0.0377(1)
C7	−0.3510(1)	0.5624(1)	0.0786(1)
C8	−0.2278(1)	0.4195(1)	−0.0117(1)
C9	−0.0943(1)	0.4550(1)	−0.1519(1)
C10	0.0324(1)	0.1343(1)	−0.2311(1)
N	0.0345(1)	0.3226(1)	−0.2521(1)
O	−0.2301(1)	0.2502(1)	0.0322(1)
Cl	−0.33565(4)	0.09450(4)	0.59858(3)
WO	−0.4792(1)	0.2215(1)	0.3002(1)
H1	0.2485	0.2602	−0.4373
H2	0.2723	0.5594	−0.5143
H3	0.0390	0.7905	−0.3509
H5	−0.2152	0.8946	−0.1249
H6	−0.4276	0.8313	0.0993
H7	−0.4463	0.5383	0.1767
H10A	0.1241	0.0786	−0.3188
H10B	0.0656	0.0824	−0.1337
H10C	−0.0959	0.1351	−0.2324
HO	−0.3168	0.2492	0.1152
WH1	−0.4264	0.1969	0.3725
WH2	−0.5298	0.1461	0.3042
Compound *2*			
C1	0.3561(3)	−0.1390(1)	0.5717(2)
C2	0.3975(3)	−0.0798(2)	0.6862(1)
C3	0.3521(3)	0.0164(2)	0.6781(1)
C4	0.2623(3)	0.0577(1)	0.5557(2)
C5	0.2205(3)	0.1591(1)	0.5455(2)
C6	0.1368(4)	0.1971(1)	0.4255(3)
C7	0.0876(3)	0.1365(1)	0.3132(2)
C8	0.1238(2)	0.0352(1)	0.3138(1)
C9	0.2182(2)	−0.0046(1)	0.4408(1)
C10	0.2417(3)	−0.1726(1)	0.3404(2)
N	0.2709(2)	−0.1022(1)	0.4543(1)
O	0.0762(2)	−0.0192(1)	0.2092(1)
WO1	0.2232(2)	0.0264(1)	−0.0130(1)
WO2	0.5392(2)	0.1374(1)	−0.0273(1)
WH1	0.1789	0.0085	0.0598
WH2	0.1294	0.0247	−0.0772
WH3	0.4248	0.1073	−0.0240
WH4	0.6149	0.0913	−0.0059
H1	0.3799	−0.2099	0.5610
H2	0.4552	−0.1140	0.7568
H3	0.3870	0.0488	0.7525
H5	0.2519	0.1952	0.6332
H6	0.1141	0.2610	0.4186
H7	0.0242	0.1585	0.2259
H10A	0.2958	−0.2337	0.3841
H10B	0.3004	−0.1425	0.2674
H10C	0.1025	−0.1781	0.3090

Table 2. Structural data.

Bond lengths (Å)	*1*	corr.	*2*	corr.
C1–C2	1.402(2)	1.405	1.401(4)	1.406
C2–C3	1.370(2)	1.372	1.353(4)	1.357
C3–C4	1.418(2)	1.420	1.420(4)	1.423
C4–C5	1.413(2)	1.415	1.416(4)	1.421
C4–C9	1.430(2)	1.433	1.431(4)	1.435
C5–C6	1.372(2)	1.374	1.368(4)	1.372
C6–C7	1.407(2)	1.410	1.401(4)	1.406
C7–C8	1.387(2)	1.389	1.407(4)	1.411
C8–C9	1.431(1)	1.433	1.459(4)	1.463
C8–O	1.350(1)	1.353	1.290(4)	1.295
C9–N	1.391(1)	1.393	1.387(4)	1.391
N–C1	1.335(1)	1.337	1.345(4)	1.348
N–C10	1.485(2)	1.488	1.484(4)	1.489

Bond angles (°)	*1*	*2*
C1–C2–C3	118.6(1)	119.3(4)
C2–C3–C4	120.2(1)	120.9(4)
C3–C4–C9	119.4(1)	118.8(4)
C4–C9–N	117.7(1)	117.8(4)
C9–N–C1	121.4(1)	121.9(4)
N–C1–C2	122.5(1)	121.2(4)
C4–C5–C6	119.8(1)	119.8(4)
C5–C6–C7	120.2(1)	120.8(4)
C6–C7–C8	122.2(1)	123.4(4)
C7–C8–C9	118.4(1)	115.6(4)
C8–C9–C4	118.9(1)	120.3(4)
C9–C4–C5	120.4(1)	120.1(4)
O–C8–C7	121.2(1)	122.3(4)
C9–C8–O	120.3(1)	122.1(4)
C10–N–C9	122.5(1)	122.7(4)
C1–N–C10	116.1(1)	115.3(4)
C3–C4–C5	120.0(1)	121.1(4)
C8–C9–N	123.4(1)	122.0(4)

Hydrogen bond lengths(Å) and angles(°)

Structure *1*		
O–HO···WO	2.631	172.4
WO–WH1···Cl	3.094	166.0
WO–WH2···Cl[a]	3.191	161.0
Structure *2*		
O···WH1–WO1	2.691	174.7
O[b]···WH2–WO1	2.712	178.0
WO1···WH3–WO2	2.777	169.3
WO1[c]···WH4–WO2	2.817	169.8

Equivalent positions
[a] (−1−x,−y,1−z)
[b] (−x,−y,−z)
[c] (1−x,−y,−z)

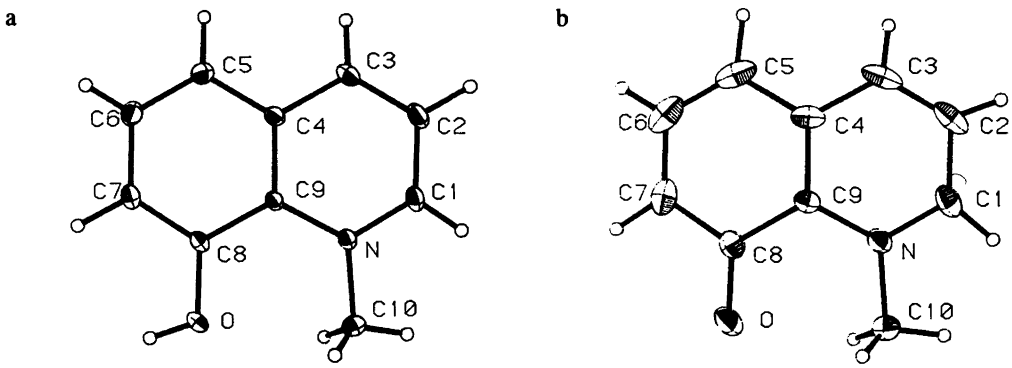

Fig. 1. ORTEP plots of 8-hydroxy-1-methylquinolinium cation (a) and of 1-methylquinolinium-8-olate zwitterion (b).

In Table 2 are listed various structural data. Estimated standard deviations are calculated from the variance–covariance matrices. Analyses of the rigid body motion were carried out; bond lengths corrected for librational motion are given in Table 2.

ORTEP drawings with the numbering schemes for the molecules are shown in Fig. 1.

DISCUSSION

Molecular structure, compound 1. Prout *et al.* have determined the structure of the 1:1 complex of 8-hydroxyquinoline with 1,3,5-trinitrobenzene.[2] The pattern of long and short C–C bonds is nearly identical to that of *1* in spite of the complex formation. The difference between the two is the methyl group attached to the nitrogen atom of the present compound. In order to obtain information of the effect of *N*-methylation of 8-hydroxyquinoline a simple Hückel calculation was performed. The results showed that the positive charge is roughly equally shared among the atoms N, C1, C3 and C9. A weakening of the N–C1 and N–C9 bonds results in a lengthening of these bonds; this is also what the experimental structural data indicate. More unexpected, however, is the observation that there is an increase in the C2–C3 and C8–C9 bond lengths and a decrease in the C6–C7 bond upon the *N*-methylation. The change in all five bond lengths mentioned is close to 0.02 Å, which seems to be significant. The other bonds are unaffected by the methylation.

The C–O bond length in *1* is 1.353 Å which is typical in hydrogen bonded phenols.[8,9] The nitrogen–methyl carbon bond length of 1.488 Å is within the range of literature values in *N*-methylpyridinium salts (1.46–1.51 Å[10,11]).

The oxygen–methyl carbon atoms are separated by 2.65 Å; the two nearest methyl hydrogen atoms are at van der Waals' distance from the oxygen atom, 2.40 and 2.44 Å, respectively. The hydroxyl hydrogen atom is pointing away from the methyl group in the molecular plane and forms a hydrogen bond to a water molecule (*cf.* Table 2).

The 8-hydroxy-1-methylquinolinium ion is, with the obvious exception of two methyl hydrogen atoms, essentially planar. The oxygen and methyl carbon atoms are slightly out on each side of the least squares plane, 0.08 and 0.12 Å, respectively.

Molecular structure, compound 2. 1-Methylquinolinium-8-olate, *2*, is derived from the 8-hydroxy-1-methylquinolinium ion, *1*, by deprotonation of the hydroxy substituent. In terms of simple bond theory this leads to a shortening of the C–O bond owing to an enhanced double bond character and an accompanying increase of the C7–C8 and C8–C9 bond lengths. Our data are in complete agreement with these predictions, the C–O bond is by 0.06 Å shorter in *2* than in *1*, the neighbouring C–C bonds by 0.02–0.03 Å longer. An analogous situation is observed for the picric acid–picrate system with a slightly larger effect.[8,12]

The zwitterion is also nearly planar, the oxygen and methyl carbon atoms being situated 0.06 and 0.09 Å, respectively, out on each side of the least squares plane.

Crystal packing. The crystal forces, apart from Coulomb forces in *1*, are dominated in both compounds by hydrogen bonds and interactions between aromatic molecular pairs.

In structure *1* the oxygen atom of the quinolinium ion acts as hydrogen donor in a hydrogen bond to the water molecule. The water molecule is hydrogen bonded to two chloride ions as indicated in Table 2. In structure *2* pairs of centrosymmetrically related molecules are linked together by pairs of water molecules through hydrogen bonds; the oxygen atom of the zwitterion is thus an acceptor for two hydrogen bonds. Each water molecule is hydrogen bonded to two other water molecules as described in Table 2.

In both compounds the aromatic moieties are arranged in centrosymmetrical pairs as indicated in Fig. 2, which shows the overlap diagrams as seen along the normal to the parallel molecular planes. The interplanar spacing is 3.35 Å in both compounds.

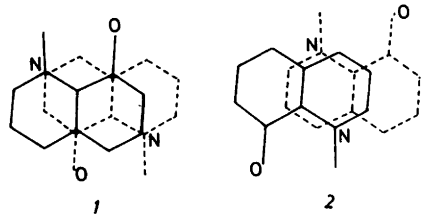

Fig. 2. π–π interaction overlap diagrams of the structures *1* and *2*.

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Enthalpy of Reaction of Grignard Reagents with Brønsted Acids

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Enthalpies of formation of the magnesium salts BMgBr of 20 Brønsted acids HB have been determined from the enthalpy of reaction of HB with pentylmagnesium bromide in ether. The enthalpy of metallation of hydrocarbons with a Grignard reagent has been shown to vary linearly with the pK_a value of the carbon acid. Metallation of especially oxygen and nitrogen acidic substances has a more negative enthalpy of reaction than found for a carbon acid of the same pK_a. The reason is assumed to be back donation of the lone pairs of the hetero atoms to empty orbitals at magnesium and an attempt is made to quantify the effect.

The acidity of very weak Brønsted acids has been estimated by various methods.[1] Equilibrium methods and acidity function procedures have been useful in determining pK_a values for hydrocarbons forming resonance-stabilized anions; acidities thus determined have been linked to the acidity of alcohols, carboxylic acids, *etc.*, in aqueous solution. For unactivated alkanes the "kinetic acidity" has been studied by measuring the rate of hydrogen isotope exchange in a suitable system and using Brønsted linear free energy plots. pK_a-Values ranging from 18–41 have been determined for hydrocarbons forming charge delocalized anions and values around 50 estimated for unactivated hydrocarbons.

An alternative to the kinetic procedures has been an enthalpic approach utilizing the apparent linear correlation between the enthalpy of protonation of the anion and the pK_a-value. Thus, Arnett *et al.*[2] used the DMSO anion deprotonation of Brønsted acids and established a linear correlation between ΔH_r and pK_a for carbon acids up to $pK_a=33$, including many types of acids with lower pK_a, even hydrogen chloride and hydrogen iodide. An apparent linear correlation was found also for the enthalpy of protonation[3] of Grignard reagents in ether with HBr and pK_a of the corresponding hydrocarbons:

$$RMgBr+HBr \rightarrow RH+MgBr_2 \qquad (1)$$

The method was especially useful for unactivated hydrocarbons of extremely low acidity. Knowing the enthalpy of formation for the Grignard reagents the enthalpy of reaction could be determined for the metallation of the various hydrocarbons by a reference Grignard reagent, *in casu* pentylmagnesium bromide:

$$C_5H_{11}MgBr+R-H \rightarrow C_5H_{12}+R-MgBr \qquad (2)$$

The enthalpy of reaction for this hypothetical reaction is likewise linearly correlated to the pK_a-value of RH.

It was found of interest to extend the thermochemical measurements of the metallation with Grignard reagents from carbon acids to Brønsted acids in general, including oxygen, nitrogen, halogen and sulfur acidic compounds; the general reaction being (solutions in ether):

$$C_5H_{11}MgBr + B-H \rightarrow C_5H_{12} + B-MgBr \qquad (3)$$

Determination of the enthalpies of formation for the magnesium salts of alcohols, thiols, amines, *etc.* was thought to be a useful contribution to the little-developed thermochemistry of Grignard reactions, and the relation between $\Delta H_r(2)$ and pK_a was interesting in the light of the results obtained with carbon acids.

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Table 1. Enthalpy of metallation of Brønsted acids HB with pentylmagnesium bromide in diethyl ether. pK_a values taken from Ref. 1 (values in parentheses are estimated). Enthalpies of formation of B-MgBr given for ~0.1 M salt in 0.5 M ethereal pentylmagnesium bromide.

Brønsted acid HB	pK_a(HB)	ΔH_r(1) kcal mol^{-1}	Number of experiments	ΔH_f^o[B–MgBr]soln in RMgBr/ether kcal mol^{-1}
HBr	–9	−59.8±0.5	(4)	−133.6
HCl	–7	−58.1±0.8	(3)	−145.7
CF_3COOH	–0.5	−65.4	(1)	−242.0
C_2H_5COOH	5	−60.0±0.5	(3)	−237.6
$C_{11}H_{23}COOH$	5	−58.1	(1)	−182.7
C_6F_5OH	5.5	−55.9[b]	(1)	
C_6H_5SH	8	−42.6	(1)	−71.4
C_6H_5OH	10	−48.4±1.0	(3)	−143.6
$CH_2(CN)_2$	11	−48.6	(1)	−59.7
$C_{12}H_{25}SH$	12	−43.8	(1)	
CF_3CH_2OH	12.5	−47.7	(1)	
CH_3OH	16	−52.5[b]	(1)	−165.2
C_2H_5OH	18	−47.7±1.0	(3)	−169.8
$(CH_3)_2CHOH$	18	−46.2	(1)	−177.9
$(CH_3)_3COH$	19	−42.5	(1)	−185.1
$C_{11}H_{23}CONHCH_3$	(25)	−44.5[b]	(1)	
$(C_6H_5)_2NH$	23	−28.4	(1)	−53.0
$C_6H_5NH_2$	28	−36.6±1.0	(2)	−84.8
CH_3NH_2	(41)	−31.2±0.6	(3)	−97.8
Cyclo-$C_6H_{11}NH_2$	41.6	−31.8	(1)	
$C_6H_5CH_3$	41.2	−7.9±1.0	(3)	−60.3
cyclo-$C_6H_{11}NHCH_3$	(43)	−29.3	(1)	
$(CH_2)_5NH$	(45)	−27.9	(1)	−104.7
$(C_2H_5)_2NH$	(46)	−26.6	(1)	−107.1
Cyclopentadiene	16	−35.5[a]	(1)	
$C_6H_5{\equiv}H$	23	−30.1[a]	(1)	−16.6
C_6H_6	43	−6.3±0.5	(4)	−49.8
Cyclopropane	46	−1.6	(1)	−50.5
Methane	48	−3.6±1.0	(3)	−79.3
Cyclopentane	51	+0.5±1.0	(3)	−80.5
Cyclohexane	52	+2.2±1.0	(3)	−90.9

[a] Metallation using dialkylmagnesium. [b] Precipitation.

Since the purpose was to include many substances and not necessarily to obtain the utmost accuracy, a simple calorimetric procedure was followed and the best available commercial quality of substrates was used after simple distillation. Though most of the measurements were single determinations, repetition and control experiments revealed an uncertainty of the results of no more than 2–3 %. Deprotonations of carboxylic acids were carried out in a flow reactor and the temperature measured after 0.01 s so that no Grignard addition to the carboxylate could interfere. Deprotonation was instantaneous with alcohols, thiols and carboxylic acids, but with amines and especially secondary amines it was slow and could last several min; for diethyl amine even between 1 and 2 h. The enthalpies of solution of the substrates in diethyl ether were determined when necessary.

The results are given in Table 1 as $\Delta H_r(3)$ and ΔH_f^o [BMgBr] and in Fig. 1 as $-\Delta H_r(3)$ *versus* pK_a for the acid HB.[1]

As seen from Fig. 1, the $\Delta H_r(3)$-values for oxygen and nitrogen acids are more negative than those of carbon acids with the same pK_a values. Thus, cyclohexylamine and toluene both have

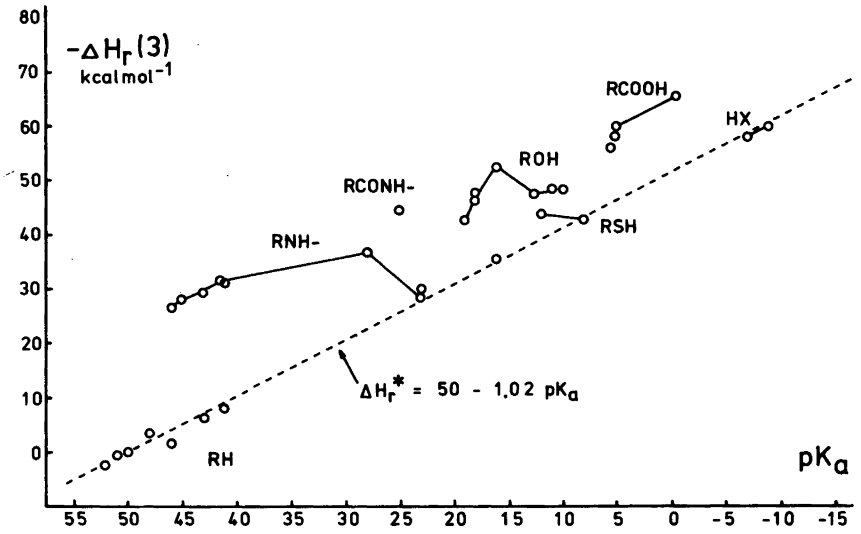


Fig. 1. Plot of $-\Delta H_r(3)$ *versus* pK_a. For explanation of the regression line, see text.

pK_a=41, but the reaction:

$$C_6H_5CH_2MgBr + C_6H_{11}NH_2 \rightarrow C_6H_5CH_3 + C_6H_{11}NHMgBr$$

is exothermic by 23.9 kcal mol^{-1}. Likewise cyclopentadiene and methanol both have pK_a=16, but the exchange reaction is exothermic by 17 kcal mol^{-1}. In analogy it is known from the literature [7] that diphenylmethyl lithium will easily metallate ammonia, while diphenylmethyl potassium will not.

In an attempt to explain these phenomena the author has chosen to abstain from an analysis of possible solvent and entropy effects and ascribe the results solely to enthalpy factors. It seems obvious that nitrogen and oxygen, in comparison with carbon, form relatively strong bonds to magnesium and lithium. The reason for this must be the Lewis acidity of *e.g.* the magnesium ion, which enables it to share the lone pairs on the hetero atoms by back donation:

$$R-\ddot{O}-MgBr \rightarrow R-\overset{+}{O}=\bar{M}gBr$$

Hydrogen and magnesium form covalent σ bonds to carbon. A more electronegative carbon atom forms a somewhat stronger bond to hydrogen, but a much stronger bond to the more electropositive magnesium. In reaction (1) which concerns carbon acids, the reaction enthalpy varies mainly with the difference D(R–H)−D(R–MgBr), and this term, which concerns σ bonds is linearly correlated to pK_a (RH). When back donation occurs, the σ bond is enforced by a donor–acceptor bond, which we may call a π-bond contribution so that:

$$D(B\text{–}MgBr) = D_\sigma(B\text{–}MgBr) + D_\pi(B\text{–}MgBr)$$

It seems to be a useful abstraction, however, to postulate that for a given pK_a of HB there will be approximately the same difference D_σ–(B–H)−D_σ(B–MgBr) regardless of the nature of B. The same linear relation between pK_a and $\Delta H_r(2)$ then is obtained for all Brønsted acids, when only the σ-bonding is considered. In Fig. 1 the correlation line has been drawn through points belonging to Brønsted acids for which the magnesium salts are not expected to be stabilized by back donation. These include the carbon acids, but also the halogen acids and sulfur acids like thiols, since the lone pairs on halogen and sulfur are not easily shared with magnesium. The best linear fit to these points is:

$$\Delta H_r^* = 50 - 1.02\ pK_a$$

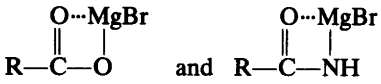
in which ΔH_r^* is the hypothetical fraction of $\Delta H_r(3)$ which pertains only to the σ bonding in the salt B–MgBr.

The extent to which a given value of $-\Delta H_r(3)$ exceeds $-\Delta H_r^*$ is ascribed to π-bonding caused by back donation within the magnesium salt:

$$D_\pi(B\text{–}MgB)=\Delta H_r^*-\Delta H_r(3) \quad (4)$$

From Fig. 1 it is seen that D_π(B–Mg) as defined in eqn. (4) varies not only with the heteroatom, but also with the substitution on the heteroatom. $\Delta H_r(3)$ is almost the same for ethanol, trifluoroethanol and phenol, with pK_a values of 19, 12.5 and 10, respectively. This means that with more electronegative substitution the increase in O-Mg σ-bond strength is offset by a decrease in the strength of the coordinative bond. In the series RNH_2, $PhNH_2$, Ph_2NH having pK_a=41, 28 and 23, respectively, the values for $\Delta H_r(2)$ are −31.8, −36.6, and −28.8 kcal mol^{-1}, indicating that the increase in σ-bond strength is more than offset by the decrease in π-bonding in the magnesium salt of diphenyl amine.

For carboxylic acids and amides the π-bonding is much greater than for amines or alcohols of the same acidity. This indicates that magnesium is coordinated to both the σ-ligand and to the carbonyl oxygen:



The π-contribution to the bonding in the magnesium enolate of malononitrile is low, but significant and may indicate that the $N^+=Mg^-$ bond is weaker for sp^2 hybridized nitrogen than for amine nitrogen.

Besides the electronic factors steric interactions also play a role for the intensity of the π-bonding. The rather considerable increase in π(O–Mg) observed on going from *tert*-butyl alcohol to ethanol and methanol seems to indicate a steric hindrance for bonding in alcohols having a bulky alkyl group.

A complication which has not yet been discussed is the possibility of a change in the amount of solvent coordination during reaction (3). This problem has not been studied in detail. The reactions of phenol and of benzenethiol with pentylmagnesium bromide were carried out in THF as well as in ether and were found to be 5 kcal mol^{-1} and 0 kcal mol^{-1}, respectively, more exothermic in THF. This indicates that either in THF or in ether the solvation of reactants and products differ. Since the enthalpies of coordination of C_2H_5MgBr and $MgBr_2$ are −16 and −26 kcal mol^{-1}, respectively, in THF, but 7.4 and 7.4 kcal mol^{-1} in ether,[4] there is some indication that differences in solvation between reactants and products are not very great in diethyl ether solution. The possibility exists, however, to investigate the problem since it is possible to study the coordination equilibrium between magnesium compounds and diethyl ether by infrared spectroscopy.[5]

EXPERIMENTAL

Materials. Pentylmagnesium bromide was prepared in the usual way from sublimed magnesium and ether distilled from lithium aluminium hydride. Substrates were distilled to obtain a center cut.

Calorimetric procedure. The calorimeter was a 60 ml spherical Dewar flask with an 8 mm Ø opening fitted to a 200 mm inlet tube, through which was passed (1) the leads for a manganin heating coil, (2) the leads for a copper/constantan thermocouple, and (3) an 0.8 mm Teflon capillary for the addition of substrate. The calorimeter was sealed with a rubber stopper. The reference junction of the thermocouple was placed in water of 20.0 °C. The calorimeter was flushed with nitrogen and 50 ml of 0.5 M ethereal pentylmagnesium bromide was added. The temperature of the closed calorimeter was adjusted to 20.0 °C, and the substrate adjusted to 20.0 °C was added in a known amount, usually as a solution in dry ether from a 5 ml Metrohm piston burette through the Teflon capillary. The calorimeter was shaken by hand and the temperature was read after thermal equilibrium was obtained, usually after 30 s. For calibration was used a current calibrator, Fluke Model 382 A and the thermocouple was read by means of a Keithley Model 177 microvoltmeter. The enthalpies of solution of the reactants were determined similarly and corrections were introduced when reactions were run on undiluted substrates.

Flow stream measurements were performed as described.[6] The specific heat of the reaction mixture was determined in the calorimeter.

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Marine Alkaloids. 7. Synthesis of Debromoflustramine B and Related Compounds

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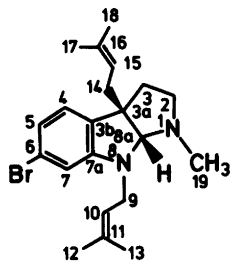
Debromoflustramine B has been synthesized and characterized. The synthesis and spectroscopic properties of a series of structurally related compounds are reported.

Recently a series of bromo-substituted alkaloids were isolated and identified from the marine bryozoan *Flustra foliacea* (L.). Most of the alkaloids are derived from the 1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole skeleton.[1] To establish a framework for the spectroscopic parameters of such compounds and also to study the chemistry and pharmacology of this interesting class of alkaloids, a synthetic study was undertaken. In this report we describe the synthesis and properties of debromoflustramine B (*5*) and related derivatives.

RESULTS AND DISCUSSION

Tryptophan derivatives with a protected N_b amino group are known to suffer electrophilic attack at the 3-position with concomitant attack of the N_b amine function at position 2 leading to derivatives of the 1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole ring system.[2]



Flustramine B

Reactions between N_b-trifluoroacetyl, N_b-acetyl, and N_b-ethoxycarbonyltryptamine (*1a, 1b* and *1c*, respectively) and γ,γ-dimethylallyl bromide (1-bromo-3-methyl-2-butene) were found to lead to the expected 1-acylated-3a-alkylated-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indoles (*2a, 2b* and *2c*) together with the 8-alkylated homologues (*3a, 3b* and *3c*). In all reactions the 1,2-dialkylated-N_b-acylated tryptamines (*6a, 6b* and *6c*) were always found as side products, presumably formed by acid-catalyzed rearrangement of *3*.[3]

On reaction with γ,γ-dimethylallyl bromide *2* could be alkylated to give *3*.

Hydrolysis of *3* to form *4* posed severe problems since *3* is sensitive to base. Acid hydrolysis only resulted in rearranged product (*6b, 6c*) and base-catalyzed as well as imidazole-mediated hydrolyses[4] gave unsatisfactory yields. Several attempts to hydrolyze *3b* and *3c* resulted only in a preparatively unacceptable yield of *4*. Finally, it was found that sodium borohydride reduction[5] of *3a* gave rise to a quantitative yield of *4*.

Several attempts to methylate *4* by standard procedures failed due to formation of large amounts of decomposition products.

However, reaction with formaldehyde and sodium cyanoborohydride[6] gave a fair yield of debromoflustramine B (*5*).

To prepare an *N*-1 methylated derivative (*7*) without *N*-8 substitution N_b-methyltryptamine

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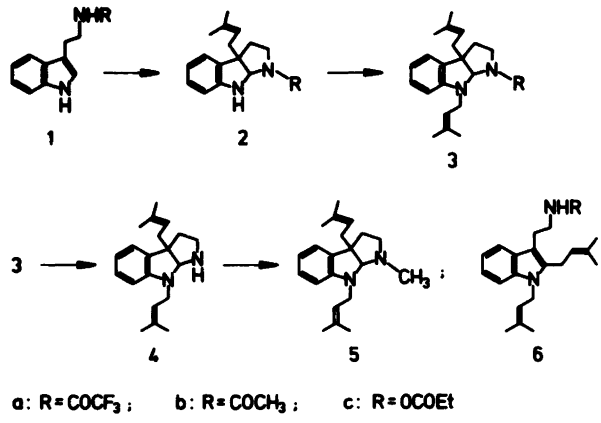

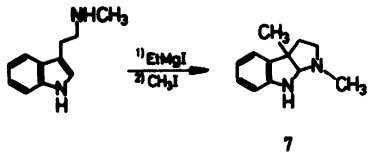

magnesium salt, prepared from the parent compound and ethylmagnesium iodide, was treated with methyliodide.[7] In our hands the analogous reaction with γ,γ-dimethylallyl bromide was not successful for the synthesis of *5*.

The ^{13}C NMR chemical shifts of the skeleton carbons of the compounds in Table 1 were assigned by comparison with the shifts of flustramine A and B,[1a,b] physostigmine[8] and related compounds. However, the aromatic carbons (particularly C–4, C–5 and C–6) were assigned partly by comparison with indoline and indoline derivatives and partly by comparison with the naturally occurring derivatives taking into account the bromo-substituent effect in the flustramines.[9,10] The latter compounds also proved to be valuable models for the assignment of the chemical shifts of the carbons in the isoprene units. All assignments, except for *2b*, were verified by off-resonance decoupling experiments.

EXPERIMENTAL

Mass spectra were recorded at 70 eV on an AEI-MS902 instrument; precise mass measurements were obtained by the peak matching method. UV spectra were recorded on a Unicam SP 18 instrument and IR spectra on a Perkin-Elmer 580 spectrometer. ^{13}C NMR spectra were recorded at 22.5 MHz and ^{1}H NMR at 90 MHz on a Jeol FX 90 Q instrument.

N_b-Trifluoroacetyltryptamine (1a) was prepared by a modified literature procedure (Ref. 11): Tryptamine (0.14 mol) was dissolved in dry CH_2Cl_2 (150 ml) and triethylamine (0.15 mol). Trifluoroacetic anhydride (20 ml) was added dropwise while the mixture was stirred and cooled in ice. After stirring for 2 h the reaction mixture was washed with Na_2CO_3 solution (200 ml) and water (200 ml). The aqueous phase was washed with CH_2Cl_2 and the combined CH_2Cl_2 phases after drying over $MgSO_4$ and evaporation left a residue which after recrystallization (MeOH: H_2O, 1:1) gave a colorless crystalline product in quantitative yield. M.p. 100–101 °C (lit. 99–100 °C).[11]

N_b-Acetyltryptamine and N_b-ethoxycarbonyltryptamine were prepared according to the literature.

1-Trifluoroacetyl-3a,8-bis(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole (3a). To a solution of N_b-trifluoroacetyltryptamine (0.02 mol) in 117 ml acetate buffer (glacial acetic acid 200 ml, water 20 ml, and sodium acetate 8 g) γ,γ-dimethylallylbromide (0.06 mol) was added dropwise (30 min) with stirring in an N_2-atmosphere. After stirring for 3 h (under N_2) water (65 ml) was added and the reaction mixture was extracted with diethylether (150 ml) which after drying over $MgSO_4$ and evaporation left 11.4 g. Extraction with hexane–ethylacetate (3:1, 100 ml), left after filtration 28 % unreacted trifluoroacetyltryptamine. The filtrate on evaporation left a crude product which on purification by column chromatography (silica gel, Lobar, Merck) with ethylacetate–hexane (1:3) gave *2a* (353 mg, 5.6 %), *3a* (364 mg, 4.8 %) and *6a* (389 mg, 5.1 %). *3a* and *6a* may be purified by

Table 1. ^{13}C NMR data of debromoflustramine B and related compounds. Spectra measured at 22.5 MHz in $CDCl_3$ (40 mg ml^{-1}). Chemical shifts are given in parts per million relative to internal Me_4Si. Assignments for values marked with the same symbols may be interchanged. The ^{13}C signal multiplicities as obtained from the off-resonance decoupled spectra are shown in parenthesis (s=singlet, d=doublet, t=triplet, q=quartet).

Position	2b	2a	7	3b	3c	3a	4	5
2	46.9	46.4(t)	52.5(t)	46.9(t)°	45.4(t)°	46.0(t)°	45.6(t)°	52.8(t)
3	35.3°	35.1(t)°	41.0(t)	37.2(t)	37.0(t)	37.1(t)	40.9(t)°	39.0(t)°
3a	56.1	55.9(s)	53.5(s)	55.8(s)	56.2(s)	55.4(s)	56.5(s)	57.1(s)
3b	131.6	135.6(s)*	137.0(s)	133.0(s)$^{\Delta}$	133.2(s)$^{\Delta}$	132.4(s)$^{\Delta}$	133.7(s)$^{\Delta}$	133.3(s)*
4	122.7	123.1(d)	122.7(d)	122.6(d)	122.6(d)	122.9(d)	123.1(d)	122.8(d)
5	118.2*	118.7(d)	118.7(d)	119.3(d)	119.5(d)	118.7(d)	120.5(d)	120.8(d)
6	127.9	128.7(d)	127.4(d)	128.2(d)	128.1(d)	128.6(d)	127.6(d)	127.5(d)
7	108.8	109.3(d)	109.0(s)	106.6(d)	106.3(d)	106.9(d)	105.2(d)	105.2(d)
7a	149.0	148.8(s)	149.5(d)	150.5(s)	150.1(s)	150.1(s)	151.0(s)	151.0(s)
8a	79.4	81.5(d)	89.8(d)	82.7(d)	84.3(d)	84.5(d)	87.0(d)	87.0(d)

Isoprene units					C-3a	N-8	C-3a	N-8	C-3a	N-8	C-3a	N-8	C-3a	N-8
1		34.3°	35.6°		37.2	44.6°	37.0	44.1°	37.1	44.6°	37.7°	43.1°	38.5°	46.8
2		118.9*	119.1		117.1	121.4	117.1	121.5	117.9	120.6	121.0	116.7	121.6	117.4
3		134.5	135.8*		134.6$^{\Delta}$	133.9$^{\Delta}$	133.7$^{\Delta}$	133.7$^{\Delta}$	135.5$^{\Delta}$	134.8$^{\Delta}$	134.7$^{\Delta}$	134.7$^{\Delta}$	135.7*	134.0*
4		25.6	25.9		25.8*	25.9*	25.7	25.7	25.9*	25.7*	25.9*	25.7*	25.9	25.9
5		17.6	17.9		18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0

N-1	C=O	169.3	C=O	156.2	$-CH_3$	37.1	C=O	169.5	C=O	175.6	C=O	156.0			$-CH_3$	38.1
Substituent	$-CH_3$	22.2	$-CF_3$	n.o.			$-CH_3$	22.9	$-CH_2$	61.1	$-CF_3$	n.o.				
									$-CH_3$	14.7						

fractional recrystallization from hexane–ethylacetate (3:1 v/v). High resolution mass measurements gave: *2a* calc. for $C_{17}H_{19}F_3N_2O$ 324.145 found 324.144; *3a* calc. for $C_{22}H_{27}F_3N_2O$ 392.208 found 392.204; *6a* calc. for $C_{22}H_{27}F_3N_2O$ 392.208 found 392.202. UV (EtOH) *2a* λ_{max} 212 nm (ε 1.3×10^4), 236 (9.0×10^3), 296 (2.4×10^3); *3a* 213 (1.6×10^4), 252 (8.3×10^3), 304 (1.5×10^3). UV (0.5 N ethanolic HCl) *2a* 212 (1.3×10^4), 236 (9.1×10^3), 296 (2.5×10^3); *3a* 212 (1.5×10^4), 253 (8.1×10^3), 310 (1.6×10^3).

1-Acetyl-3a-(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole (2b). N_b-Acetyltryptamine (3 mmol) dissolved in 6 ml acetate buffer (glacial acetic acid 100 ml, water 20 ml and sodium acetate 8 g) was mixed with γ,γ-dimethylallylbromide, a slow stream of N_2 being passed through the solution. After 2 h at room temperature water (10 ml) was added. The reaction mixture was extracted with ether, which after drying ($MgSO_4$) and evaporation left 400 mg. Column chromatography (silica gel, Lobar, Merck, ethylacetate) gave *2b* (14.3 %). Calc. for $C_{17}H_{22}N_2O$ 270.173 found 270.174. UV(EtOH) λ_{max} 213 nm (ε 8.1×10^3), 244 (2.1×10^3), 294 (7.5×10^2). UV (0.5 N ethanolic HCl) 213 (5.5×10^3), 244 (1.1×10^3), 294 (3.7×10^2).

The same method was used for preparation of *2c* (yield 16 %).

1-Acetyl-3a,8-bis(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole (3b). To a mixture of *2b* (0.25 mmol) and K_2CO_3 (2 mmol) in acetone (2 ml) was added γ,γ-dimethylallylbromide (0.25 mmol) in acetone (1 ml) with a nitrogen stream being passed through the reaction mixture. Leaving overnight (with N_2), addition of water (2 ml) and extraction with CH_2Cl_2 left on evaporation of the CH_2Cl_2 solution 84.5 % of *3b*. Purification by column chromatography (silica gel, Lobar, Merck, ethylacetate) afforded 69 % pure *3b*, calc. for $C_{22}H_{30}N_2O$ 338.236 found 338.238. UV(EtOH) λ_{max} 216 nm (ε 1.4×10^4), 258 (7.6×10^3), 314 (1.9×10^3). UV (0.5 N ethanolic HCl) 212 (1.3×10^4), 254 (6.0×10^3), 308 (1.4×10^3).

Compound *3c* was prepared by the same method, yield 75 %, calc. for $C_{23}H_{32}N_2O_2$ 368.246 found 368.245. UV(EtOH) λ_{max} 212 nm (ε 1.6×10^4), 253 (8.8×10^3) 304 (2.9×10^3). UV (0.5 N ethanolic HCl) 212 (1.6×10^4), 255 (7.6×10^3), 304 (2.4×10^3).

3a,8-Bis(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole (4). a. Alkaline hydrolysis of *3a:* A solution of *3a* (0.12 mmol in an equimolar amount of ethanolic (40 %) 0.1 N NaOH (0.612 ml) was heated to 90 °C for 2.5 h. The reaction mixture was extracted with diethylether, which after drying ($MgSO_4$) and evaporation left a crude product. Column chromatography (silica gel, Lobar, Merck, MeOH:$CHCl_3$, 3.5:6.5) gave 3.3 % pure *4*. UV(EtOH) λ_{max} 212 nm (ε 1.1×10^4), 258 (6.8×10^3), 310 (1.4×10^3). UV (0.5 N ethanolic HCl) 212 (1.1×10^4), 248 (5.4×10^3), 304 (1.3×10^3).

b. Imidazole mediated hydrolysis of *3a:* To a solution of *3a* (0.1 mmol) and imidazole (0.1 mmol) in MeOH (2 ml) was added water (2 ml) dropwise. The resulting colloidal solution was cleared by addition of MeOH (7 ml). After 10 h at room temperature, 4 N NaOH was added to adjust to pH 11 followed by extraction with ether. Drying ($MgSO_4$) and evaporation gave a mixture of two products which after column chromatography (silica gel, Lobar, Merck, $CHCl_3$:MeOH, 6.5:3.5) gave 17 % of *4*.

c. $NaBH_4$ reduction: An initial cooled solution of *3a* (0.75 mmol) in abs. EtOH (375 ml) was treated with pulverized $NaBH_4$ (3 mmol). After stirring at room temperature for 1 h the excess $NaBH_4$ was removed by addition of acetone and the reaction mixture was stirred for an additional 15 min. Evaporation in vacuum left a crude product, which on purification by column chromatography (silica gel, Lobar, Merck, $CHCl_3$: ethylacetate, 6.5:3.5) gave a quantitative yield of *4*, calc. for $C_{20}H_{28}N_2$ 296.225 found 296.229.

d. Acid hydrolysis of *3b:* A solution of *3b* (3.52 mmol) in ethanol (119 ml) and 1 N HCl (119 ml) was kept at 60 °C for 1 h. After neutralization (Na_2CO_3), extraction with ether, drying over $MgSO_4$ and evaporation, the crude product was subjected to column chromatography (silica gel, Lobar, Merck, ethylacetate). A yield of 53 % *6b* was secured, calc. for $C_{22}H_{30}N_2O$ 338.236 found 338.234.

Compound *6c* was prepared analogously.

1-Methyl-3a,8-bis(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole, debromoflustramine B (5). To a stirred solution of *4* (0.39 mmol) and aqueous formaldehyde (37 %, 1.96 mmol) in CH_3CN (1.18 ml) $NaBH_3CN$ (0.63 mmol) was added. After the vigorous exothermic initial reaction had ceased, the mixture was stirred for 15 min and glacial acetic acid was added until neutral reaction. Stirring was continued for 1.45 h while the neutral reaction was maintained by addition of glacial acetic acid. After evaporation of the solvent in vacuum the residue was adjusted to pH 9 with 0.5 KOH. Extraction with ether (3×15 ml), washing with 0.02 N KOH, drying over K_2CO_3 and evaporation left a crude product which yielded to column chromatography (silica gel, Lobar, Merck, $CHCl_3$: ethylacetate, 6.5:3.5) to give *5* (57 %) calc. for $C_{21}H_{30}N_2$ 310.241 found 310.239; UV(EtOH) λ_{max} 211 nm (ε 1.1×10^4), 254

(2.0×10^3), 306 (5.4×10^2); UV (0.5 N ethanolic HCl) 211 (1.3×10^4), 246 (1.9×10^3), 295 (6.1×10^2); IR ($CHCl_3$) 2965 (s), 2940 (s), 2860 (s), 1605 (s), 1490 (s), 1460 cm^{-1} (s).

1-Methyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole (7). Prepared according to Ref. 7. UV(EtOH) λ_{max} 214 nm (ε 8.5×10^3), 244 (1.0×10^4), 299 (3.7×10^3). UV (0.5 N ethanolic HCl) 210 (7.2×10^3), 238 (9.8×10^3), 193 (3.4×10^3).

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The Nucleophilic Reactivity and Tautomerism of the Imidazole Nitrogens of N^2-Acetyl-histidine Methylamide and N^2-Acetyl-histidine

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The second order reaction rate constants for the reactions of N^2-acetyl-histidine methylamide (*1*) and N^2-acetyl-histidine (*2*) with a series of alkylating agents were determined at pH=7.4 and 37 °C. The nucleophilicities, *n*, in the Swain-Scott scale, of *1* and *2* were estimated to 3.35. The $k_N\pi/k_N\tau$-ratio was found to decrease with the constant, *s*, in the Swain-Scott equation and with the molecular size of the alkylating agent. Agents of low molecular size with low *s*-values produced a $k_N\pi/k_N\tau$-ratio higher than one, since the concentration of the N^τ-H-tautomer is higher than that of the N^π-H-tautomer, whereas bulky agents with high *s*-values reacted preferentially with the N^π-H-tautomer which is sterically less hindered and a stronger nucleophile, thus producing $k_N\pi/k_N\tau$-ratios lower than one.

The imidazole ring of histidine is an important group in proteins and enzymes which is implicated to play a role as a general base and acid catalyst in chymotrypsin,[1] ribonuclease[2] and many other enzymes. In the phosphotransferase system histidine residues act as nucleophilic catalysts transferring phosphoryl groups between enzymes.[3] Furthermore, as exemplified by hemoglobin and myoglobin, imidazole nitrogens may be ligands in the coordination of metals. The versatility of histidine residues as catalysts is primarily explained by the fact that they are endowed with p*K*-values in the range of the pH of many important biological fluids.

Unprotonated histidine residues exist in two tautomeric forms (Fig. 1), the N^τ-H-tautomer (a) and the N^π-H-tautomer (c), being named by the nitrogen with a hydrogen atom.* The interconversion between the tautomeric forms is mediated by protonation (b) of the imidazole ring.[4] In ^{13}C-magnetic resonance spectroscopy[5] and Raman scattering[6] studies it has been estimated that the N^τ-H-tautomer of L-histidine is four times more common than the N^π-H-tautomer in basic solutions. In two peptide-bound histidines it was estimated that roughly 70 % existed in the form of the N^τ-H-tautomer.[5]

* IUPAC terminology is used [*J. Biol. Chem. 247* (1972) 977]. The N^π-nitrogen is proximal to the substituted carbon of the imidazole ring, whereas the N^τ-nitrogen is more distant from that carbon.

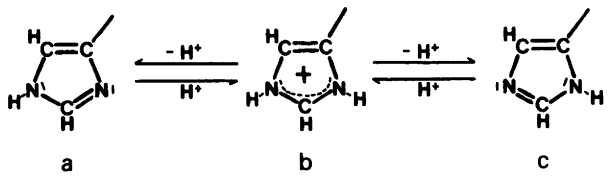

Fig. 1. The tautomerism of histidine residues. (a) N^τ-H-tautomer, (b) protonated histidine, (c) N^π-H-tautomer.

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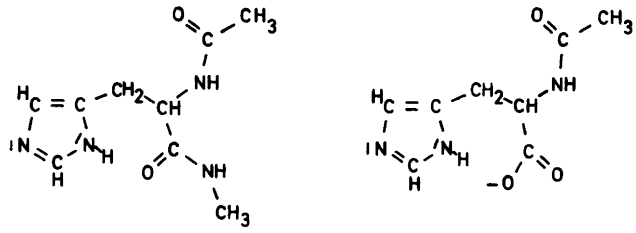

1 *2*

Fig. 2. N^2-Acetyl-histidine methylamide (*1*) and N^2-acetyl-histidine (*2*).

In seeming contradiction to this, alkylations performed with the purpose of probing the catalytic mechanisms of enzymes have sometimes been shown to yield exclusively the N^{τ}-alkylated isomer of histidine. Thus, for instance, His-57 in the active site of chymotrypsin is alkylated at the N^{τ}-nitrogen both by methylating agents[7] and substrate analogues.[8] Moreover, in a number of synthetic preparations[2,9–13] the yield of N^{τ}-alkylated isomer has been higher than that of the N^{π}-alkylated isomer, and it has been claimed that the N^{τ}-nitrogen of histidine derivatives is the most reactive in nucleophilic substitution reactions.[2,9,12]

In order to come to an understanding of the factors governing the distribution of alkylations between the imidazole nitrogens in histidine derivatives and in order to determine their intrinsic nucleophilicities, *n*, we have reacted N^2-acetyl-histidine methylamide (*1*, Fig. 2), which was chosen as the most suitable model compound for histidine residues in proteins, with a series of alkylating agents at 37 °C and pH=7.4. For comparison, the reactivity of N^2-acetyl-histidine (*2*, Fig. 2) *versus* a few alkylating agents has been included.

Our results are in direct contradiction to claims that nucleophilic substitution reactions at the imidazole nitrogens of histidine derivatives invariably produce higher yields of the N^{τ}- than of the N^{π}-alkylated isomer and it is our aim to show that the distribution of alkylations between the two imidazole nitrogens is a function of both the constant *s* in the Swain-Scott equation and sterical properties of the alkylating agent.

MATERIALS AND METHODS

Chemicals. N^2-Acetyl-L-histidine (*2*) was obtained from Sigma Chemical Co., St. Louis, Mo. N^2-Acetyl-L-histidine methylamide (*1*) was prepared by esterification of *2* with 1.25 M HCl in methanol for 15 h at room temperature. After evaporation to dryness and repeated evaporations with methanol the ester was dissolved in 40 % methylamine (Merck *p.a.*) and left to react at room temperature for 15 h. The reaction product was then applied to a Dowex 1 (20×2 cm, OH^--form) column which was eluted with water and rinsed with HCl. After evaporation of the water fraction, *1* was recrystallized twice from a minimal amount of hot methanol. The compound was chromatographically pure on TLC ($CHCl_3$–CH_3OH–NH_3, 40:40:20, Silica gel) developed with Pauly's reagent and had an m.p. of 260 °C. MS gave |*m/z* (interpr.)|: 210 (M), 180 (M−$HNCH_3$), 167 (M−$COCH_3$), 152 (M−$CONHCH_3$) and 151 (M−H_2NCOCH_3) upon electron impact on a HP 5930A quadrupole instrument. N^{im}-Methyl-L-histidines were from Sigma Chemical Co., S:t Louis, Mo. and N^{τ}- and N^{π}-(2-hydroxyethyl)-L-histidines were synthesized as in *14*. N^{τ}- and N^{π}-ethyl-L-histidines were kind gifts of Dr. M.S.S. Murthy.[13]

[U-^{14}C]Ethylene oxide (414 MB_q/mmol), [^{14}C-methyl]methanesulfonate (2200 MBq/mmol) and [2-^{14}C]iodoacetic acid (1900 MBq/mmol) were from The Radiochemical Centre, Amersham, England. [^{3}H-Methyl]nitrosourea (60 GBq/mmol) and [1-^{14}C-ethyl]nitrosourea (550 MBq/mmol) were from New England Nuclear, Boston, Mass. [^{14}C-dimethyl]-2,2-dichlorovinyl phosphate (DDVP) (4200 MBq/mmol) and [^{3}H-

ethyl]-methanesulfonate were kind gifts of Prof. B. Holmstedt and Dr. S. Osterman-Golkar, respectively.

Reaction conditions. Preheated 0.05 M solutions of *1* or *2* were adjusted to pH=7.4 by the addition of hydrochloric acid and the reactions between the alkylating agents and *1* or *2* were carried out in a thermostated water bath at 37 °C. About 37 kBq of each radiolabeled alkylating agent was used, corresponding to concentrations of less than 0.1 mM. The reaction time was 90 min.

The reactions of *1* were terminated by the addition of an equal volume of 1 M HCl and applied to Dowex 50 (12×1 cm, Na^+-form) columns which were first washed with 100 ml H_2O to remove excess of radiolabeled reagents and then eluted with 30 mM sodium phosphate buffers at pH=6.75. In this separation system, *1* eluted after 120 ml of buffer. The retention volumes of the N^{τ}- and N^{π}-methylated, N^{τ}- and N^{π}-ethylated and N^{τ}- and N^{π}-(2-hydroxyethyl-ated) derivatives of *1* were 100, 180, 135, 215, 60 and 100 ml of buffer, respectively. The identities of the alkylation products with *1* were checked by comparison with the synthetic N^{im}-alkyl-histidines on a Biotronic LC 6000E amino acid analyzer following hydrolysis of the radiolabeled peaks in 2 M HCl at 100 °C for 15 h.

The reactions of *1* with iodoacetate ion and those of *2* were terminated by the addition of an equal volume of 4 M HCl. The reaction mixtures were hydrolyzed for 15 h at 100 °C. The iodoacetate ion reaction mixtures were then freed from excess radiolabeled reagent on a 5×2 cm Dowex 50W×4 exchanger in its NH_4^+-form which was washed with water and eluted with ammonia. The ammoniacal eluates were evaporated and injected into the amino acid analyzer. The positions of the radioactive peaks were compared to those of the common amino acids according to Ref. 2. Following hydrolysis of the acetyl group under the same conditions as for *1*, the reactions of *2* were applied directly to an Aminex A-5 ion exchanger (20×0.9 cm, Na^+-form) which was washed with water and then eluted with a 0.05 M sodium phosphate buffer at pH=7.0. In this separation system histidine, N^{τ}- and N^{π}-methyl-histidine, N^{τ}- and N^{π}-ethyl-histidine, N^{τ}- and N^{π}-(2-hydroxyethyl)histidine eluted with 120, 100, 165, 145, 195, 65, and 100 ml of buffer, respectively.

The fractions taken out from the separations were counted for radioactivity in an Intertechnique SL 30 scintillation spectrometer after mixing with an equal volume of Instagel and counting efficiency was determined by automatic external standardization.

Reaction kinetics. For the reactions of the alkylating agents the second order rate constants, k, were calculated according to the formula:

$$k = \frac{|RY|\ k'}{|Y|_0 |RX|_0 t\ (1-e^{-k't})} \qquad (1)$$

where $|RY|$ is the concentration of product determined from the radioactivity in the corresponding peaks and $|RX|_0$ and $|Y|_0$ the starting concentrations of alkylating agent and nucleophile (*1* or *2*), respectively. The term $k'/(1-e^{-k't})$, where k' is the first-order rate constant for disappearance of the alkylating agent estimated from its rate constant for hydrolysis as given in Ref. 15 and its uncorrected rate of reaction with *1* or *2*, gives a correction for the decay of alkylating agent during the course of the reaction. For methyl methanesulfonate k' was estimated to 35 10^{-6} s^{-1}, whereas for the other alkylating agents it was considerably lower. Due to the excess of *1*

Table 1. Second order reaction rate constants, k±S.D.×10^6($M^{-1}s^{-1}$) for *1* and *2* at 37 °C and pH=7.4.

	1			*2*		
	$k_N\tau$	$k_N\pi$	$k_N\tau+k_N\pi$	$k_N\tau$	$k_N\pi$	$k_N\tau+k_N\pi$
Methyl methanesulfonate	136±7	191±20	327±28(n=4)[a]	150	137	287
Ethyl methanesulfonate	7.8±0.5	6.5±0.1	14.3±0.4(n=4)[a]	9.9	5.6	15.5
Ethylene oxide	27±3	32±4	59±7(n=5)[a]	27	22	49
Iodoacetate	39	18	57			
Dichlorvos	3.3	4.4	7.7			

[a] n is the number of determinations.

Table 2. Nucleophilicity constants, n, for the imidazole group of *1* and *2* calculated from rate data of a few directly alkylating agents at 37 °C.

	1	*2*
Methyl methanesulfonate	3.4	3.4
Ethyl methanesulfonate	2.5	2.6
Ethylene oxide	3.3	3.3
Iodoacetate	4.2	

and *2* in relation to the alkylating agents, the secondary alkylations of the mono-N^{im}-alkylated compounds could be neglected in the kinetic treatment.

RESULTS

The observed second order rate constants of the two imidazole nitrogens of *1* and *2* and the sums of these rate constants for the reactions with a few alkylating agents at pH=7.4 and 37 °C are presented in Table 1.

Except where indicated the rate constants are based on duplicate experiments. The absolute values of the rate constants of *N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea were not determined since they are transformed to reactive intermediates after base-catalyzed decompositions, the rates of which under the reaction conditions are unknown.

The sums of the rate constants of the two imidazole nitrogens in Table 1 were divided by the degrees of dissociation of the nucleophiles (calculated from the pK-values given in Refs. 16 and 17) at pH=7.4 to obtain the pH-independent rate constants which were used for the calculation of the nucleophilicity constants, n, of *1* and *2* in the equation of Swain and Scott [18]

$$\log (k_y/k_{H_2O})=sn \qquad (2)$$

where k_y and k_{H_2O} are the second order rate constants for the reactions of an alkylating agent with the nucleophiles Y and water, respectively, and s is a constant expressing the sensitivity of the alkylating agent to the nucleophilic strength, n, of the nucleophile. The values for k_{H_2O} were taken from Ref. 15. The calculated n-values of *1* and *2* are shown in Table 2. No estimate of the nucleophilicity could be based on the reactivity of DDVP since its reactivity with water is not known.

Table 3. The relative reactivities ($k_N\pi/k_N\tau$) of the imidazole nitrogens of a few histidine derivatives *versus* a number of alkylating agents together with their substrate constants. Unless otherwise stated at 37 °C and pH=7.4.

Alkylating agent	*1*	*2*	Ac-His methyl ester	His	*s*
Methyl nitrosourea	1.79				0.32[a]
Methyl methanesulfonate	1.37	0.91			0.89[b]
Dichlorvos	1.33				0.9[c]
Ethyl nitrosourea	1.49				0.0[a]
Ethyl methanesulfonate	0.86	0.53			0.69[b]
Ethylene oxide	1.22	0.81	1.1[d]		0.96[b]
Styrene oxide		very low[e]	very low[e]		0.81[e]
Bromoacetate				0.33[f]	
Iodoacetate	0.48	0.33[g]		0.50[h]	1.33[i]
Methyl iodoacetate			0.26[j]		
Iodoacetonitrile			0.1[k]		
2-Acetylaminoacrylic acid		very low[e]			

[a] Ref. 19. [b] Ref. 15. [c] Ref. 20. [d] Ref. 14, 1.25 M HCl in MeOH, 25 °C. [e] No N^{π}-alkylated isomer was detected, H_2O:MeOH 1:1, Osterman-Golkar, S. personal communication. [f] Ref. 12, Cu^{2+}-His at pH=6.0–6.1, 22 °C. [g] Ref. 2, pH=8.1, 100 °C. [h] Ref. 2, pH=5.5, 25 °C. [i] Ref. 21. [j] Ref. 10, acetone + K_2CO_3, 25 °C. [k] Ref. 10, acetone + K_2CO_3, 25 °C. [l] Ref. 9, N^{π}-alkylated isomer present only as impurity, H_2O, 50 °C.

In Table 3 the ratios between the observed rate constants of the N^{π}- and N^{τ}-nitrogens for a few histidine derivatives are listed. Some values from other sources with different reaction conditions have been included in Table 3. These are sometimes given in the literature as ratios between products, but since it was indicated in these cases that the formation of di-N^{im}-alkylated compounds was small, the ratios between product yields should be close to ratios between rate constants.

Since the conditions were not the same as in our experiments, these ratios are not strictly comparable to those obtained in this study. The *s*-values of the alkylating agents are also included in Table 3.

DISCUSSION

The mean of the *n*-values in Table 2 calculated from the rate constants for their reactions with methyl methanesulfonate and ethylene oxide is 3.35 for both model compounds, *1* and *2*. This value lies close to the *n*-values estimated for imidazole, 3.58 (*22*), pyridine, 3.6 (*18*) and pyrimidine 3.30,[22] all of which are heterocyclic nitrogen compounds with reactive "pyridine" nitrogens.

When comparing the *n*-values of *1* and *2* with that of imidazole, the Brønsted dependence on basicity has to be considered. If the values 0.32 for ethylene oxide[23] and 0.2 for methyl methanesulfonate[15] are used for β, *1*, whose p*K*-value at 25 °C is 6.5[16] compared to 7.0 of imidazole,[17] should display a 0.16 and 0.1 *n*-units lower nucleophilicity constant than imidazole in its reactivity *versus* these agents. The nucleophilicity of *2* (pK=7.1 at 25 °C)[17] should be roughly the same as that of imidazole. Thus, it can be seen that for these two alkylating agents the *n*-values in Table 2 agree well with expectation, meaning that the side chains of *1* and *2* present relatively little steric hindrance against reactions of these agents with the imidazole group.

The *n*-values of *1* and *2*, if calculated from their reactivities towards ethyl methanesulfonate are 0.85 and 0.75 units, respectively, below the above-mentioned mean value. This parallels the low nucleophilicity, n=2.9, of the *N*-7 group of guanosine *versus* the same agent as compared to 3.7 if calculated from its reactivity with methyl methanesulfonate (*24*). (The *N*-7 atom of guanosine is part of an imidazole moiety of the guanosine molecule in which tautomerism has been eliminated due to the sugar bonding of the *N*-9-atom.) Ethyl methanesulfonate may thus be characterized by a lower reactivity *versus* nitrogens than expected from eqn. (2). (See also Ref. 25.)

The *n*-value of *1* calculated from the rate constant with the iodoacetate ion is based on a comparison with a series of rate constants with negatively charged nucleophiles.[21] Since the rate constants of the iodoacetate ion with negatively charged nucleophiles will be lowered by anion–anion repulsion, its reactivity *versus* *1* probably appears higher than it would in a comparison with uncharged nucleophiles.

The nucleophilic reactivity of *1* may be regarded as the intrinsic reactivity of histidine residues in proteins. Differences between the reactivity of a protein histidine residue and *1* should be ascribed either to the microenvironment of the protein histidine residue and/or to the distribution of the alkylating agent within the protein.

It has been directly shown that the N^{τ}-H-tautomer is the predominating form in *2*[5] (*i.e.* that the equilibrium constant of the tautomerism, K_T, is higher than one). There are several indications that this is the case also for *1*; Firstly, NMR-studies in two small histidine-containing peptides have revealed that the proportion of N^{τ}-H-tautomer was about 70 %.[5]

Secondly, N^{π}-alkylated histidines, including a tripeptide,[26] have higher p*K*-values than do their N^{τ}-alkylated isomers[14,27] pointing to the N^{π}-H-tautomer as a stronger base and thus a stronger nucleophile than the N^{τ}-H-tautomer. Assuming the p*K*-difference of 0.65 between the two N^{im}-methylated histidine residues of this tripeptide to be valid also for the p*K*-difference between the two tautomers of *1*, it may be estimated that the proportion of N^{τ}-H-tautomer is about 80 % in *1*. Thirdly, ethylnitrosourea, which has an *s*-value close to zero (*19*), and thus is virtually insensitive to the nucleophilicity of nucleophiles, produces a $k_N\pi/k_N\tau$-ratio higher than one in its reaction with *1*. An approximation of the proportion of N^{τ}-H-tautomer in *1* may be obtained by estimating the ratio between the rate constants of the two tautomers from the Brønsted equation

$$\log \frac{k_{N^{\pi}\text{-H-tautomer}}}{k_{N^{\tau}\text{-H-tautomer}}} = \beta \times (pK_{N^{\pi}\text{-H-tautomer}} - pK_{N^{\tau}\text{-H-tautomer}}) \quad (3)$$

by assuming the same pK-difference as above, 0.65, and using the value 0.32 for β of ethylene oxide.[23] This ratio between rate constants may then be used together with the ratio between oberved rate constants in Table 3 to estimate the equilibrium constant, K_T, according to

$$K_T = \frac{|N^{\tau}\text{-H-tautomer}|}{|N^{\pi}\text{-H-tautomer}|} = \frac{k_{N^{\pi}\text{-H-tautomer}}}{k_{N^{\tau}\text{-H-tautomer}}} \times \frac{k_{N^{\pi}}}{k_{N^{\tau}}} \quad (4)$$

indicating a proportion of about 60 % of N^{τ}-H-tautomer in *1*. Due to the sterical hindrance against reaction of alkylating agents at the N^{π}-nitrogen, the proportion of N^{τ}-H-tautomer is, however, probably slightly underestimated by this method.

Although the above estimates of the proportion of N^{τ}-H-tautomer in *1* are relatively consistent, a discussion in quantitative terms of the difference in nucleophilicity between the two tautomers of *1* and *2* would require values for K_T determined directly by means of physical methods. Thus only a few general remarks will be made about this difference in nucleophilic reactivity.

The influence of the *s*-value on the relative reactivity of the two tautomers and consequently on the observed ratios between rate constants, is evident when different alkylating agents donating the same alkyl group are intercompared (Table 3). Compounds with low substrate constants react relatively more with the predominating N^{τ}-H-tautomer, whereas compounds with higher substrate constants are more sensitive to the nucleophilicity of the nucleophile, thus favouring reaction with the N^{π}-H-tautomer, the stronger nucleophile.

The difference in reactivity between the two tautomers increases with the molecular size of the alkylating agent. This is probably because the side chains on imidazole in *1* and *2* present greater sterical obstacles against reactions of bulky agents with the N^{τ}-H-tautomer than with the N^{π}-H-tautomer. The sterical effect on the reactivity appears to be a major factor determining ratios between observed rate constants ($k_N\pi/k_N\tau$) and consequently between product yields of mono-N^{im}-alkylated histidines in synthetic preparations. An especially clear example of this effect on the product yield is presented by the reaction of styrene oxide with *2*, which despite the fact that the agent has a relatively low substrate constant produces a very low amount of the N^{τ}-alkylated isomer (Osterman-Golkar, S., personal communication). Previous claims that synthetic preparations of mono-N^{im}-alkylhistidines always produce a higher yield of the N^{τ}-alkylated product[9] may be explained by the fact that they were based on reactions with relatively bulky reagents.

The observed $k_N\pi/k_N\tau$-ratios (Table 3) for the compounds studied are about fifty per cent higher for *1* than for *2*. This is presumably explained by a lower equilibrium constant, K_T, in *2* as compared to *1* (this is indicated in the study of Reynolds *et al.*[5] where *2* had a lower K_T-value than glycyl-histidyl-glycine), which in turn may be explained by a stabilization of the N^{π}-H-tautomer in *2* by the formation of a hydrogen bond to the α-carboxylate ion.

High yields of both mono-N^{im}-alkylated histidine isomers are sometimes difficult to obtain in synthetic preparations. This has especially been true when mono-N^{im}-alkylhistidines are synthesized from *2* with bulky, electron-repelling groups like the ethyl or carboxymethyl group where low yields of the N^{π}-alkylated isomer have been obtained.[2,12,13] The reason for this is mainly that the apparent reactivity of an imidazole nitrogen (*i.e.* the rate constant for the formation of di-N^{im}-alkylated imidazolium ions) is increased when the other nitrogen has been alkylated, which if the $k_N\pi/k_N\tau$-ratio is different from one leads to a progressive decrease in the ratio between product yields of the minor and major N^{im}-alkylated isomer as the reaction time is prolonged.

Thus, for instance, in the carboxymethylation of Cu^{2+}-His, Wieghardt and Goren[12] observed a 6–7-fold increase in the reactivity of either imidazole nitrogen when the other nitrogen had been carboxymethylated, and the authors ascribed this to the electron-repelling effect of the carboxymethyl group introduced. (For a kinetic model of extensive alkylation of histidine compounds confer the article quoted above[12]).

In the synthesis of mono-N^{im}-(2-hydroxyethyl)histidines[14], Calleman and Wachtmeister observed, however, a corresponding doubling in the reactivity upon 2-hydroxyethylation of either imidazole nitrogen also with an electron-withdrawing group such as the 2-hydroxyethyl group (unpublished data). This indicates that part of the explanation to the increase in the observed rate constant of an imidazole nitrogen when the other has been alkylated is that tautomerism is eliminated in mono-N^{im}-alkylated histidine derivatives.

The relationships derived in this study may be of value in planning synthetic preparations where a high yield of mono-N^{π}-alkylated histidine product is desired. In such cases *1* rather than *2* should be used as starting material, and when there is a choice between different alkylating agents, the reagent with the smallest size and lowest *s*-value should be used.

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The Nucleophilic Reactivity of Valine Methylamide. A Model of the *N*-Terminal Valine Residues of Hemoglobin

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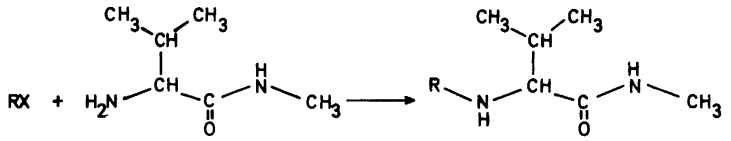
Valine methylamide (*1*) was synthesized as a model of the *N*-terminal valine residues of hemoglobin and a separation system was devised for its reaction products with a few mutagenic/carcinogenic alkylating agents. The pK'_a of *1* was 7.65 at 37 °C or about one pH unit higher than those previously determined for the *N*-terminal valine residues of liganded hemoglobin, in line with the existence of salt bridges between these residues and negatively charged amino acids. The nucleophilicity, *n*, of *1* in the Swain-Scott scale was about 4.35, which in combination with a relatively low pK_a-value in comparison to other aliphatic amines makes it the most reactive model of nucleophilic amino groups in proteins at physiological pH. The reactivity *versus* the *N*-terminal valine residues of hemoglobin of the few, relatively small, alkylating agents studied to date were slightly lower than predicted from their reactivity *versus* *1* also when the Brønsted dependence of the nucleophilicity on the basicity was taken into account.

The primary amino groups of the *N*-terminal valine residues of the α- and β-chains are two reactive nucleophilic sites of the hemoglobin molecule. Reactions at these sites with monosaccharides lead to the formation of glycosylated hemoglobins,[1] which are believed to be formed by thermal (enzymatically uncatalyzed) reactions.[2] Moreover, reactions with carbon dioxide at these sites play an important role in the allosteric interactions of hemoglobin[3] and their carbamoylation by potassium cyanate has been suggested as a cure for sickle cell anemia. Also electrophilic compounds with mutagenic/carcinogenic effects react with the *N*-terminal valine residues of hemoglobin, and a method for dose monitoring of such compounds *in vivo* by determining their reaction products with the *N*-terminal valine residues of hemoglobin, has been suggested.[4] Due to this link to dose monitoring *in vivo* we are projecting the use of valine methylamide (*1* in Fig. 1) as a dose monitor (trapping agent) for electrophilic reagents, RX, in mutagenicity test systems *in vitro*.

In order to investigate the suitability of *1* as a dose monitor in *in vitro* test systems we have determined its nucleophilic reactivity *versus* a few mutagenic/carcinogenic electrophiles and compared it with the corresponding reactivity of the *N*-terminal valine residues of hemoglobin.



1

Fig. 1.

0302-4369/83 $2.50

MATERIALS AND METHODS

Chemicals. *1* was prepared by dissolving 9 g of L-valine methyl ester (Sigma, S:t Louis, Mo.) in 25 ml of 40 % methylamine (Merck, *p.a.*) and leaving it to react for 15 h at room temperature. The reaction mixture was evaporated to dryness in a rotatory evaporator at 60 °C and the syrup dissolved in 5 ml of water, which was applied to a 3.5×10 cm Dowex 1 column in the OH^--form. The uncharged *1* was collected in 200 ml of water effluent, whereas the negatively charged valine, produced by hydrolysis of the valine methyl ester, was retained on the positively charged column. Upon evaporation, the water effluent yielded a residue which resisted attempts of recrystallization in different organic solvents, but was free from ninhydrin-positive contaminants when analyzed on TLC (1-propanol—H_2O, 7:3, SiO_2). Mass spectra were obtained using the direct inlet probe of a Finnigan 4021 mass spectrometer. The CI spectrum with methane as the reagent gas revealed ions with m/z=131, 159, 171 as expected for a compound of molecular weight 130. The electron impact spectrum displayed fragments corresponding to M, M−CH_3, M−$CONHCH_3$, and M−C_3H_7, and gave a larger peak of M+1 than of M, which at moderate sample pressures is characteristic of amines and amides.[5] [^{14}C]Methyl methanesulfonate (2.2 GBq/mmol), [U-^{3}H]ethyl methanesulfonate (925 MBq/mmol), [U-^{14}C]ethylene oxide (800 MBq/mmol) and [7-^{3}H]styrene oxide (3.3 GBq/mmol) were purchased from the Radiochemical Centre, Amersham, England. *N*-Acetoxy-*N*-acetyl-9-aminofluorene-[ring-^{3}H] (AAAF) (36.9 GBq/mmol) was a kind gift from Dr. R. Pero.

Determination of rate constants. The reactions between the alkylating agents and *1* were performed in 1 ml of water solution in sealed pyrex tubes equipped with screw caps in a thermostated water bath at 37 °C. The 0.05 M solutions of *1* were adjusted to pH=7.4 by addition of hydrochloric acid. About 37 kBq of the electrophiles were used providing for at least a thousandfold excess of the concentration of *1* in relation to the electrophiles. The reaction time was 60 min for all electrophiles studied. The reactions were terminated by addition of 1 ml of 1 M hydrochloric acid.

The reaction mixtures with methyl methanesulfonate, ethyl methanesulfonate, ethylene oxide and styrene oxide were applied to Dowex 50 ion exchange columns (12×1 cm, Na^+-form), which were washed with 100 ml of water to remove excess radiolabeled reagent followed by elution with a 0.03 M sodium phosphate buffer at pH=8.25 with a flow rate of about 85 ml per hour. Unreacted *1* eluted after 180 ml of buffer and was detected by ninhydrin on TLC. The relative retention volumes of the different alkylated derivatives of *1* are shown in Table 1.

The reaction mixtures with AAAF were separated on a reverse phase column (Spherisorb ODS, 5μ, Jones Chromatographs Ltd.) on a high performance liquid chromatograph (Laboratory Data Control) with a 45 min gradient from 20 to 100 % methanol in water. The reaction product of *1* with AAAF eluted after 22 min.

The fractions taken out from the separations were counted for radioactivity in an Intertechnique SL 30 scintillation spectrometer after mixing 1 ml from each fraction with 2 ml of Lumagel.

Reaction Kinetics. The concentrations of the reaction products, |RY|, between *1* and the different alkylating agents were determined by radioactivity counting. The second order rate constants, k, were calculated according to the formula

$$k = \frac{|RY|\ k'}{|Y|_0|RX|_0 t\ (1-e^{-k't})}$$

where the starting concentrations of the alkylating agent and *1* are denominated $|RX|_0$, and $|Y|_0$, respectively, and the term $k'/(1-e^{-k't})$ corrects for the decrease of the concentration of the alkylating agent during the reaction. The first order rate constant for the disappearance of the alkylating agent, k', was estimated from the sum of its uncorrected rate of reaction with *1*, and with water.[6] For methyl methanesulfonate k' was estimated to 75×10^{-6} s^{-1}, whereas it was considerably lower for the other alkylating agents.

The values for the nucleophilicity, n, of *1* were calculated according to the equation of Swain and Scott[7] $\log (k_Y/k_{H_2O}) = sn$ where k_Y and k_{H_2O} are the second order rate constants for the reactions between the alkylating agents and Y and water, respectively, and s, is the substrate constant which expresses the sensitivity of the alkylating agent to the nucleophilicity, n, of the nucleophile.

Determination of the pK_a'-value of 1. The pK_a' of *1* was determined at 37 °C with a Titrator TTT2

Table 1. Retention volumes relative to that of valine methylamide on a 12×1 cm Dowex 50 column eluted with 0.03 M phosphate buffer at pH=8.25.

Valine methylamide	1.00 (=def.)
N^2-Methylvaline methylamide	1.17±0.03
N^2-Ethylvaline methylamide	1.20±0.03
N^2-(2-Hydroxyethyl)valine methylamide	0.29±0.01
N^2-(2-Hydroxy-2-phenylethyl)-valine methylamide	1.17±0.01

pH-meter (Radiometer, Copenhagen) equipped with an autoburette. A solution of 0.025 mmol of *1* was prepared in 25 ml of 1 mM HCl and the pH was measured after additions of small volumes of a solution of 0.1 M NaOH (P.H. Tamm, Uppsala, Sweden). The pK'_a-value 7.65 of *1* was obtained graphically by plotting ΔV NaOH/ΔpH as a function of pH.

RESULTS AND DISCUSSION

The retention volumes of a few alkylated derivatives of *1* relative to that of the compound itself are shown in Table 1. Since the α-amino group is the only ionizable group in the alkylated derivatives of *1*, the only factors which are expected to influence the chromatographic behavior of these derivatives are the p*K*-values of their amino groups and their lipophilicity. This makes it possible to obtain good separations between compounds with small differences in p*K*-values by eluting them with buffers which have a pH slightly above these p*K*-values and a low ionic strength.

The electron-affinity of the alkyl group introduced onto *1* seems to explain the chromatographic behavior observed. Electron-donating groups like methyl and ethyl groups increase the p*K*-values of the amino groups and consequently the retention volumes on cation exchangers of the corresponding derivatives of *1*, whereas electron-attracting groups like the 2-hydroxyethyl group have an inverse effect on the retention. The (2-hydroxy-2-phenylethyl) group introduced onto *1* by the reaction with styrene oxide is an electron-attracting group, but the corresponding derivative of *1* is, similarily to the common aromatic amino acids, retarded on Dowex ion-exchangers due to its lipophilicity.

The reaction product between *1* and the carcinogen AAAF was chromatographed on a reverse phase column since it was irreversibly retained on the Dowex ion exchanger due to its aromatic structure. Despite the fact that its rate constant with the *N*-terminal residues of hemoglobin has yet to be determined, it was included in the study to demonstrate the feasibility of monitoring also aromatic reaction products with *1*.

The second order rate constants for the reactions of a few electrophilic reagents with *1* at pH=7.4 and 37 °C are listed in Table 2. Also included in Table 2 are the mean values of the rate constants for the *N*-terminal valines of the α- and β-chains of hemoglobin. The rate constants of *1* were determined as described above, whereas those of the hemoglobins, taken from other sources, were determined in red blood cells treated *in vitro* at 37 °C with the electrophilic reagents. Since the pH within the red blood cells has been determined to 7.40[8] the rate constants should be strictly comparable.

Table 2. Second order reaction rate constants, $10^3 \times (k \pm \text{S.D.})$ ($M^{-1}s^{-1}$) at 37 °C and *pH*=7.4 for the reactions of a few electrophilic reagents with valine methylamide and the mean of the rate constants of the *N*-terminal valine residues of the α- and β-chain of hemoglobin, respectively.

Electrophile	Valine methylamide	Hemoglobin		
		Mouse	Rat	Human
Methyl methanesulfonate	1.28±0.16 (n=3)[a]		0.9[b]	0.5–1.0[b]
Ethyl methanesulfonate	0.040±0.004 (n=3)[a]	0.030[c]		
Ethylene oxide	0.20±0.0006 (n=4)[a]	0.1[d]		0.2[e]
Styrene oxide	0.28 (n=2)[a]			
N-Acetoxy-*N*-acetyl-aminofluorene	0.065±0.019 (n=10)[a]			

[a] n=number of determinations. [b] Ref. 9. [c] Ref. 10. [d] Ref. 11. [e] Ref. 12.

The pK-values at 25 °C of the N-terminal valine residues of the α- and β-chains of human hemoglobin have been estimated to about 6.8 and 7.0 in the liganded state and 7.8 and 6.8, respectively, in the deoxy state,[13] to be compared with 7.65 of the model nucleophile *1* at 37 °C or 8.0 of valine amide at 25 °C.[14] It can thus be concluded that the tertiary structure of hemoglobin decreases the pK of its N-terminal groups by about 1 unit except for in the case of valine 1α in the deoxy state. This observation lends additional support to the suggestion that the N-terminal valine residues are electrostatically bound to negatively charged groups in the tetrameric hemoglobin complex and may play important roles in the allosteric properties of this protein.[15]

If the rate constants in Table 2 are divided by the degrees of dissociation of the nucleophiles at pH=7.4 (0.36 for *1* and 0.85 for the oxyhemoglobins assuming a decrease in the pK-value of 0.3 units when the temperature is increased to 37 °C from 25 °C) the values for the pH-independent, intrinsic, rate constants, k_{int}, in Table 3 are obtained. If the intrinsic rate constants of *1*, k_{int}, are inserted together with k_{H_2O}, the second order rate constants of water and s, the substrate constant, into the equation $\log k_{int}/k_{H_2O}=sn$ of Swain and Scott (7) then it is possible to calculate the nucleophilicity, n, of *1*. The values for k_{H_2O} and s of the compounds studied have been taken from Refs. 6 and 16. The nucleophilicity of *1* calculated from its rate of reaction with methyl methanesulfonate, ethylene oxide and styrene oxide gave similar results (4.48, 4.26 and 4.35), whereas when estimated from its rate of reaction with ethyl methanesulfonate it was about 0.6 units lower, 3.7 (Table 3). This lower nucleophilicity of *1* displayed in the reaction with ethyl methanesulfonate parallels that of other amines in their reactions with the same agent.[17,18]

The determination of the nucleophilicity, n, and pK-value of *1* permits us to compare its nucleophilic reactivity at pH=7.4 with that of a few other models for nucleophilic groups in proteins. The nucleophilicity and pK-value of N^2-acetyl-histidine methylamide, a model for histidine residues in proteins, have been determined to n=3.35[18] and pK=6.31[20] at 37 °C. Despite the higher degree of dissociation of this nucleophile in comparison to *1*, its lower nucleophilic strength makes it roughly four times less reactive than *1* at pH=7.4 in reactions with alkylating agents with substrate constants close to one, such as methyl methanesulfonate and ethylene oxide.[18] In this comparison the nucleophilic reactivity of N^2-acetyl-histidine methylamide was based on the sum of the reactivities of its two imidazole nitrogens. Data are lacking for the nucleophilic reactivity of N^2-acetyl-lysine methylamide, but butylamine with an n-value of 5.13 and a pK-value of 10.63[21] also provides a good model for the ε-amino groups of lysine residues in proteins. Due to its high pK-value, the nucleophilic reactivity of this compound at pH=7.4 is about six hundred times less than that of *1* in reactions with alkylating agents with substrate constants close to one, despite the fact that it is a stronger nucleophile than *1* in the base form. Likewise, the high pK-value of the guanidino group of arginine residues (>12, Ref. 22) gives this group a poor reactivity at pH=7.4. This comparatively high reactivity of *1* at pH=7.4 makes it a promising dose monitor for mutagenic electrophilic reagents in *in vitro* test systems.

It can be concluded that the amino groups of N-terminal valine (or other amino acid) residues

Table 3. Intrinsic rate constants, $k_{int} \times 10^3$ ($M^{-1}s^{-1}$) at 37 °C for the reactions between valine methylamide and a few alkylating agents together with their substrate constants, s, rate constants of hydrolysis, $k_{hydrolysis} \times 10^3$ (s^{-1}) and the nucleophilicities, n, estimated from the reactions with the respective nucleophiles.

Electrophile	k_{int}	$k_{hydrolysis}$	s	n
Methyl methanesulfonate	3.56±0.44	0.020[a]	0.89[b]	4.48
Ethyl methanesulfonate	0.112±0.011	0.017[a]	0.69[b]	3.70
Ethylene oxide	0.56±0.018	0.0025[a]	0.96[b]	4.26
Styrene oxide	0.78	0.013[a]	0.81[c]	4.35

[a] Ref. 16. [b] Ref. 6. [c] Ref. 19.

Table 4. pH-independent rate constants, (k_{int}±S.D.) × 10^3 ($M^{-1}s^{-1}$) at 37 °C for valine methylamide and the *N*-terminal valine residues of oxyhemoglobin with a few electrophilic reagents.

Electrophile	Valine methylamide	Hb		
		Mouse	Rat	Human
Methyl methanesulfonate	3.56±0.44		1.0	0.6–1.2
Ethyl methanesulfonate	0.112±0.011	0.035		
Ethylene oxide	0.56±0.018	0.1		0.25

are the most reactive nucleophilic amino groups in proteins at physiological pH, except in situations where the tertiary structure of a protein lends exceptional reactivities to specific nucleophilic amino acid residues. Differences in the reactivity of alkylating agents with, on the one hand, *N*-terminal valine residues such as in hemoglobin and, on the other hand *1*, should be ascribed to the microenvironment of the *N*-terminal valine residue and/or to the distribution of the alkylating agent within the protein.

As can be seen in Table 4, the mean pH-independent rate constants of the *N*-terminal valine residues of hemoglobin are 4–5 times lower than that of the model compound *1*. Assuming a slope, β, in the Brønsted equation $\log (k_{Y'}/k_{Y''})=\beta(pK_{Y'}-pK_{Y''})$ of 0.2 for the alkyl methanesulfonates [6] and 0.32 for the epoxides [23] a difference in rate constants of a factor of two would be expected for the alkyl methanesulfonates and ethylene oxide, respectively.

It thus seems that the reactivity *versus* electrophilic reagents of the *N*-terminal valine residues of oxyhemoglobin is slightly lower than predicted from their reactivity *versus 1* even if the Brønsted dependence of the nucleophilicity upon the basicity of amino groups is taken into account. Due to the paucity of reaction kinetic data for the *N*-terminal valine residues of hemoglobin this comparison is limited to relatively small alkylating agents and the difference in reactivity may be even more pronounced for bulky reagents. In fact, albeit the *N*-terminal valine residues of human hemoglobin have been shown to be reactive *versus* small electrophilic reagents such as carbon dioxide,[3] potassium cyanate,[13] methyl methanesulfonate[9] and ethylene oxide,[4] the absence of α-chains glycosylated in that position seems to indicate a steric hindrance against reactions on the *N*-terminal valine of the α-chain. This sterical hindrance is presumably exerted by a salt bridge to the *C*-terminal carboxylate group of arginine 141 of the opposite α-chain.[15]

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Syntheses and Properties of Hexa- and Heptachlorostyrenes with Fully Chlorinated Aromatic Nuclei

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All six possible hexa- and heptachlorostyrenes with fully chlorinated aromatic nuclei have been synthesized. Their ultraviolet and infrared spectra are discussed, fragmentation in the mass spectrometer described and from the ^1H and ^{13}C NMR spectra all parameters have been determined, including $^1J_{CH}$, $^2J_{CH}$ and $^3J_{CH}$ coupling constants. UV and NMR data indicate that the styrene system is nonplanar.

Chlorinated styrenes together with chlorinated benzenes and polychlorinated biphenyls (PCB) have been found in water, sediment and fish samples from the Norwegian fjord, Frierfjorden.[1] The source of these pollutants seems to be the production of magnesium, where a high temperature process is used involving chlorine gas and graphite electrodes. The pollutants have also been detected in the bloodstream of workers in this magnesium plant. Several isomers of hexa- and heptachlorostyrenes were identified without the precise knowledge of their stereochemical identity.

The uptake and bioaccumulation of the styrenes were as effective as for chlorinated benzenes and PCB. The biological effects of the latter are well known, while effects of the highly chlorinated styrenes are unknown. In order to gain some information of this nature and to help identify the different isomers analytically, a synthetic study of these styrenes was initiated.

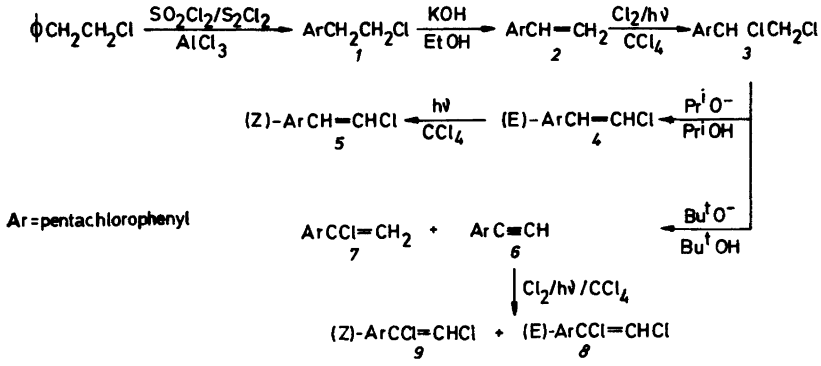
SYNTHESES

Most of the six possible title compounds have been synthesized before. Thus *α,2,3,4,5,6-hexachlorostyrene* (*7*) was obtained in 31 % yield by pyrolysis of a highly chlorinated bicycloheptadiene.[2] *β,2,3,4,5,6-Hexachlorostyrene* (*4* or *5*) was made by dehydrobromination of 1-bromo-2-chloro-1-pentachlorophenylethane.[3] *β,β,2,3,4,5,6*-Heptachlorostyrene (*13*) was obtained as a minor component in the electrochemical reduction of octachlorostyrene,[4] while the same author obtained both *(E)- and (Z)-α,β,2,3,4,5,6-heptachlorostyrene* (*8*) *and* (*9*) by chlorination of acetylene *6*.

Since only one of the hexachlorostyrenes *4* and *5* was known, its stereochemistry was uncertain and since *7* was obtained in a rather complicated way, it was decided to see if a unifying synthetic strategy could be followed. Since much is known about the rather simple chemistry involved in preparing side chain halogenated styrenes,[5] analogue routes were tried.

It was soon realized that the pentachlorophenyl group exerted a large influence on the chemistry of the two-carbon chain. For instance, the easy route from acetophenone through 1,1-dichloro-1-phenylethane to α-chlorostyrene must be abandoned due to complete unreactivity of the carbonyl group in 2,3,4,5,6-pentachloroacetophenone towards any chlorinating agent. Treatment of pentachlorobenzonitrile (commercially available) with Grignard reagents or methyl lithium to build up the side chain, unexpectedly led to rupture of the aryl-nitrile bond to give

0302-4369/83 $2.50



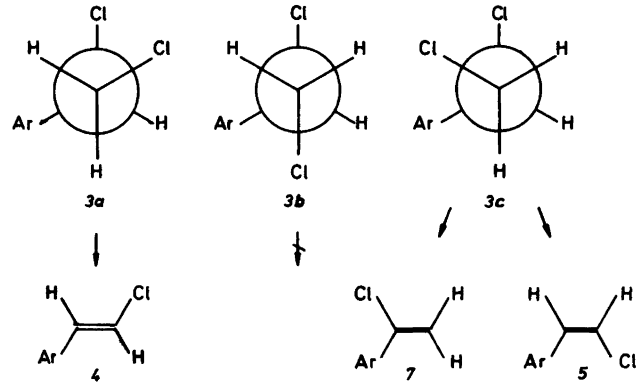
Scheme 1.

pentachlorobenzene.

The extreme ease whereby the aromatic nucleus was chlorinated without any attack on the side chain was then utilized,[6] with slight modification. Thus 1-chloro-2-phenylethane was smoothly chlorinated to 1-chloro-2-pentachlorophenylethane in excellent yield.[7] The reactions leading to the three isomeric hexachlorostyrenes (*4*, *5* and *7*) and the (*E*)- and (*Z*)-heptachlorostyrenes *8* and *9* are summarized in Scheme 1 and detailed in Experimental.

It must be mentioned that in most of the synthetic steps mixtures of compounds were obtained. Since all compounds contain the dominating pentachlorophenyl group, this will make the different compounds physically almost indistinguishable. In column chromatography all compounds follow the solvent front due to their lipophilicity; likewise the solubility is almost the same for all compounds rendering recrystallisation a poor tool for achieving the high purity required for the aims intended, *viz.* biological testing and analytical standard-making. Preparative GLC proved to be the only method to get the high purity required. Even then large head and tail fractions had to be discarded.

The choice of base applied in the dehydrohalogenation of 1,2-dichloro-1-pentachlorophenylethane *3* was important. The less hindered methoxide or ethoxide ion led to formation of considerable amounts of β-alkoxy compounds. They could be formed either by nucleophilic substitution in the starting material and subsequent dehydrohalogenation of the intermediate 1-alkoxy-2-chloro compound, by an addition–elimination reaction of a first formed β-chlorostyrene derivative or by addition to the acetylene *6*, as discussed elsewhere.[3,8] As expected, no alkoxy derivative was found when isopropoxide or *tert*-butoxide was used. The outcome of the



Scheme 2.

Table 1. Dehydrohalogenation of *3* (1 mol) by base (*q* mol) at *t* °C. Yields corrected for unreacted *3*. Traces of *5* formed in all experiments.

		Yield (%) of		
q	*t*	*4*	*6*	*7*
Base Me_2CHO^-				
1	108	41	40	19
1	60	82	7	10
Base Me_3CO^-				
1	83	35	53	12
1	60	38	51	10
2	83	5	84	9

elimination reaction (Table 1) could be rationalized on a conformational basis (Scheme 2).

Of the three staggered rotamers only *3a* and *3c* can give *trans* elimination products. *3a* is probably the most stable rotamer. Elimination would lead to excess of *4* (*E*-isomer) with isopropoxide at the lower temperature. Increasing temperature leads to increasing double elimination to form acetylene *6* and also to increased population of conformer *3c* which on elimination would give the α-isomer *7*. *3c* would also theoretically give the (*Z*)-β-isomer *5*. But steric hindrance imposed by the pentachlorophenyl group would effectively shield the β-hydrogen to be removed in an E2-elimination, thus only traces of the *Z*-isomer *5* were observed. However, one cannot exclude the possibility that *5* most easily could undergo a *trans* elimination to the acetylene *6*.

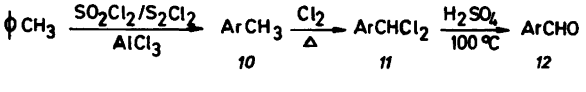
From Table 1 it can be seen that increasing base–substrate ratio expectedly led to increasing yield of acetylene *6* at the expense of *4*. The α-isomer *7* seems to be unaffected by base, a result that is understandable both from steric and electronic considerations. Molecular model considerations reveal that the vinyl hydrogen *cis* to the aromatic group in *7* (*i.e.* the hydrogen lost in a *trans* elimination) is sterically more hindered than the α-hydrogen in *4*. In addition the latter hydrogen must be more acidic as the substituent effect of five chlorine atoms on the aromatic ring has been estimated to σ=3.49,[9] stabilizing a negative charge on the α-carbon of *4* much better than on the β-carbon of *7*.

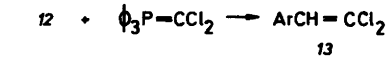
β,β,2,3,4,5,6-Heptachlorostyrene *13* was prepared by a Wittig reaction of pentachlorobenzaldehyde with dichloromethylene triphenylphosphorane (Scheme 3).[10]

ULTRAVIOLET SPECTRA

Data from the absorption spectra in the region from 200 nm and upwards for the chlorostyrenes and some other compounds pertinent to the discussion are given in Table 2.

First of all it should be mentioned that the auxochromic effect of chlorine atoms on the benzene chromophore is not at all additive, as demonstrated by a survey of the absorption maxima of polychlorinated benzenes in the *K*-region.[11] Thus the highest λ_{max}, 224 nm, is observed for 1,4-dichloro- and 1,2,3-trichlorobenzene; log ε 4.11 and 3.91, respectively. It is not surprising, since electron diffraction data show that already in 1,2-dichlorobenzene the chlorine atoms are twisted out of the plane of the aromatic ring, one to each side, torsion angle 15°.[12] And in hexachlorobenzene a chair-like conformation is observed where the chlorine atoms are displaced alternatively over and below the mean plane of the benzene ring.[12] This lack of coplanarity reduces the overlapping of the non-bonded electrons of chlorine atoms with the aromatic π-system and may also be responsible for the large positive σ-value of the pentachlorophenyl group (see above); the +M-effect of the chlorine atoms being reduced.





Scheme 3.

Table 2. Absorption maxima (λ_{max}, nm) and molar absorptivity (log ε) of chlorostyrenes *2*, *4–5*, *7–9*, *13* and some related compounds.

Compound	*E*-band	*K*-band	*B*-band	Solv.	Ref.
C_6H_6	184(4.83)	204(3.94)	254(2.31)	MeOH	13
C_6HCl_5		219(4.33)	289(2.87);298(2.59)	Cyclohexane	14
C_6Cl_6		222(4.41)	291(2.40);298(2.36)	Cyclohexane	14
$C_6H_5CH{=}CH_2$		248(4.24)	282(2.87);291(2.70)	MeOH	13
$C_6H_5CH{=}CHCl$		254(4.22)		N.i.[a]	11
2	221(4.64)	240(4.24)	297(2.50)	EtOH	[b]
4	227(4.55)	244(4.30)	310(2.60);325(2.13)	EtOH	[b]
5	213(4.65)	240(4.06)	290(2.47);298(2.46)	EtOH	[b]
7	216(4.80)	239(3.99)	292(2.70);297(2.73)	EtOH	[b]
8	216(4.81)	240(4.08)	293(2.77);300(2.81)	EtOH	[b]
9	216(4.81)	242(4.11)	295(2.82);304(2.80)	EtOH	[b]
13	217(4.70)	240(4.15)	290(2.62);300(2.58)	EtOH	[b]
$C_6H_5CH{=}CCl_2$		260(4.20)		N.i.[a]	11

[a] No information. [b] This work.

Table 3. IR absorption (ν_{max}, cm^{-1}) of chlorostyrenes.

Vibration	*2*	*4*	*5*	*7*	*8*	*9*	*13*
=C–H	3093 (w)	3079 (w)	3080 (vw)	2921 (w)	3083 (w)	3067 (w)	3015 (vw)
Olef. C=C	N.o.[a]	1614 (m)	1623 (w)	1634 (s)	1652 (vw)	1616 (w)	1616 (w)
Arom. C=C	1362 (s)	1371 (m)	1371 (m)	1400 (m)	1373 (m)	1369 (m)	1377 (m)
	1345 (m)	1344 (s)	1346 (s)	1346 (s)	1350 (s)	1346 (s)	1340 (s)
	1330 (s)	1308 (m)	1319 (m)	1324 (s)	1326 (s)	1319 (m)	1319 (m)
Others	1417 (m)	1241 (m)	1240 (m)	1418 (w)	1270 (m)	1242 (m)	1239 (w)
	1232 (m)	1125 (w)	1168 (m)	1231 (s)	1126 (m)	875 (s)	918 (s)
	1122 (m)	924 (m)	1120 (m)	1147 (s)	653 (m)	647 (m)	663 (w)
	936 (m)	855 (s)	667 (m)	1080 (m)			
	660 (w)	684 (m)		920 (m)			
				897 (s)			
				674 (s)			
				657 (s)			

[a] N.o.: Not observed.

This great steric demand of the chlorine atoms also makes it difficult to obtain coplanarity in the styrene system. The bathochromic effect observed for styrene as compared to benzene (44 nm) is greatly reduced when the benzene ring is perchlorinated ($C_6HCl_5 \rightarrow$ *2*: $\Delta\lambda_{max}$=21 nm). Similar blue shifts upon perchlorination of the aromatic ring is observed for the pair *trans* β-chlorostyrene *vs.* *4* (10 nm) and β,β-dichlorostyrene *vs.* *13* (20 nm).

INFRARED SPECTRA

The infrared spectra of the chlorostyrenes are summarized in Table 3.

The C–H stretching vibration is very weak in all styrenes; likewise the C=C stretch in all compounds apart from α-hexachlorostyrene *7* where this vibration gives rise to a rather strong absorption at 1634 cm^{-1}; in fact, the absorption band in this region for *2* is completely absent.

The terminal methylene group in *2* and *7* is represented by the characteristic scissoring vibration band at 1417 and 1418 cm^{-1}, respectively. The unifying structure element, the pentachlorophenyl group, is represented with three medium to strong bands which have been assigned to the C=C stretching in the aromatic ring.[2,15] The *trans* position of the hydrogens in *4* is confirmed[16] by the absorption at 924 cm^{-1}, absent in the *cis* compound *5*. The strong absorption at 918 cm^{-1} in *13* is characteristic for the $=CCl_2$ group.[17] The out-of-plane hydrogen vibration for 1,1-disubstituted terminal olefin *7* gives rise to the strong absorption at 897 cm^{-1}.

MASS SPECTRA

Some of the features from the mass spectra of the chlorostyrenes are given in Table 4.

All the chlorine-containing fragments show the correct intensities (within a few percent) for the isotope peaks. The base peak in all compounds is either M^+ or M^+–Cl. The three most abundant fragments in all spectra are M^+, M^+–Cl and M^+–2Cl and account for 46–60 % of the total ion current. Since also pentachlorostyrene *2* follows this fragmentation pattern, one may conclude that these fragments have lost their chlorine atoms from the ring. There are no differences in the fragmentation of the (*E*)- and (*Z*)-heptachlorostyrenes as judged from the relative abundance of consecutive chlorine expulsions, and apart from a switch in intensities of the M^+ and M^+–Cl peaks, the (*E*)- and (*Z*)-hexachlorostyrenes follow a similar pattern.

Table 4. Relative abundance of ions M^+-n Cl produced by chlorostyrenes on electron impact.

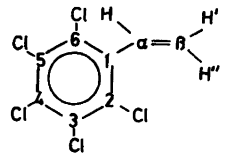
n	*2*	*4*	*5*	*7*	*8*	*9*	*13*
0	100	100	96	50	70	71	100
1	87	79	100	100	100	100	65
2	59	34	55	47	75	73	68
0–2[a]	52	60	46	48	51	53	50
3	6	3	9	7	15	14	12
4	9	4	5	4	12	11	10
5		4	5	3	8	8	7
6					11	11	7
0–6[a]	55	65	50	51	61	63	58

[a] Per cent of total ion current.

NMR SPECTRA

A comparison of the vinyl protons in pentachlorostyrene *2* with those of styrene itself shows that only for the proton *trans* to the aromatic group a rather large deshielding ($\Delta\delta$~0.6 ppm) is observed (Table 5).This deshielding is probably caused by the large electron withdrawing effect from the pentachlorophenyl group (see above).

If one uses the observed chemical shifts for the olefinic protons in *2* one can calculate the



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empirical substituent constant $S(\delta)$[18] for the pentachlorophenyl group in ethylenes to be: $S(\delta)$: +1.37 (*gem.*), +0.44 (*cis*) and +0.54 (*trans*) ppm. These values together with the values for chlorine[18] are then used to calculate the shifts for the vinyl protons in the hexa- and heptachlorostyrenes given in Table 5. The deviations from the experimental values are negligible (<0.1 ppm) for the *E*-isomer *4*, but $\delta_{calc}-\delta_{exp}$ increases as the deviation from coplanarity increases (see discussion of UV-data). Changes in geometry of the system may effect both the diamagnetic term

Table 5. Calculated (see text) and experimental ^1H NMR chemical shifts (δ) for chlorostyrenes (*14*) in $CDCl_3$.

Compound	H	H'	H''
Styrene	6.63	5.18	5.72
2, exp	6.62	5.79	5.69
4, calc	6.80		6.77
4, exp	6.80		6.71
5, calc	6.75	6.87	
5, exp	6.57	6.63	
7, calc		5.97	5.82
7, exp		5.88	5.48
8, calc		7.05	
8, exp		6.53	
9, calc			6.90
9, exp			6.33
13, calc	6.93		
13, exp	6.64		

Table 6. Coupling constants ($J_{H,H}$, Hz) for chlorostyrenes and -ethylenes in $CDCl_3$.

Compound	J_{gem}	J_{trans}	J_{cis}
Styrene[19]	1.3	17.5	10.7
2	0.9	17.8	11.6
4, *5* and *7*	2.1	13.9	7.7
CH_2=CHCl[20]	1.4	14.6	7.3
ClCH=CHCl[21]	—	12.1	5.3

(σ^{dia}) and the neighbour anisotropic term (σ^{N}). If the pentachlorophenyl group reduces the electron density of the vinyl system by resonance (and not by inductive effect alone), this effect will decrease with gradual loss of coplanarity, thereby reducing the size of σ^{dia}. The large value of the Hammett substituent constant (+3.49) points to a substantial −M effect, as inductive effect alone can hardly be responsible for this value.

Without precise knowledge of the geometry of this system, it is difficult to assess the change in σ^{N}.

The fully chlorinated aromatic ring does not seem to have any great influence on the proton–proton coupling constants in our styrenes (Table 6). Thus in *2* the values are about the same as in styrene,[19] while the coupling constant for the hexachlorostyrenes *4*, *5* and *7* are close to those of vinyl chloride[20] and further away from those of 1,2-dichloroethylene.[21] As conjugation is claimed to have little effect on the vicinal coupling constants,[21] this again points to the operation of resonance effects as the main electronic effects from the pentachlorophenyl group.

The ^{13}C chemical shifts and the observed $^{1}J_{CH}$, $^{2}J_{CH}$ and $^{3}J_{CH}$ coupling constants are given in Table 7 and 8 respectively. The former parameters were obtained from the broad-band decoupled spectra, while the coupling constants were obtained from the undecoupled spectra combined with selective ^{1}H decoupling.

The calculated chemical shifts of the aromatic carbons of *2* were obtained by applying the empirical substituent constants[22] of chlorine atoms on the reported shift values of styrene.[23] The agreement with the observed values for *2* is rather good with the largest deviation at C4 ($\Delta\delta$=3.9 ppm). The introduction of one or two chlorine atoms in the side chain hardly affects the chemical shifts of the *ortho*- and *meta*-carbon atoms ($\Delta\delta_{max}$=1.3ppm).* On the other hand, the effect on C1 and C4 is larger, which is not at all surprising. These two carbon atoms will experience changes in resonance effect more than the

* Compared with the observed values for *2*.

Table 7. ^{13}C NMR chemical shifts (δ) for chlorostyrenes (*14*) in $CDCl_3$. Calculated (see text) shifts in italics.

C	*2*	*4*	*5*	*7*	*8*	*9*	*13*
α	131.3	127.1 *125.3*	125.8 *123.3*	137.3 *134.3*	127.3 *128.5*	128.6 *128.5*	123.9 *119.3*
β	124.2	128.2 *127.2*	125.2 *127.2*	120.5 *118.2*	120.7 *121.2*	122.9 *121.2*	128.5 *130.5*
1	136.6 *138.0*	133.1	133.2	133.2	133.5	134.5	132.6
2+6	131.8 *132.7*	132.1	132.3	132.3	132.6	132.4	132.5
3+5	132.1 *134.1*	132.4	132.0	132.4	132.4	133.4	132.1
4	132.5 *136.4*	133.3	133.5	134.7	135.5	135.5	134.1

Table 8. Coupling constants ($J_{C,H}$, Hz) for chlorostyrenes (*14*) and some reference compounds.[25]

Compound	^1J α,H	β,H'/H''	^2J 1,H	α,H'	α,H''	β,H	^3J 2–6,H	1,H'	1,H''
2	163.7	160.8	2.2	3.8	2.1	3.2	4.0	6.0	12.6
4	168.7	197.2			2.7	9.7	3.6		2.8
5	167.5	199.2		7.5		6.0	3.4	8.8	
7		164.2		2.6	8.6			6.7	5.6
8		201.4		1.7				7.1	
9		196.9			13.5				2.5
13	171.5					0.9	2.8		
CH_2=CHCl	162	198							
CHCl$\overset{c}{=}$CHCl		198			16.5				
CHCl$\overset{t}{=}$CHCl		199		1.8					
CHCl=CCl_2		201				1.3			

other ring carbons; a change caused by the reduced coplanarity of the hexa- and heptachlorostyrenes.

The calculated values for the side chain carbons were obtained by applying the empirical increments of chlorine in ethylenic compounds[24] on the observed values for 2,3,4,5,6-pentachlorostyrene *2*. The agreement with the observed values is rather good ($\Delta\delta_{max}$=4.6 ppm).

The $^1J_{CH}$ values for the side chain chlorinated styrenes are consistent with those for chloroethylenes (Table 8).[25] The lack of effect from the pentachlorophenyl group on the vicinal proton coupling constants is thus repeated for $^1J_{CH}$, and also to a large extent for $^2J_{CH}$. Thus the observed $^2J_{CH}$ for the heptachlorostyrenes *8, 9* and *13* are close to those of chloroethylenes. For the hexachlorostyrenes the situation is not that clear. For *7* similar patterns as for the heptachlorostyrenes are observed; $^2J_{\alpha H'}$ being smaller than $^2J_{\alpha,H''}$, while for *4* and *5* it is opposite. Also for *2* the same situation prevails ($^2J_{\alpha,H'}>^2J_{\alpha,H''}$). The $^3J_{CH}$ coupling constants also follow a somewhat inconsistent pattern. It is stated that the *cis* coupling constant across a double bond is smaller than the *trans* constant.[26] From Table 8 it can be seen that for the coupling between C1 and the β-protons this is true for the pair *4* and *5*, in compound *7* and for the pair *8* and *9*; for *2* it is the other way around. The constants for coupling between C2+C6 and the α-proton are between 2.4 and 4.0 Hz for all compounds (*2, 4, 5* and *13*).

EXPERIMENTAL

General. Melting points (uncorrected) were determined on a micro hotstage. IR spectra (KBr) were recorded on a Perkin-Elmer 281 B Infrared spectrophotometer, UV spectra on a Cary model 14 Recording spectrophotometer, NMR spectra on a Bruker WM 400 spectrometer and mass spectra on a VG-Micromass 7070 F instrument. Analytical GLC were performed on a Hewlett packard 5710A instrument and preparative GLC on Varian Aerograph model 711, both used with FID-detectors.

1-Chloro-2-pentachlorophenylethane (1). In a slightly modified and improved procedure[7] a mixture of 1-chloro-2-phenylethane (70.2 g, 0.5 mol) and disulfur dichloride (4.1 g, 30.4 mmol) was added dropwise to a mixture of sulfuryl chloride (405 g, 3.0 mol) and dry aluminium chloride (2.5 g, 18.7 mmol) kept at 80 °C. After 3 h at this temperature gas evolution (HCl, SO_2) had subsided and benzene (400 ml) was added. After washing with dilute hydrochloric acid, sodium bicarbonate solution and finally water, the benzene layer was dried ($MgSO_4$) and solvent evaporated to give white crystals (150 g, 96 %). M.p. 92–93 °C (EtOH) (lit.[7] 89–90 °C). ^{1}H NMR (CCl_4): δ 3.65 (4H,s).

2,3,4,5,6-Pentachlorostyrene (2) was made from *1* according to literature procedure[7] in 95 % yield. M.p. 112–114 °C (EtOH) (lit.[7] 113–114.5 °C).

1,2-Dichloro-1-pentachlorophenylethane (3). To a solution of chlorine (10.6 g, 0.15 mol) in tetrachloromethane (500 ml) was added *2* (30 g, 0.11 mol). After UV-irradiation for 2 h at room temperature, the solution was washed with sodium thiosulfate solution and water. After drying ($MgSO_4$) the organic solvent was evap-

orated to give 28.8 g (75 %) yellowish material. M.p. 94–96 °C (EtOH) (lit.[3] 97–99 °C). ^{1}H NMR (CCl_4): AMX-spectrum: δ_A 4.03, δ_M 4.57, δ_X 6.04, J_{AM}=11.1 Hz, J_{AX}=6.3 Hz, J_{MX}=10.1 Hz.

(E)-*β,2,3,4,5,6-Hexachlorostyrene (4).* To a solution of *3* (10.0 g, 28.8 mmol) in 2-propanol (400 ml) kept at 60 °C, was added dropwise a solution of sodium (0.7 g, 30.4 mmol) in 2-propanol (100 ml). The dropping funnel must be kept warm to avoid precipitation of sodium isopropoxide. The addition lasted for 3.5 h and the solution was stirred overnight at 60 °C, after which it was diluted with tetrachloromethane and washed with water to remove inorganic salts. After drying ($MgSO_4$) and evaporation of solvents, 8.7 g white residue was obtained. GLC (10 % SP 2100 at 250 °C) showed the presence of starting material (<1 %), *4* (72 %), *5* (10 %) and *6* (12 %). Two recrystallizations (EtOH) gave 4.6 g of *4* (95 % purity, GLC). The impurity (alkyne *6*) was removed by preparative GLC (10 % Apiezon L at 260 °C). Purity of 4: 99.8 %. M.p. 120–120.5 °C (lit.[3] 115–116 °C).

(Z)-β,2,3,4,5,6-Hexachlorostyrene (5). (*E*)-isomer *4* (1.0 g, 3.2 mmol) was dissolved in tetrachloromethane (100 ml) and irradiated with UV-light. GLC-monitoring showed a very slow isomerization (~1.4 %/h). After 65 h the reaction was stopped and GLC showed a composition of (*Z*):(*E*)=89:11. Crystallization from mesitylene gave *5* of purity 99.9 % (GLC, SP 2100 at 250 °C). M.p. 126–129 °C.

(Pentachlorophenyl)acetylene (6) and α,2,-3,4,5,6-hexachlorostyrene (7). Compound *3* (8.0 g, 23 mmol) was dissolved in *tert*-butylalcohol (160 ml) and a solution of potassium *tert*-butoxide (5.26 g, 47 mmol) in *tert*-butylalcohol (160 ml) was added. After refluxing for 15 min tetrachloromethane was added and the mixture washed with water. Evaporation of the solvents (after drying) gave 5.7 g of a crude product which by GLC was found to contain 82 % of *6* and 10 % of *7*. Two recrystallizations from ethanol gave *6* (3.5 g) of 98 % purity (GLC). M.p. 182–184 °C (lit.[8] 185–186 °C). ^{1}H NMR (CCl_4): 3.67 (s) ppm.

The mother liquors were evaporated. After two recrystallizations from methanol, the residue gave a product containing 67 % of *7*. Preparative GLC (10 % Apiezon L at 260 °C) gave *7* in 97.7 % purity. M.p. 88–94 °C (lit.[2] 83 °C).

(E)- and (Z)-α,β,2,3,4,5,6-Heptachlorostyrenes (8) and (9). (Pentachlorophenyl)-acetylene (*6*) (3.5 g, 12.8 mmol) was dissolved in tetrachloromethane (100 ml), *cf.* Ref. 4. Chlorine (1.36 g, 19.2 mmol) dissolved in tetrachloromethane (30 ml) was added and the mixture was irradiated (UV) for 90 min. Longer irradiation time increases the amount of impurities. After evaporation of solvent, a product (3.8 g) was obtained which contained 58 % of *8* and 39 % of *9*. After two recrystallizations from acetone *8* (0.96 g) of 96 % purity (GLC) was obtained. Column chromatography (SiO_2 with hexane as eluent) increased the purity to 98.8 %. M.p. 114.5–115 °C (lit.[4] 109–110 °C). Attempts to further increase the purity using preparative GLC led to partial isomerization to *9*.

From the mother liquors was obtained a product (2.3 g) which contained 72 % of *9*. Preparative GLC (10 % Apiezon L, 220–260 °C, 2 °/min) gave *9* of 98.7 % purity. M.p. 49–51 °C (lit.[4] oil).

2,3,4,5,6-Pentachlorotoluene (10) was made in 58 % yield according to literature procedure.[6] M.p. 217–218 °C (lit. 217.5 °C) ^{1}H NMR(CCl_4): δ 2.57 (s).

α,α,2,3,4,5,6-Heptachlorotoluene (11).[27] *10* (10.0 g, 37.8 mmol) was heated to 240 °C. Chlorine gas was slowly passed through the melt for 5 h. The obtained crude product (10.1 g) was shown to contain 90 % of *11* and traces of α,2,3,4,5,6-hexachlorotoluene (^{1}H NMR (CCl_4): δ 4.87 (s, 2H) ppm). It was, however, considered pure enough to use in the next synthetic step. ^{1}H NMR (CCl_4): δ 7.50 ppm (s).

2,3,4,5,6-Pentachlorobenzaldehyde (12) was made through reaction of *11* with conc. sulfuric acid at 100 °C for 18 h.[27] The conversion was quantitative. ^{1}H NMR (CCl_4): δ 10.23 (s).

β,β,2,3,4,5,6-Heptachlorostyrene (13). Triphenylphosphine (11.35 g, 43.3 mmol) was dissolved in benzene (15 ml) and bromotrichloromethane (5.12 g, 25.8 mmol) was added. After stirring in nitrogen atmosphere for 1 h at 0 °C, pentachlorobenzaldehyde (*12*) (2.0 g, 7.2 mmol) dissolved in benzene (20 ml) was added in one portion. After stirring overnight, the solvent was evaporated and the residue exhaustively extracted with hexane. Purification using column chromatography (SiO_2, hexane) gave 1.9 g product which contained 90 % *13*. Recrystallization did not improve the purity. Preparative GLC (SP 2100 at 220 °C) gave a product of 99.7 % purity. M.p. 112–113 °C (lit.[4] 108–109 °C).

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Separation and Characterization of Mononitro Derivatives of Phenanthrene, Pyrene, Chrysene, Fluoranthene and Triphenylene

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Mononitro derivatives of selected polycyclic aromatic hydrocarbons with 3 and 4 condensed rings have been synthesized to obtain samples for measurements of mutagenic properties. Crude purification of the compounds was carried out on gravity columns prior to final purification by HPLC to a purity of approximately 99.9 %. Structural isomers were identified from NMR, MS and UV spectra.

It is well known that nitrated polycyclic aromatic hydrocarbons (nitro-PAH) which are found in carbon black,[1–3] in combustion emissions from diesel engines,[4–10] and in airborne particulate matter[11–14] may be mutagenic. However, when literature data on mutagenicity are compared, it appears that erroneous results have been published. Most probably this is due to the use of impure test substances. The preparation of nitrated PAH has mainly been reported by Dewar *et al.*,[15–19] but their work was carried out prior to the development of modern separation techniques and of powerful spectroscopic methods. Consequently, their purification procedures could not be expected to be adequate for the preparation of samples of sufficient purity for Ames tests. Small amounts of dinitro derivatives and other impurities may seriously affect the mutagenic response, and even the presence of small amounts of structural isomers can cause large deviations from the essential effect of a pure compound.[20] Furthermore, their structure elucidation was generally not supported by spectroscopic and/or spectrometric data, but mostly by predictions based on molecular-orbital theory.[21–24] It was therefore of interest to prepare nitro-PAH of high purity and to determine the structure of these compounds beyond doubt by UV, MS and NMR studies. The results for nitro-PAH with three and four condensed rings are reported here.

RESULTS AND DISCUSSIONS

Synthesis and purification. The preparation and crude purification of 9-nitrophenanthrene (9-nitro-Phe) and 1-nitropyrene (1-nitro-Pyr) were performed according to literature procedures.[15] Final purification was subsequently carried out by HPLC on silica columns. However, after storage for 3 months in the dark at −20 °C, a purity test of 1-nitro-Pyr revealed the appearance of two decomposition products, of which one was strongly mutagenic. These compounds were effectively removed by repeated purification on silica.

According to Dewar,[18] nitration of *chrysene* results in approximately 90 % of 6-nitrochrysene (6-nitro-Ch) plus one additional isomer, tentatively identified as 1-nitro-Ch from electron density calculations according to which the 1-position is more reactive than positions 4 and 5.[23,24]

The synthetic procedure of Dewar *et al.*[18] was modified in order to increase the yield of the mononitro fraction and the initial crude purification was carried out on silica instead of alumina, because silica resulted in a better group separa-

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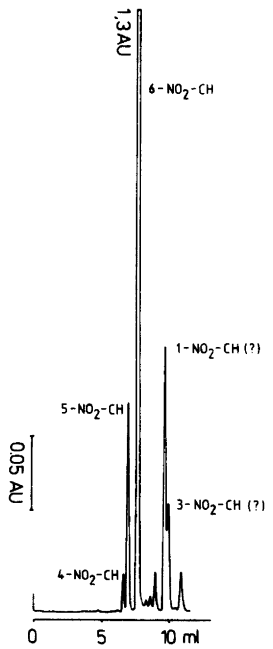


Fig. 1. Distribution of isomers of nitrochrysene (8μg) after the modified synthesis and work-up procedure, on 3μm Hypersil silica (250×4.6 mm) with 7.5 % dichloromethane and 0.1 % acetonitrile in hexane, at 2 ml/min, with UV-detection at 280 nm. The nitro group position of the three isolated isomers is marked on the figure (4,5,6), while the position of two other isomers is suggested (1 and 3).

tion of the mononitro fraction. This fraction was then analyzed by HPLC. The fraction contained five isomers of nitro-Ch. Three of the isomers were isolated, purified and identified as 6-nitro-Ch, 5-nitro-Ch and 4-nitro-Ch. The position of the nitro group in the two remaining isomers has not been determined, but based on the expected reactivity, the second largest peak in Fig. 1 is assumed to be the 1-nitro-Ch and the co-eluting shoulder peak 3-nitro-Ch. The retention of the assumed 1-nitro-Ch relative to 6-nitro-Ch on alumina was in agreement with the observations of Dewar.[18]

Nitration of *fluoranthene* (Fl) has been reported by Streitwieser to yield only four of the five possible nitro-Fl derivatives (1-, 3-, 7- and 8-nitro-Fl)[22] because position 2 should be non-reactive and impossible to nitrate directly.[6,25] When our nitration procedure was employed, however, crude purification on silica and further purification by HPLC gave Streitwieser's four products as well as a fifth nitro-Fl, which was identified (by MS) as 2-nitro-Fl (Fig. 2). Conceivably Streitwieser also got 2-nitro-Fl which, however, was not isolated due to insufficient resolution of the separation methods employed.

1-Nitrotriphenylene (1-nitro-Tri) and 2-nitrotriphenylene (2-nitro-Tri) which are the only mononitro derivatives of *triphenylene,* have been synthesized by Dewar[19] in 46 % yield and by Zinke[26] in 50 % yield as 1:1 isomeric mixtures. When our method was employed, a 59 % yield of a 1:1 mixture of 1-nitro-Tri and 2-nitro-Tri was obtained after purification on silica. Recently Radner reported that nitro-Tri was obtained in 92 % when triphenylene was nitrated with dinitrogentetroxide.[27] The nitrotriphenylene isomers comprise an outstanding example of the importance of isomer purity in tests for mutagenic effects, since the number of revertants (in the TA 98 assay) per microgram of 2-nitro-Tri is of the order of 10 000 times higher than for 1-nitro-Tri.[20] Thus, a purity of 99.9 % will not necessarily be sufficient as 0.1 % impurity of the former in the latter would lead to grossly

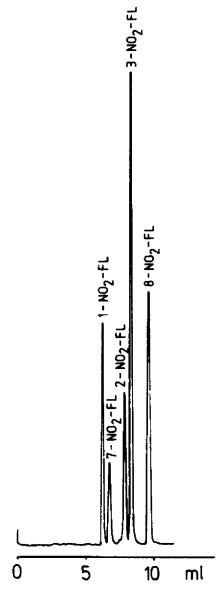


Fig. 2. Distribution of isomers of nitrofluoranthene (6μg totally). Column and conditions as in Fig. 1. The nitro group position is marked on the figure.

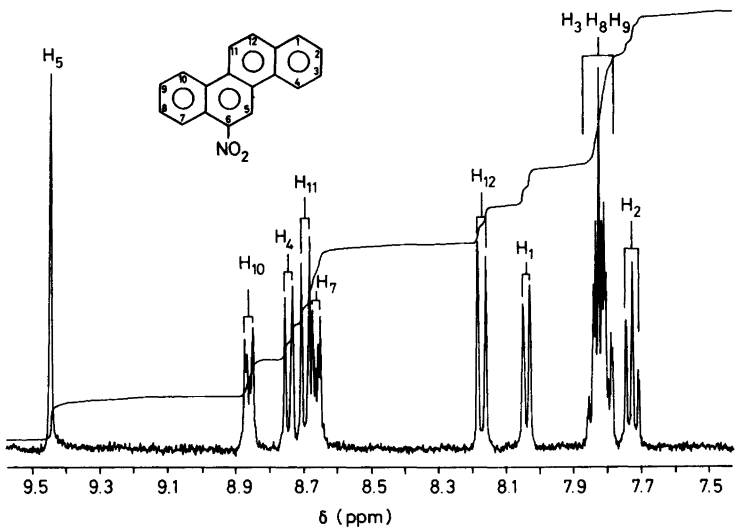


Fig. 3. The 400 MHz ^{1}H NMR spectrum of 6-nitrochrysene in $CDCl_3$ at 24 °C relative to internal TMS.

erroneous data for 1-nitro-Tri.

NMR spectroscopy. Except for 4-nitrochrysene which was identified from MS and UV spectra, and 1-nitropyrene, the structure of which was elucidated by ^{13}C NMR spectroscopy,[28] all the mononitro derivatives were conveniently identified by ^{1}H NMR spectroscopy. 1-Nitrotriphenylene, and 2-nitrotriphenylene gave proton spectra equivalent to those reported in the literature.[29] The ^{1}H NMR spectrum of the nitro-Phe isomer consisted of a sharp singlet at 8.61 ppm and a complex multiplet at 8.15–7.46 ppm in a ratio of 1:8 which is compatible only with the structure 9-nitrophenanthrene for this compound.

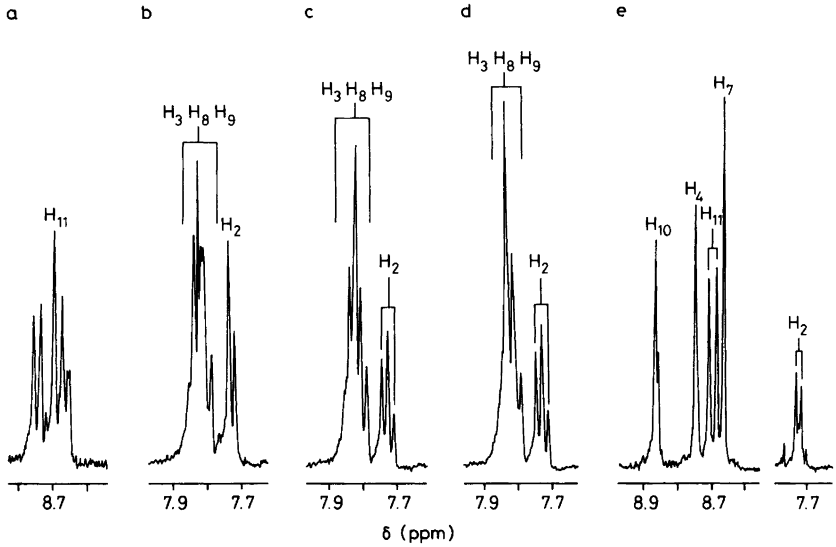


Fig. 4. Decoupling experiments with 6-nitrochrysene. *a,* Irradiation of H-12. *b,* Irradiation of H-1. *c,* Irradiation of H-7. *d,* Irradiation of H-10. *e,* Irradiation of H-3, H-8 and H-9.

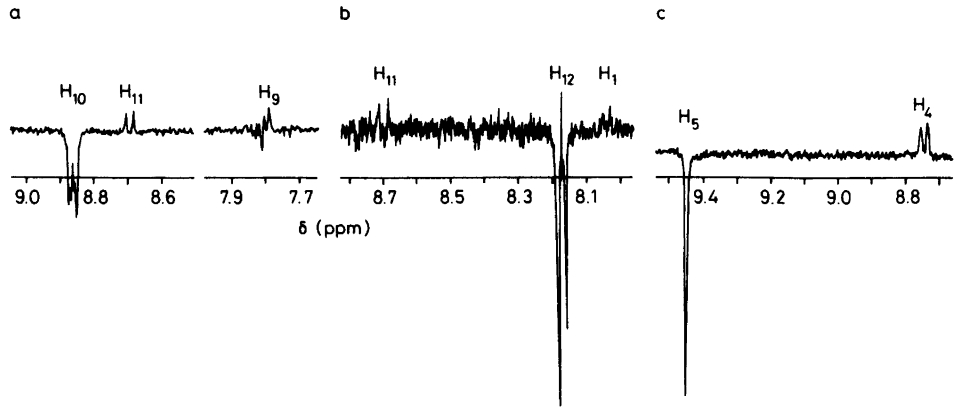


Fig. 5. ^{1}H- [^{1}H] NOE difference spectra of 6-nitrochrysene. *a*, Irradiation of H-10. *b*, Irradiation of H-12. *c*, Irradiation of H-5.

The main product from nitration of *chrysene* gave a proton NMR spectrum (Fig. 3) which turned out to be due to 6-nitrochrysene. This structure was partly deduced by double resonance studies. Irradiation at the frequencies of H-12 and H-1 causes the H-11 signal to collapse to a singlet (Fig. 4a) and the multiplet due to H-2, H-3, H-8 and H-9 to simplify considerably (Fig. 4b). Simplification of the H-3, H-8 and H-9 multiplets also results when the frequencies of H-7 (Fig. 4c) and H-10 (Fig. 4d) are saturated. Finally, irradiation of the complex multiplet from H-3, H-8 and H-9 simplifies the H-2, H-4, H-7 and H-10 signals (Fig. 4e). These experiments clearly show that this spectrum consists of one singlet and three subspectra of which there is one AB, one ABMX, and one AA'MX system. The nitro group is therefore attached to either of the positions 5 and 6, and to determine the substitution pattern NOE studies were carried out. Fig. 5 shows NOE difference spectra of the compound which confirm that (1) H-10 and H-11 belong to the same bay area of the molecule (Fig. 5a), (2) H-12 is in a *peri* position relative to H-1 (very little nuclear Overhauser enhancement) (Fig. 5b), and (3) the singlet is due to a hydrogen atom

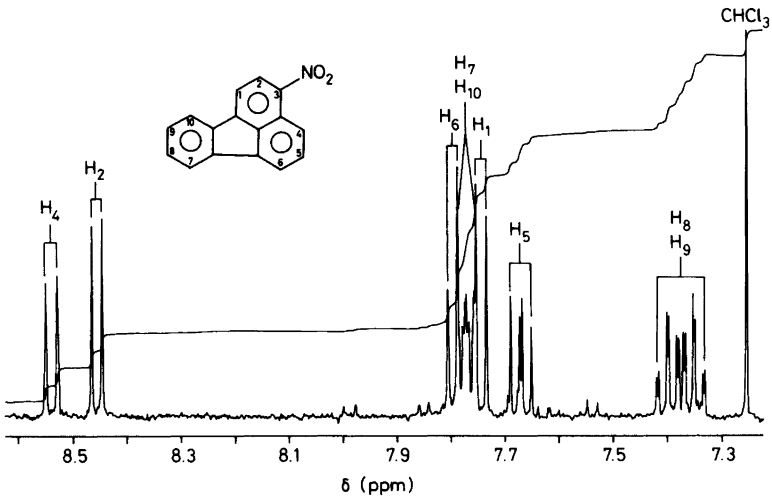


Fig. 6. The 400 MHz ^{1}H NMR spectrum of 3-nitrofluoranthene in $CDCl_3$ at 24 °C relative to internal TMS.

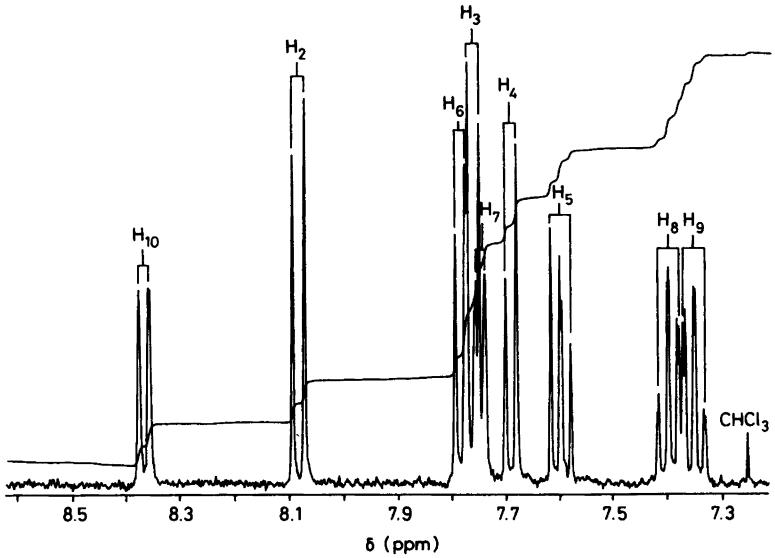


Fig. 7. The 400 MHz ^{1}H NMR spectrum of 1-nitrofluoranthene in $CDCl_3$ at 24 °C relative to internal TMS.

which belongs to the same "bay" as H-4 (a strong nuclear Overhauser effect) (Fig. 5c). The most abundant mononitro-chrysene derivative is therefore 6-nitrochrysene.

^{1}H NMR studies were also carried out on one of the minor mononitrochrysene derivatives, but the compound did not permit a complete analysis of the spectrum due to decomposition under the experimental conditions. However, a sharp singlet at 9.33 ppm strongly indicates that the isomer is 5-nitrochrysene which is the only nitrochrysene isomer, except 6-nitrochrysene, that will give rise to a singlet in the proton spectrum. This conclusion is also supported by the good agreement between the proton spectrum of this compound and that of 5-acetylchrysene (Table 1).[30]

Four of the five mononitro isomers obtained by nitration of *fluoranthene* were analyzed by ^{1}H NMR spectroscopy.

The most and the least abundant isomers gave rise to the spectra shown in Figs. 6 and 7, respectively. The structures of these compounds were partly deduced by double resonance experiments. For the most predominant isomer separate irradiation at the frequence of H-4 or H-6 causes the H-5 double doublet to collapse to a doublet. Similarly, the H-1 doublet collapses to a singlet when the H-2 frequency was saturated.

Table 1. The proton NMR spectra of 5-acetylchrysene (5-Ac-Ch)[30] and 5-nitrochrysene (5-nitro-Ch) in ppm in $CDCl_3$ relative to internal TMS.

5-Ac-Ch	5-nitro-Ch
7.4–8.0 (m,7H)	7.6–8.2 (m,7H)
8.07–8.27 (m,H-10, H-11)	8.35–8.53 (m,2H)
8.66 (s,H-6)	8.84 (m,1H)
8.82 (m,H-4)	9.33 (s,1H)

Finally, partial saturation of the H-7, H-10 multiplet almost converts the H-8, H-9 multiplet into an AB quartet. Analogous experiments for the least predominant isomer gave the following results: Partial saturation of the double doublet from H-5 causes the H-4 and H-6 doublets to collapse essentially to singlets. Furthermore, irradiation at the H-10 frequency removes a *para* coupling to H-7, a *meta* coupling to H-8 and an *ortho* coupling to H-9. Similarly, when the frequency of H-7 is saturated, a *para* coupling to H-10, a *meta* coupling to H-9 and an *ortho* coupling to H-8 are eliminated. Finally, irradiation of the doublet from H-2 causes the H-3 doublet to collapse to a singlet. It is therefore evident that both NMR spectra consist of one

Table 2. Chemical shifts in ppm relative to internal TMS of the hydrogen atoms in the naphtalene moiety of fluoranthene, 1-nitrofluoranthene and 3-nitrofluoranthene.

Proton	Fl[a]	1-nitro-Fl[b]	3-nitro-Fl[b]
H-1	7.82	–	8.00
H-2	7.52	8.30	8.35
H-3	7.72	7.84	–
H-4	7.72	7.87	8.51
H-5	7.52	7.74	7.87
H-6	7.82	7.99	8.02

[a] From Ref. 32. [b] These values have been calculated from the observed values of fluoranthene by using the deshielding parameters of Wells.[31]

AB, one AMX and one four-spin subspectrum.

Since J_{AB} (>7.5 Hz) is an *ortho* coupling constant in both cases, the two isomers are 1-nitrofluoranthene (1-nitro-Fl) and 3-nitrofluoranthene (3-nitro-Fl). In order to determine which isomer corresponds to which structure, the chemical shifts of the hydrogen atoms in the naphtalene moiety of the alternative structures were calculated[31–33] (Table 2) and compared with the experimental data (Figs. 6 and 7). This comparison clearly reveals that only 3-nitro-Fl is expected to give signals matching the two doublets observed at low field in the spectrum of the most abundant nitrofluoranthene derivative (Fig. 6). Consequently, this isomer is 3-nitrofluoranthene, whereas the least abundant one is 1-nitrofluoranthene. This conclusion is supported by the different four-spin subspectra observed in the NMR spectra of the two compounds. The nitro group in 3-nitro-Fl has very little influence on the disubstituted benzene moiety which therefore appears as an ABMM′ subspectrum (Fig. 6). In 1-nitro-Fl, on the other hand, the nitro group deshields H-10 considerably more than H-7, H-8 and H-9 and the four-spin system results in an ABMX.

The isomer with the longest retention time on Hypersil silica gave the ^{1}H NMR spectrum shown in Fig. 8. As evidenced by a simple decoupling experiment this spectrum contains an AMX subspectrum which is characteristic for a 1,2,4-trisubstituted benzene derivative. This isomer is therefore 8-nitrofluoranthene.

The fourth nitrofluoranthene isomer gave the ^{1}H NMR spectrum shown in Fig. 9a. Unfortunately, the compound was unstable and decomposed before a complete series of decoupling experiments had been performed, rendering a detailed analysis of the spectrum impossible.

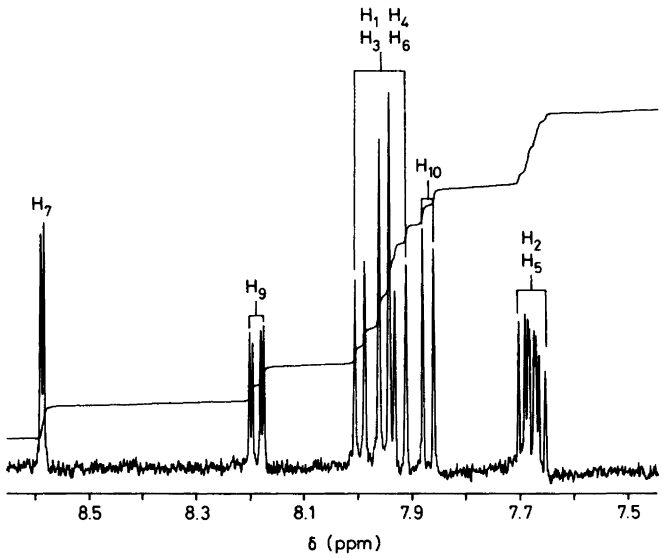


Fig. 8. The 400 MHz ^{1}H NMR spectrum of 8-nitrofluoranthene in $CDCl_3$ at 24 °C relative to internal TMS. The interpretation is based on decoupling experiments and by taking the relative positions of H-1–H-6 in fluoranthene into consideration.[32]

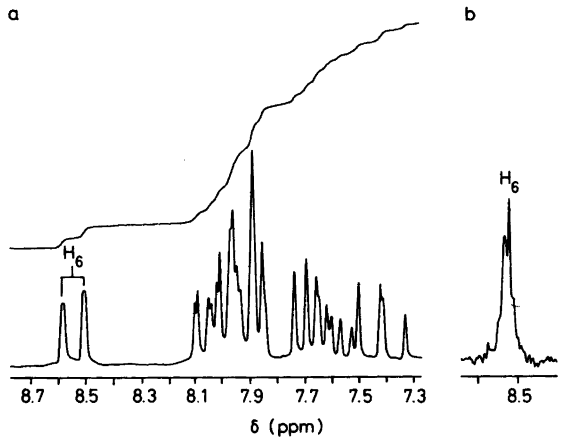


Fig. 9. a, The 90 MHz ^{1}H NMR spectrum of 7-nitrofluoroanthene in $CDCl_3$ at 29 °C relative to internal TMS. *b,* Irradiation at 7.7 ppm, decoupling at 8.54 ppm.

However, partial saturation of the multiplet at 7.7 ppm caused the two low-field signals to collapse essentially to a broad singlet (Fig. 9b) which proves that a doublet arising from an *ortho* coupling (*J* 7.3 Hz) is situated at 8.54 ppm. This isomer is therefore 7-nitrofluoranthene which is the only remaining alternative that will give rise to such a doublet at low field.

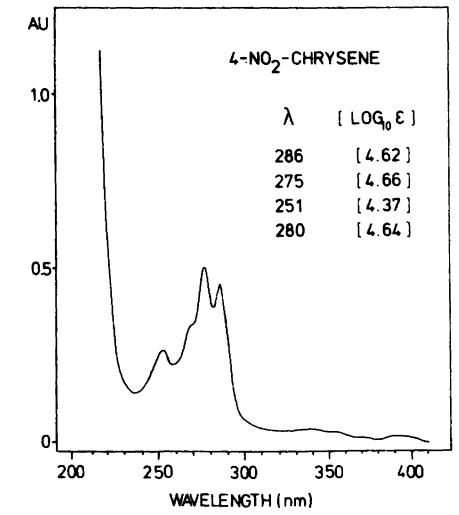
Mass spectroscopy. Two of the most common fragmentation processes after electron impact ionization of aromatic nitro compounds are loss of NO and NO_2. The loss of NO may occur after rearrangement of the nitro group to a nitrite moiety, or through a three-centered cyclic transition state.[34,35] Furthermore, loss of HNO_2 may also take place, most probably as NO_2+H. The same cleavage reactions are also observed in the mass spectra of nitro-PAH, *e.g.* 9-nitroanthracene, 2-nitropyrene, 6-nitrobenzo(a)pyrene and 3-nitroperylene,[36,37] and these processes can be used to elucidate the structures of the nitro-PAH derivatives reported here from characteristic peaks in their mass spectra (Table 3).

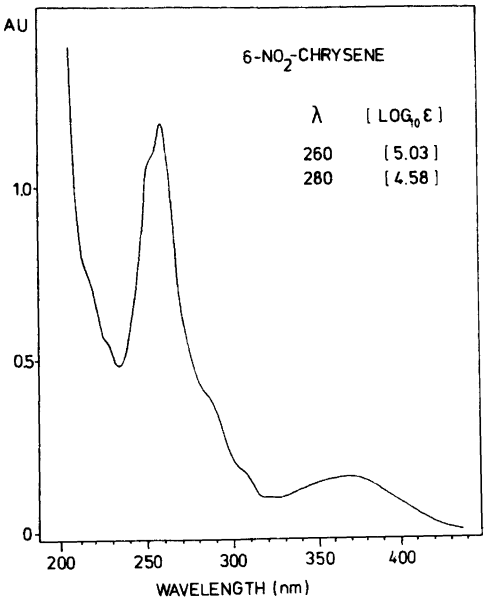
The M–OH fragment was exclusively found in the spectra of nitro-PAH with the nitro group in a *bay* position, *e.g.* of 5-nitro-Ch and 1-ni-

Table 3. Mass spectrometric fragments, in % of the base peak.

Compound	M	M−17[a]	M−30[b]	M−31[c]	M−46[d]	M−47[e]	M−58[f]	Substituent position
9-NO_2-Phe	100	–	63	1	57	68	59	*peri*
1-NO_2-Pyr	100	–	76	5	96	55	53	*peri*
4-NO_2-Ch	56	7	58	100	57	90	54	*bay*
5-NO_2-Ch	100	2	47	3	44	80	91	*bay*
6-NO_2-Ch	100	–	19	1	48	78	62	*peri*
1-NO_2-Fl	90	–	30	3	100	53	29	"*bay*"
2-NO_2-Fl	81	–	100	7	89	48	39	*peri*
3-NO_2-Fl	100	–	39	1	81	67	48	*peri*
7-NO_2-Fl	78	–	12	–	100	47	22	"*bay*"
8-NO_2-Fl	100	–	27	1	89	56	29	*peri*
1-NO_2-Tri	21	6	100	12	17	58	90	*bay*
2-NO_2-Tri	100	–	5	–	51	70	29	–

[a] M–OH, [b] M–NO, [c] M–HNO, [d] M–NO_2, [e] M–HNO_2, [f] M–NOCO, not adjusted for the ^{13}C-isotope contribution.





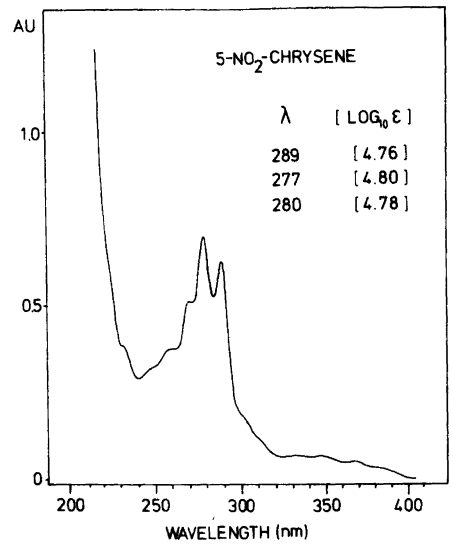


Fig. 10. The UV-spectra of 4-, 5- and 6-nitrochrysene in acetonitrile.

troperylene, 1-nitrobenzo[*e*]pyrene and 5-nitrobenzo[*ghi*]perylene.[38] The appearance of such a fragment is in accordance with the M–OH peak in the mass spectrum of *o*-nitrobiphenyl,[34] another compound with a nitro group in a *bay* position. It was therefore reasonable to believe that 4-nitrochrysene was the third nitro-Ch isomer isolated after nitration of chrysene. The intensity of the M–OH fragment is generally low and this explains the lack of M–OH fragmentation in 1-nitro-Fl and 7-nitro-Fl where abstraction of a *bay* hydrogen atom is significantly less probable due to the longer distance between the nitro group and the hydrogen atom across the *bay*.

Fragments due to loss of HNO were found in the mass spectra of all the nitro-PAH derivatives, but with higher intensity in the spectra of the compounds with the nitro group in a *bay* position. It is interesting to note that the M–HNO fragments are more intense than the corresponding M–OH fragments. When the temperature in the ion source was increased, remarkably intense peaks due to oxygen loss appeared in the mass spectra of the compounds with a nitro group in a *bay* position. So far only preliminary experiments have been performed, but a thermal degradation and rearrangement of the nitro derivatives to carbazoles accommodates the observations.

UV-spectroscopy. The UV-spectra of the isomers of nitro-Ch (Fig. 10), nitro-Fl (Fig. 11) and nitro-Tri (Fig. 12) demonstrated the lack of a strong high-wavelength band in the spectra of isomers with a *bay* region nitro group. This is suggested to be caused by a less efficient π-electron overlap from a nitro group forced out-of-plane in a *bay* position. The effect is less marked in the fluoranthenes, where the distance between two "bay"substituents is increased, due

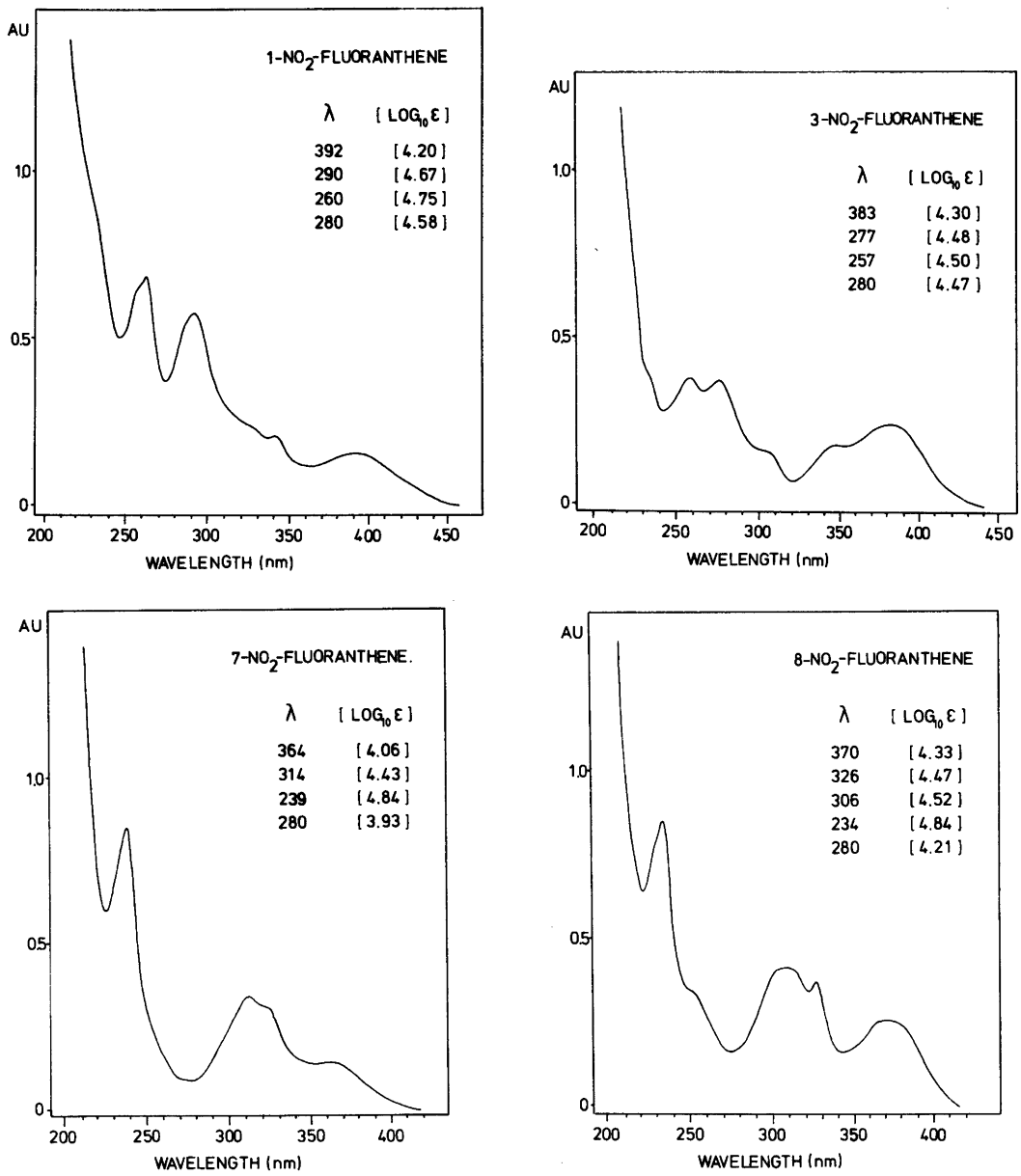
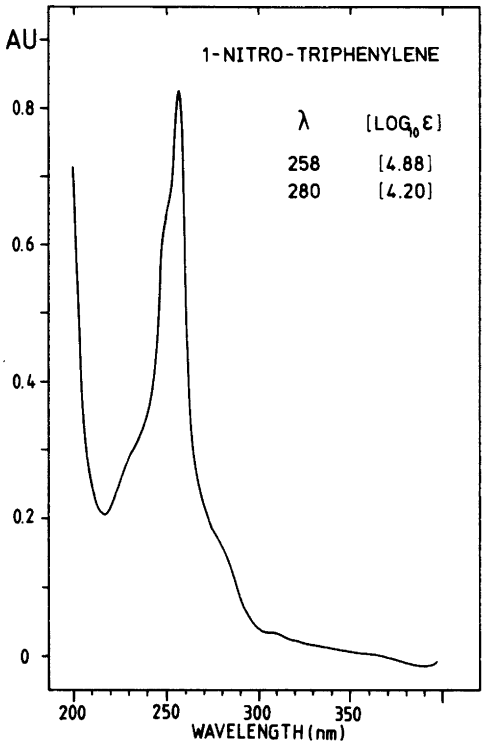


Fig. 11. The UV-spectra of 1-, 3-, 7- and 8-nitrofluoranthene in acetonitrile.

to the five-membered central ring. The UV-spectra therefore support the conclusion that 4-nitro-Ch is a minor product when chrysene is nitrated with nitric acid in acetic anhydride.

EXPERIMENTAL

The HPLC equipment consisted of Waters M 6000 A pumps, a Waters U6K injector, a Waters 440 UV detector with 2 flow cells, a Perkin Elmer LC-55 variable wavelength UV detector and a



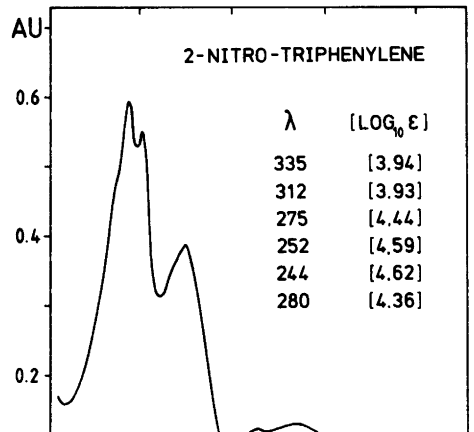


Fig. 12. The UV-spectra of 1-nitro- and 2-nitro-triphenylene in acetonitrile.

Kontron SFM 23LC spectrofluorometric detector.

The organic solvents used for the crude purification on gravity columns were of *pro analysi* quality (Merck). All the HPLC work was performed with solvents of HPLC quality (Rathburn), except chloroform (Fluka, *p.a.*).

The UV spectra were recorded on a Cary Recording Spectrophotometer, model 14, with a scan speed of 6.25 A/s. The molar absorbances at the maxima were measured on a Cary Spectrophotometer, model 16.

The mass spectra (70 eV) were obtained on a Micromass 7070 F double focusing magnetic instrument with electron impact ionization and a ion-source temperature of 200 °C.

The ^{1}H NMR spectra were recorded on a Bruker WH 400 (400.13 MHz) spectrometer at 24 °C or on a Jeol FX 90 Q (89.55 MHz) instrument at 29 °C. The samples were 0.1–0.3 % by weight in $CDCl_3$ which provided the deuterium signal for the NMR field lock. The spectra were run with a spectral width of 4000 Hz, a pulse width of 3 μs (25°) and a repetition time of 1 s. A Lorentzian-Gaussian conversion was applied to the FID before Fourier transformation. The computer operation conditions gave a digital resolution of 1.0 Hz at 400 MHz and of 0.5 Hz at 90 MHz.

Procedure for nitration and crude purification. 10–50 mg of the aromatic hydrocarbon were dissolved in acetic anhydride (20–200 ml) at room temperature or by careful heating, and cooled to 0 °C. Nitric acid (100 %, density 1.52) in acetic anhydride was added dropwise with stirring at 0 °C and the reaction mixture was stirred in the dark. Details are given in Table 4. The reactions were followed by TLC on silica (Merck Kieselgel 60–F_{254}), with dichloromethane–hexane (30:70) as eluent. When the mononitro fraction appeared to be at a maximum concentration, the reaction was quenched by adding water (40 ml) and concentrated (95–97 %) sulfuric acid (0.5 ml) at 0 °C. The hydrolysis of the acetic anhydride was completed by stirring for approximately 9 h at 0 °C. The solution or suspension was extracted with dichloromethane and the organic phase was washed with an aqueous sodium bicarbonate solution (4 g in 60 ml water). The organic phase was evaporated to dryness under vacuum. The crude product was dissolved in dichloromethane (5–10 ml), diluted with hexane (10–20 ml) in order to reduce the elution strength and applied on a silica (Merck Kieselgel 60, 0.063–0.200 mm) column. The first fractions contained unreacted starting material (if present). The next fractions contained mononitro derivatives (Table 4). Further elution resulted in small amounts of

Table 4. Experimental data on synthesis and crude purification of mononitro PAH.

PAH	mg PAH	mmol HNO_3	molar ratio HNO_3 : PAH	Nitration Time (h)	Temp. (°C)	Column size (cm)	Ch_2Cl_2/ hexane	Elution volume (ml)	Yield %
Ch	18.0	0.7	9	48	0				
				120	20	26×1.5	30/70	110–250	77
Fl	20.7	0.05	0.6	168	0	26×1.5	30/70	110–250	38
Tri	50.0	2.4	11	48	60	25X2.5	40/60	160–210 [a]	
								211–290 [b]	59
								291–340 [c]	

[a] 1-nitro-Tri. [b] Mixture of 1- and 2-nitro-Tri. [c] 2-nitro-Tri.

dinitro derivatives.

Purification by HPLC. A 99.9 % purity was considered the practical limit for the preparative purification procedures, and this purity has turned out to be necessary for some compounds since the mutagenic response in TA-98 assays may vary with a factor of up to 10 000 within one group of isomers.[20]

The purity measurements were carried out with two different HPLC systems, one by adsorption chromatography and one by reversed phase chromatography, with UV-detection at 2 wavelengths. The 280 nm wavelength was chosen as a standard for all compounds, while a second higher wavelength was chosen according to the various absorption maxima. Thus, the purity measured was based on the assumption of approximately equal molar absorbance of possible contaminants, an assumption which turned out to be essentially valid when controlled by glass capillary gas chromatography. Gas chromatography alone was found less satisfactory for purity testing, since the amounts of thermal decomposition products (of the higher nitro-PAH) increased with increasing temperature and increasing time on the columns. A fluorescence detector was found to be valuable to spot minor amounts of certain decomposition products formed by evaporation of solutions in the light or by prolonged storage.

Three HPLC columns were utilized in the purification procedures: A. 250×10 mm, packed with 3 μm Hypersil silica (Shandon). B. 250×7.7 mm, packed with 5 μm Spherisorb silica (Phase Sep.). C. 250×10 mm, packed with 5 μm Hypersil-ODS (Shandon).

The nitro-PAH were applied in CH_2Cl_2 or in CH_2Cl_2–hexane (1:1). The application volumes were 25–150 μl. The flow rates were 4–5 ml/min.

9-Nitro-Phe was purified first on column B with 5 % CH_2Cl_2 in hexane, than rechromatographed on column A with 3 % CH_2Cl_2 in hexane.

1-Nitro-Pyr was purified on column A with 5 % CH_2Cl_2 in hexane. Decomposition products were removed on Kieselgel 60 with 40 % CH_2Cl_2 in hexane.

4-, 5- and 6-Nitro-Ch were separated on column A with 5 % CH_2Cl_2 in hexane. 6-Nitro-Ch was purified on column A with 0.05 % isopropanol in hexane. 4- and 5-Nitro-Ch were purified on column A with 3 % CH_2Cl_2 in hexane.

1-, 2-, 3-, 7- and 8-Nitro-Fl were separated on column B with 5 % CH_2Cl_2 in hexane. 7- and 3-Nitro-Fl needed rechromatography on column B with 3 % CH_2Cl_2 in hexane.

1- and 2-Nitro-Tri separated on column A with 0.8 % acetonitrile in hexane (2-nitro-Tri eluted prior to 1-nitro-Tri). With 10 % CH_2Cl_2 in hexane the order of elution was reversed. An even better resolution was obtained on column C with 75–80 % methanol in water.

The purity of the products was examined on 5 μm Hypersil silica (300×4,6 mm) with 3–5 % CH_2Cl_2 in hexane and on Hypersil-ODS (250×4,6 mm) with 80 % methanol in water.

Proton NMR spectra. The proton spectra of 6-nitrochrysene and four of the nitrofluoranthene derivatives are summarized below. The chemical shifts are given in ppm relative to internal tetramethylsilane (TMS).

6-Nitro-Ch. ^{1}H-NMR (400 MHz): δ 7.69–7.76 (1H,m), 7.78–7.85 (3H,m), 8.03 (1H,d,*J* 7.8 Hz), 8.17 (1H,d,*J* 8.8 Hz), 8.64–8.69 (1H,m), 8.69 (1H,d,*J* 8.8 Hz), 8.72–8.76 (1H,m), 8.83–8.88 (1H,m), 9.44 (1H,s).

1-Nitro-Fl. ^{1}H NMR (400 MHz): δ 7.31–7.42 (2H,m), 7.60 (1H,dd,*J* 6.9 Hz, *J* 8.2 Hz), 7.69 (1H,d,*J* 8.2 Hz), 7.74 (1H,d,*J* 7.3 Hz), 7.76 (1H,d,*J* 8.8 Hz), 7.78 (1H,d,*J* 6.9 Hz), 8.08 (1H, d, *J* 8.2 Hz), 8.36 (1H, m, *J* 7.6 Hz).

3-Nitro-Fl. ^{1}H NMR (400 MHz): δ 7.31–7.42 (2H,m), 7.67 (1H,dd,*J* 7.0 Hz, *J* 8.6 Hz), 7.74 (1H,d,*J* 7.7 Hz), 7.75–7.79 (2H,m), 7.79 (1H,d,*J*

7.0 Hz), 8.45 (1H,d,*J* 7.7 Hz), 8.54 (1H,d,*J* 8.6 Hz).

7-Nitro-Fl. ^{1}H NMR (90 MHz): δ 7.32–7,73 (3H,m), 7.85–8.10 (5H,m), 8.54 (1H,d,*J* 7.3 Hz).

8-Nitro-Fl. ^{1}H NMR (400 MHz): δ 7.64–7.71 (2H,m), 7.87 (1H,d,*J* 8.3 Hz), 7.92 (1H,d,*J* 8.2 Hz), 7.95 (2H,d,*J* 7.5 Hz), 7.99 (1H,d,*J* 7.0 Hz), 8.19 (1H, dd,*J* 2.2 Hz, *J* 8.3 Hz), 8.59 (1H,d,*J* 2.2 Hz).

Acknowledgements. Financial support from The Norwegian Council for Scientific and Industrial Research (NTNF), The Norwegian Research Council for Science and the Humanities (NAVF) and from *Norges Vassdrags og Elektrisitetsvesen-Statskraftverkene* is gratefully acknowledged. Thanks are also due to Unni Bingen, Arnfinn Kvarsnes, Geomil Pekov and Tore Skjetne for skillful technical assistance.

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Stereoselective Syntheses of Lignin Model Compounds of the β-0-4 and β-1 Types

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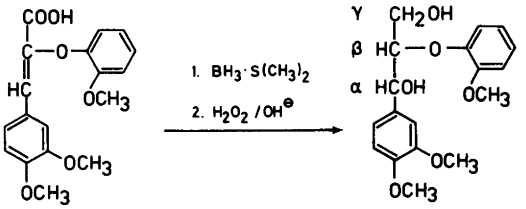
The preparation of *erythro* forms of lignin model compounds of the β-0-4 type by hydroboration of α-aryloxycinnamic acids, using a borane dimethyl sulfide complex as a reagent, has been studied. The *erythro* form of 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol was obtained in a yield of about 50 %. Acid-catalyzed rearrangements of chalcone oxides result in the formation of α-formyldesoxibenzoins which give 1,2-diaryl-1,3-propanediols on borohydride reduction. The synthesis of the *erythro* forms of 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol and 1,2-bis(4-hydroxy-3,5-dimethoxyphenyl)-1,3-propanediol by this reaction route is described; both 1,2-diaryl-1,3-propanediols represent lignin model compounds for the β-1 type of structure.

The use of adequate lignin model compounds is often crucial in lignin research. Lignin model compounds are not commercially available and therefore have to be prepared in the laboratory. In general only small amounts of model compounds are required. Thus the obvious criteria for choosing suitable synthetic routes for such compounds would seem to be simplicity of preparation and ease of purification rather than high yield. In this paper stereoselective syntheses of some lignin model compounds of the β-0-4 and β-1 types are described.

β-0-4 model compounds. The synthesis of lignin model compound *2* by hydroboration of acid *1* has been described in previous studies.[1,2] In this paper a commercially available reagent – borane dimethyl sulfide complex – has been used for hydroboration (Scheme 1). It was found that acid *1* reacted comparatively slowly with this reagent. However, the rate of reaction could be increased by raising the temperature. After 2 h treatment at 35 °C compound *2* (*erythro* form) could be isolated in a yield of 60 %. In connection with attempts to optimize the formation of β-0-4 compounds, it was found that long-term treatment with the borane complex at a lower temperature (20 °C) gave a product consisting primarily of α-(2-methoxyphenoxy)-3,4-dimethoxycinnamyl alcohol. This suggests that this compound is an intermediate in the synthesis of compound *2*. Long-term treatment with the borane reagent also resulted in a partial isomerization which led to the occurrence of minor amounts of the *threo* form of compound *2* in the reaction mixture.



1 2

Scheme 1.

Hydroboration of α-aryloxycinnamic acid 5 with borane dimethyl sulfide complex gave compound *6* (yield: 56 %). Removal of the benzyl groups by catalytic hydrogenation of this com-

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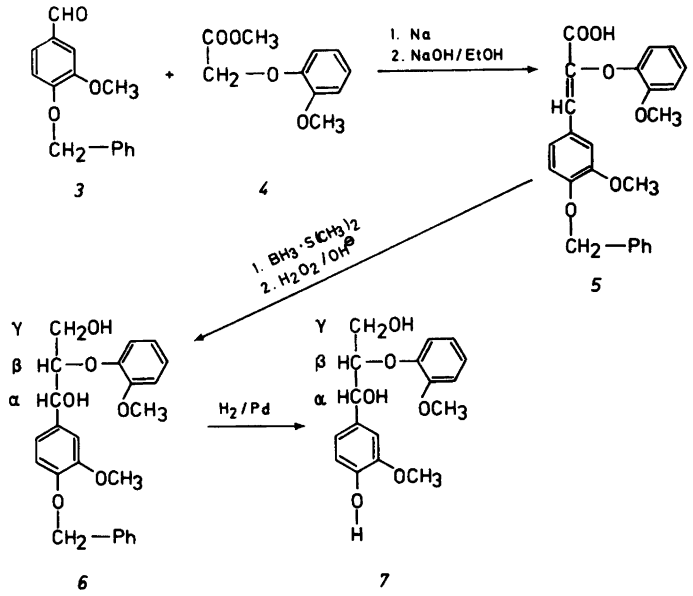
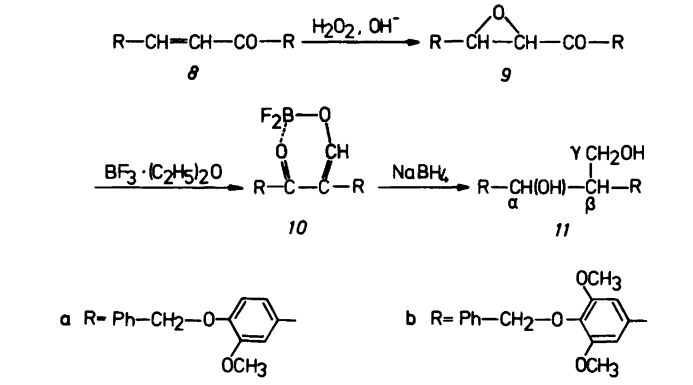


Scheme 2.

pound gave β-0-4 model *7* (*erythro* form). Acid *5* was prepared by the condensation of methyl (2-methoxyphenoxy)ethanoate (*4*) with 4-benzyloxy-3-methoxybenzaldehyde (*3*) (Scheme 2). It was found that the sodium salt of acid *5* could be extracted from an aqueous solution with dichloromethane. This finding was used in the work-up procedure for the isolation of acid *5*. For recent work on the synthesis of β-0-4 model compounds, see Refs. 3, 4 and 5.

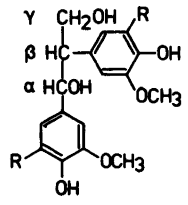
β-1 model compounds. The reaction route shown in Scheme 3 has been used for the synthesis of the lignin model compounds of the β-1 type.[5,7,8] Some chalcones (*e.g.* chalcone *8a*) are very slightly soluble in reaction media suitable for the epoxidation step. Phase-transfer catalysis was found to be useful for the oxidation of such chalcones. Model compound *12* (*erythro* form) was prepared in a high yield. β-1 model compounds have been prepared by a variety of synthetic methods.[9–13] The reaction route in Scheme 3 has the advantage over other methods of being stereoselective. The *erythro* form of compound *13* was prepared analogously. This compound has not been synthesized previously, but has been isolated from wood hydrolysates.[14,15]



Scheme 3.

EXPERIMENTAL

NMR spectra were recorded using chloroform-*d* as a solvent. *Thin layer chromatography (TLC)* was performed with SiO_2 using benzene–dioxane acetic acid (90:25:4) as eluent. Spots were made visible by first spraying with formalin



12 R=H
13 R=OCH_3

-H_2SO_4 (1:9) and then heating. R_F values: (2-methoxyphenoxy)ethanoic acid, 0.20; *7*, 0.21; *2*, 0.28; *1*, 0.32; *6*, 0.32; *5*, 0.34; 4-benzyloxy-3-methoxybenzoic acid, 0.50; *3*, 0.63.

Extraction of the sodium salt of acid 5 from an aqueous solution by dichloromethane. Acid *5* (100 mg) was dissolved in 12 ml 0.5 M NaOH and the solution extracted with 20 ml dichloromethane. The organic layer was dried over Na_2SO_4 and the solvent removed with film evaporation. A crystalline product (75 mg, m.p. >300 °C) was obtained. The product exhibited a strong IR band at 1610 cm^{-1} (carboxylate); the strong band found at 1680 cm^{-1} in the spectrum of acid *5* (C=O) was absent. Sodium content: 5.45 % (determined by flame-ionization spectrometry). Calc. for the sodium salt of acid *5*: 5.37 %.

Methyl (2-methoxyphenoxy) ethanoate (4) was prepared according to a procedure described previously.[2] This compound was obtained in a modification melting at 47 °C.

α-(2-Methoxyphenoxy)-4-benzyloxy-3-methoxycinnamic acid (5) (cf. Freudenberg and Müller[16]). A mixture of benzylvanillin (9.1 g) and methyl (2-methoxyphenoxy) ethanoate (*4*) (7.4 g) were melted together in a 100 ml flask equipped with a cooler. Ether (25 ml) and 50 % sodium in paraffin (1.7 g) were added. As indicated by the evolution of hydrogen, the sodium dissolved fairly rapidly (the temperature was kept below 30 °C to avoid a too vigorous reaction). When the evolution of hydrogen ceased, the reaction mixture was refluxed for 5.5 h (oil bath). Five ml of ethanol were added, whereupon the ether was evaporated. NaOH (1.25 g) in 25 ml ethanol was added and the mixture set aside overnight. The reaction product was transferred to a separatory funnel with the help of 200 ml 0.1 M NaOH and 150 ml ether. The aqueous layer was separated and washed with 75 ml ether. One half of the aqueous layer was extracted with 2 × 100 ml dichloromethane. The extract was dried over Na_2SO_4, treated with 60 ml 0.7 M hydrochloric acid, washed with H_2O (40 ml), and again dried over Na_2SO_4. Removal of the solvents gave a product weighing 5.0 g. [On acidification of the dichloromethane-treated aqueous layer, crystals precipitated (m.p. ≈ 160 °C) which were identified as 4-benzyloxy-3-methoxybenzoic acid (IR, m.p.; lit.[17] m.p. 168–169 °C). Subsequent extraction with trichloromethane gave a product identified as (2-methoxyphenoxy)ethanoic acid (IR, TLC).] The product was chromatographed on silica gel (100 g) with dichloromethane–ethyl acetate (9:1) as eluent. Eluate 200–500 ml gave crystals (3.46 g) of m.p. 142 °C. Recrystallization from methanol–water (9:1) gave 2.6 g acid *5* of m.p. 147 °C (yield: 34 %). ^1H NMR (270 MHz): δ 3.71 (3 H, s; OCH_3), 3.90 (3 H, s; OCH_3), 5.13 (2 H, s; CH_2), ≈7 (13 H, m; aromatic protons and vinyl proton). The IR spectrum exhibited a strong band at 1680 cm^{-1} (C=O). The second half of the original aqueous layer was acidified to pH 2 with hydrochloric acid and extracted with 2×100 ml ether. Evaporation of the solvents gave a residue weighing 5.9 g. Chromatographic purification (see above) gave acid *5*.

α-(2-Methoxyphenoxy)-3,4-dimethoxycinnamic acid (1) was prepared according to the procedure for the synthesis of acid *5* described above. For the isolation of acid *1* from the reaction mixture, the work-up procedure from a previous study[2] was used. Yields were about the same as in the synthesis of acid *1* described in that study.[2]

erythro-*1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol.* Acid *1* (2.98 g) was dissolved in 36 ml tetrahydrofuran in a 100-ml three-necked flask (nitrogen atmosphere). Borane dimethyl sulfide complex (4.5 ml) was injected over a 15 min period (magnetic stirring). The reaction mixture was kept at 35 °C for 2 h (oil bath). Excess reagent was decomposed by the addition of 13.5 ml H_2O. H_2O_2 (1.8 ml 30 % H_2O_2) was added and then 18 ml 3 M NaOH was added over a 15 min period. The reaction mixture was stirred vigorously at 35 °C for a 1 h period and transferred to a separatory funnel with 90 ml H_2O and 180 ml $CHCl_3$. The layers were separated and the aqueous layer extracted with 2×50 ml $CHCl_3$. The organic layers were combined, dried over Na_2SO_4, and the solvent was removed by film evaporation. A residue weighing 3.2 g was obtained. The crude product was chromatographed on SiO_2 (150 g)

with CH_2Cl_2—ethyl acetate (1:1) as eluent. Compound *2* (1.80 g) was obtained from eluate 525–1000 ml. Yield: 60 %. ^1H NMR (270 MHz) of the acetate derivative: δ 2.02 (3 H, s; CH_3–C), 2.07 (3 H, s; CH_3–C), 3.79 (3 H, s; CH_3–O), 3.86 (3 H, s; CH_3–O), 3.87 (3 H, s; CH_3–O), 4.23 (1 H, dd, J=4 and 12 Hz; Hγ), 4.42 (1 H, dd, J=6 and 12 Hz; Hγ), 4.70 (1 H, m; H$_\beta$), 6.02 (1 H, d, J=5 Hz; H$_\alpha$), ≈ 7 (6 H, m; aromatic protons).

erythro-*1-(4-Benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol* (6). Acid *5* (1.22 g) was dissolved in 12 ml tetrahydrofuran under magnetic stirring (nitrogen atmosphere). Borane dimethyl sulfide complex (1.5 ml) was injected over a 10 min period. The reaction mixture was kept at 35 °C (oil bath) for 3 h. Excess reagent was decomposed by the addition of 4 ml H_2O. First hydrogen peroxide (0.6 ml 30 % H_2O_2), and then 6 ml 3 M NaOH were added over a 15 min period. The reaction mixture was then stirred vigorously for 1 h (35 °C). The reaction mixture was transferred with water (30 ml) and chloroform (60 ml) to a separatory funnel. The layers were separated and the aqueous layer extracted with 2×20 ml $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and the solvents removed by film evaporation. A residual oil (1.25 g) was obtained. The product was chromatographed on SiO_2 (58 g) with CH_2Cl_2–ethyl acetate (9:1) as eluent. Compound *6* was found in eluate 500–700 ml (TLC). Removal of the solvents gave 0.69 g of an oil. Yield: 56 %. ^1H NMR (270 MHz) of the acetate derivative: δ 2.00 (3 H, s; CH_3–C), 2.06 (3 H, s; CH_3–C), 3.76 (3 H, s; CH_3–O), 3.86 (3 H, s; CH_3–O), 4.23 (1 H, dd, J = 4 and 12 Hz; Hγ), 4.41 (1 H, dd, J = 6 and 12 Hz; Hγ), 4.68 (1 H, m; H$_\beta$), 5.12 (2 H, s; CH_2), 6.02 (1 H, d, J = 5 Hz; H$_\alpha$), ≈7 (11 H, m; aromatic protons).

erythro-*1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol* (7). Compound *6* (0.28 g) was hydrogenated in 15 ml dioxane with 0.1 g 10 % Pd/C as a catalyst. When the calculated amount of hydrogen had been consumed, the catalyst was filtered off and the dioxane removed by film evaporation. A residue (0.20 g) was obtained which crystallized on inoculation with authentic *7* (m.p. 87–89 °C, lit.[18] 90–92 °C). ^1H NMR (270 MHz) of the acetate derivative (*cf.* Ref. 3): δ 2.02 (3 H, s; CH_3–C), 2.09 (3 H, s; CH_3–C), 2.30 (3 H, s; CH_3–C), 3.77 (3 H, s; CH_3–O), 3.81 (3 H, s; CH_3–O), 4.25 (1 H, dd, J = 4 and 12 Hz; Hγ), 4.46 (1 H, dd, J = 6 and 12 Hz; Hγ), 4.67 (1 H, m; H$_\beta$), 6.08 (1 H, d, J = 5.5 Hz; H$_\alpha$), ≈7 (6 H, m; aromatic protons).

1,3-Bis(4-benzyloxy-3-methoxyphenyl)-2-propene-1-one (8a). A solution of 20 g potassium hydroxide in 20 ml water was dropped into a stirred solution of the benzyl ethers of 4-hydroxy-3-methoxybenzaldehyde (2.42 g) and 1-(4-hydroxy-3-methoxyphenyl) ethanone (2.56 g) in 80 ml ethanol. After 2 h the yellow precipitate was filtered off and washed with ethanol and water. Recrystallization from chloroform–ethanol gave 4.2 g (87 %) crystals of m.p. 145–147 °C. ^1H NMR (60 MHz): δ 3.96 (3 H, s; CH_3O), 3.98 (3 H, s; CH_3O), 5.20 (2 H, s; CH_2), 5.25 (2 H, s; CH_2), 6.8–7.7 (18 H, m; aromatic and vinylic protons).

1,3-Bis(4-benzyloxy-3,5-dimethoxyphenyl)-2-propene-1-one (8b) was prepared from the benzyl ethers of 4-hydroxy-3,5-dimethoxybenzaldehyde and 1-(4-hydroxy-3,5-dimethoxy) ethanone analogously with the preparation of chalcone *8a*, m.p. 147–150 °C (from dioxane-ethanol).

1,3-Bis(4-benzyloxy-3-methoxyphenyl)-2,3-epoxy-1-propanone (9a). Chalcone *8a* (3.4 g, 7.1 mmol) was dissolved in 18 ml dichloromethane. To this a mixture of 3.5 ml hydrogen peroxide (35 % aqueous solution) and 11 ml 3 M sodium hydroxide was added while stirring at 4 °C. At a constant temperature of 4 °C, 0.72 g (2.1 mmol) of tetrabutylammonium hydrogen sulfate was added in small portions for 10 min. The rapid stirring was continued for 6 h, during which time the temperature of the mixture was allowed to rise to 16 °C. The organic phase was separated and washed with water containing a few crystals of ammonium chloride until the aqueous phase was neutral. The organic phase was dried ($MgSO_4$) and the solvent evaporated. Recrystallization from ethyl acetate–light petroleum yielded pale yellow crystals (2.8 g, 79 %) that melted at 144.5–146 °C. ^1H NMR (60 MHz): δ 3.87 (3 H, s; CH_3O), 3.92 (3 H, s; CH_3O), 4.16 (1 H, d, J = 2.0 Hz; –CH<), 4.20 (1 H, d, J = 2.0 Hz; –CH<), 5.13 (2 H, s; CH_2), 5.20 (2 H, s; CH_2), 6.8–7.5 (16 H, m; aromatic protons).

1,2-Bis(4-benzyloxy-3-methoxyphenyl)-1,3-propanediol (11a). Chalcone oxide *9a* (2.0 g) was dissolved in 200 ml dry ether and 17.2 g freshly distilled $BF_3{\cdot}(C_2H_5)_2O$ was gradually added to the solution. The mixture was refluxed for 30 min, washed with water and dried over Na_2SO_4. The residue was reduced with sodium borohydride (0.35 g) in 5 ml tetrahydrofurane. The mixture was stirred for 4 h and set aside for 48 h. It was then acidified with dilute hydrochloric acid and extracted with ether. The ether was dried (Na_2SO_4) and evaporated. The crystalline residue was recrystallized from ethyl acetate–light petroleum to yield 1.55 g (77.5 %) white crystals that melted at 105–109 °C (lit.[11] 106–108 °C). ^1H NMR (270 MHz) of the acetylated product: δ 1.92 (3 H, s; CH_3–C), 1.94 (3 H, s; CH_3–C), 3.32 (1 H, m; H$_\beta$), 3.82 (6 H, s; CH_3–O), 4.09 (1 H,

dd, J = 6.8 and 11.2 Hz; Hγ), 4.27 (1 H, dd, J = 6.4 and 11.2 Hz; Hγ), 5.12 (2 H, s; CH_2), 5.13 (2 H, s; CH_2) 6.02 (1 H, d, J = 7.3; H_α), ≈7 (16 H, m; aromatic protons).

erythro-*1,2-Bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (12).* The protecting benzyl groups were removed by catalytic hydrogenation in an ethanol solution using 5 % Pd/C as a catalyst. Filtration of the catalyst and evaporation of the solvent gave a quantitative yield, m.p. 151–154 °C (lit.[12] 158–159 °C). The ^{1}H NMR spectrum of the acetate derivative agreed with published ^{1}H NMR data[12] for this compound.

*1,3-Bis(4-benzyloxy-3,5-dimethoxyphenyl)-2,3-epoxy-1-propanone (*9b*).* Chalcone *8b* (1.2 g, 2.3 mmol) was dissolved in 6 ml dichloromethane. To this was added 1.2 ml hydrogen peroxide (35 % aqueous solution) and 3.5 ml 3 M sodium hydroxide while stirring at 4 °C. After the addition of 0.23 g tetrabutylammonium hydrogen sulfate and a reaction time of 5 h, the work-up was performed as for epoxide *9a*. A chromatographically homogeneous oil, 1.17 g, was obtained. ^{1}H NMR (60 MHz): δ 3.83 (12 H, s; CH_3O), 4.00 (1 H, d, J = 2.0 Hz, −CH<), 4.10 (1 H, d, J = 2.0 Hz; −CH<), 5.02 (2 H, s; CH_2O), 5.12 (2 H, s; CH_2O), 6.5–7.5 (14 H, m; aromatic protons).

erythro-*1,3-Bis(4-hydroxy-3,5-dimethoxyphenyl)-1,3-propanediol (*13*).* Crude chalcone oxide *9b* (1.25 g) in 110 ml dry diethyl ether was treated with boron trifluoride diethyl etherate (9.4 g) and refluxed for 30 min. Work-up, reduction with sodium borohydride and catalytic hydrogenation as in the previous synthesis gave 1.09 g crude compound *13*. Acetylation gave an acetate derivative which after purification melted at 165–167 °C (lit.[14] 169–170 °C). ^{1}H NMR (270 MHz) of the acetate derivative: δ 1.99 (3 H, s; CO CH_3), 2.01 (3 H, s; CO CH_3), 2.30 (3 H, s; CO CH_3), 2.31 (3 H, s; CO CH_3), 3.36 (1 H, m; H_β), 3.81 (3 H, s; OCH_3), 3.82 (3 H, s; OCH_3), 4.21 (1 H, dd, J = 6.5 and 11.4 Hz; Hγ), 4.43 (1 H, dd, J = 7.0 and 11.4 Hz; Hγ), 6.06 (1 H, d, J = 7.3 Hz; H_α), 6.34 (2 H, s; aromatic protons), 6.35 (2 H, s; aromatic protons). ^{13}C NMR: δ 20.4, 20.8, 21.0 (acetyl CH_3), 50.4 (C_β), 56.2 (CH_3O), 63.9 (Cγ), 75.2 (C_α), 103.7, 105.7 (C-2, C-6), 135.1, 136.2 (C-1), 151.8, 151.9 (C-3, C-5), 128.0, 128.4 (C-4), 168.5 (C=O, aromatic acetyl), 169.6 (C=O, secondary acetyl), 170.8 (C=O, primary acetyl).

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Ion Radical Cleavage Reactions. IV. The Effect of the Halogen in Determining the Mechanism of Cleavage of Halide Ion During the Reduction of 9-Cyano-10-haloanthracenes

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9-Cyano-10-bromoanthracene anion radical undergoes unimolecular cleavage in the expected manner in a number of aprotic solvents. The rate of cleavage was affected significantly by the nature of the solvent, increasing by nearly a factor of 15 in going from butyronitrile to acetonitrile. The activation enthalpies were not sensitive to the solvent (12.2±0.7 kcal/mol) and the activation entropies in three solvents were equal to −8.0±1.5 cal/K mol. In acetonitrile the activation entropy was close to zero which allowed the cleavage reaction to proceed much more rapidly in that solvent. Under the same conditions, unimolecular cleavage of chloride ion from 9-cyano-10-chloroanthracene anion radical was observed to be a minor reaction pathway. The major product of the reactions of the anion radical was 9,9′-bianthryl-10,10′-dicarbonitrile which forms by a higher order reaction. The activation parameters in this case cannot be related to the cleavage of carbon–halogen bonds.

In previous papers in this series[1–3] the unimolecular cleavage reactions of some haloaromatic anion radicals have been studied and the activation parameters and rate constants were reported. The activation energies for the cleavage of bromoanthracene[1] and bromobenzophenone[3] anion radicals were observed to be significantly lower than those for the corresponding chloro-substituted anion radicals and the rate constants, at 298 K, were of the order of 10^3 larger for the bromo-substituted anion radicals. We were therefore puzzled by the kinetic data reported by Heinze and Schwart[4] for the cleavage reactions of 9-cyano-10-haloanthracene anion radicals. They reported activation enthalpies of 3.4 kcal/mol for the chloro-substituted anion radical and 7.3 kcal/mol for the anion radical of 9-cyano-10-bromoanthracene anion radical. The corresponding activation entropies were observed to be −49 and −28 cal/K mol, respectively. This reversal in reactivity and activation energetics appeared to be completely inconsistent with the patterns that were beginning to emerge from the other related studies. We have re-investigated the cleavage reactions in order to attempt to resolve the inconsistencies. In this paper we report the results of kinetic studies on the reactions of both anion radicals and of a product study on the reduction of 9-cyano-10-chloroanthracene.

RESULTS AND DISCUSSION

The cleavage of bromide ion from 9-cyano-10-bromoanthracene anion radical. The cyclic voltammogram of 9-cyano-10-bromoanthracene in propionitrile recorded at 100 mV/s is shown in Fig. 1a. A reversible redox couple (R_2/O_2) was observed following the main reduction peak (R_1). The relative peak height, $I(R_1)/I(R_2)$ was observed to be equal to 1.44. The voltammogram for an equimolar solution of 9-cyano-10-bro-

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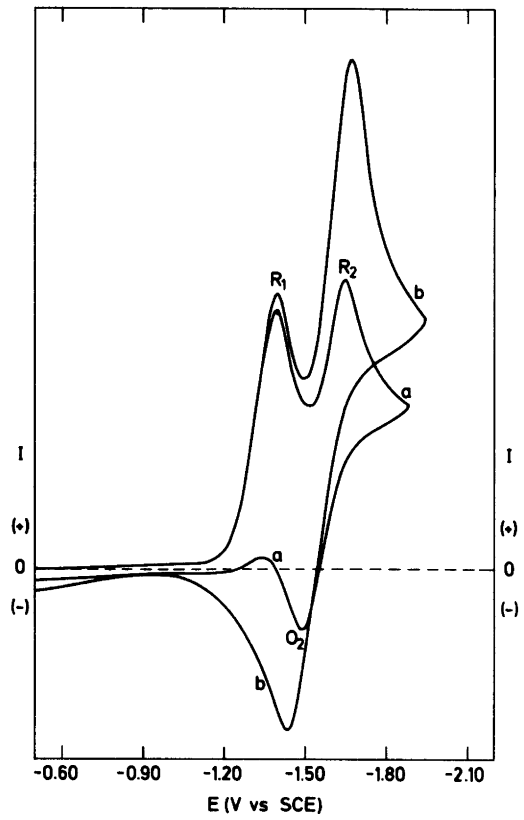


Fig. 1. Cyclic voltammograms of 9-cyano-10-bromoanthracene (2 mM) in propionitrile containing Bu_4NPF_6 (0.05 M). a, Before and b, after the addition of 9-cyanoanthracene (2 mM). Sweep-rate: 100 mV s^{-1}, t=22 °C.

moanthracene (2 mM) and 9-cyanoanthracene (2 mM) is shown in Fig. 1b. In this case the reversible couple (R_2/O_2) is enhanced relative to R_1 with $I(R_1)/I(R_2)$ equal to 0.71. These voltammograms can be accounted for by reactions (1)–(4) with (1)–(3) taking place at R_1 and (4) at R_2/O_2.

$$A-Br+e^- \rightleftharpoons (A-Br)^{\cdot -} \quad (A=9\text{-cyanoanthracenyl}) \qquad (1)$$

$$(A-Br)^{\cdot -} \xrightarrow{k_2} A^{\cdot}+Br^- \qquad (2)$$

$$A^{\cdot}+S-H \rightarrow A-H+S^{\cdot} \text{ (fast)} \qquad (3)$$

$$A-H+e^- \rightleftharpoons (A-H)^{\cdot -} \qquad (4)$$

An alternative to reaction (3) is that $A^{\cdot}$ is first reduced to A^- and then A–H is produced by proton transfer. We do not rule this possibility out nor do we specify the nature of S–H since no information is available regarding these steps. What the data do indicate is that under the time scale of the experiment, reaction (2) is complete and that the yield of 9-cyanoanthracene (A–H) appears to be quantitative under the reaction conditions. These observations justify relating observed rate constants for the decomposition of $(A-Br)^{\cdot -}$ to k_2. The uncertainty as to whether A–H forms in hydrogen atom transfer reaction (3) or by the alternative pathway involving A^- affects only the magnitude of k_2 evaluated.

The kinetics of the decomposition were studied by derivative cyclic voltammetry.[5] Rate constants were evaluated assuming mechanism (1)–(3) using theoretical data reported previously.[6] Kinetic and activation parameters are summarized in Table 1 for reactions carried out in a number of solvents. The data are further illustrated by the Arrhenius plot (Fig. 2) for the experiments carried out in butyronitrile. In this case, rate constant measurements were made at 5 temperatures ranging from 0–40.5 °C. The linear regression correlation coefficient was 0.999.

An unexpected feature of the data in Table 1 is that the rate of the cleavage reaction was observed to depend on the solvent. The value of k_2 (at 298 K) was about 15 times greater in acetonitrile than in butyronitrile as solvent and about 5.5 times greater in acetonitrile than in DMF. These results should be compared to those reported for the cleavage of 9,10-dichloroanthracene anion radical.[1] In that case very little difference could be detected in either rate con-

Table 1. Kinetic and activation parameters for the cleavage of bromide ion from 9-cyano-10-bromoanthracene anion radical.

Solvent	k_{298}/s^{-1}	$\Delta H^{\ddagger}_{298}$/kcal mol^{-1}	$\Delta S^{\ddagger}_{298}$/cal K^{-1} mol^{-1}
DMF[a]	327	12.0	−6.8
DMF[b]	306	11.0	−10.2
AN[a]	1725	13.0	−0.1
Propionitrile[a]	210	12.5	−7.8
Butyronitrile[b]	128	12.4	−7.3

[a] Substrate concentration equal to 1.0 mM. [b] Substrate concentration equal to 2.0 mM.

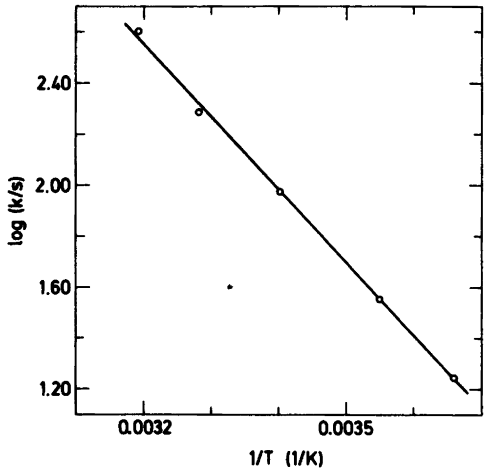


Fig. 2. Arrhenius plot for the cleavage of bromide ion from 9-cyano-10-bromoanthracene anion radical in butyronitrile.

stants or activation parameters in acetonitrile and DMF. A feature of further interest is that the enthalpies of activation, equal to 12.2±0.7 kcal/mol, appear to be independent of the solvent within experimental error. The errors in $\Delta H^{\neq}$ and $\Delta S^{\neq}$ in related cases[1] have been estimated to be of the order of 1 kcal/mol and 2 cal/K mol taking into account a ±5 % error in the rate constants. Thus, the variation in $\Delta H^{\neq}_{298}$ observed (Table 1) is within experimental error. The values of $\Delta S^{\neq}_{298}$ also appear to be the same, −8.0±1.5 cal/K mol, if the value obtained for the data in acetonitrile are not included. On the other hand, $\Delta S^{\neq}_{298}$ was observed to be near zero, −0.1 cal/K mol, in acetonitrile. This value is clearly not within experimental error of the others. Therefore, the only unusual feature of the data in Table 1 is that $\Delta S^{\neq}_{298}$ is considerably less negative in acetonitrile than expected based on the other data.

The entropy of activation for the cleavage reactions has previously been discussed[1-3] in terms of the differences in solvation of the anion radicals and the transition states leading to the cleavage of the carbon–halogen bond. The standard entropies of the halo-aromatic anion radicals are expected to be, in analogy to the entropies for the formation of hydrocarbon anion radicals,[7] significantly less negative than those for the formation of halide ions in aprotic solvents.[8] Assuming the latter to be the case, a $\Delta S^{\neq}$ near zero can be taken to indicate little solvation change in going from the anion radical to the transition state. This implies an early transition state in which little bond cleavage has taken place. If we apply these arguments to the data in Table 1 we arrive at the conclusion that the transition state for the cleavage of bromide ion from 9-cyano-10-bromoanthracene anion radical involves less bond breaking than those for the same reaction in the other solvents. There do not appear to be any trends in the physical properties of the solvents that would predict such solvation differences. On the contrary, DMF and acetonitrile are very similar in such properties as dielectric constant and ion solvating power. Furthermore, no such differences in activation entropies were observed in the related cleavages.[1,3] Thus, an explanation of the near zero $\Delta S^{\neq}$ when acetonitrile is solvent is not apparent.

It is interesting to compare the kinetic and activation parameters observed for the decomposition of 9-cyano-10-bromoanthracene anion radical with those for 9-bromo and 9,10-dibromoanthracene anion radicals. Data are now available for all three processes in DMF. For the cleavage of Br^- from structure *1* where X is either H, Br or CN, the rate constant at 298 K was observed to be 2.4×10^5, 3.5×10^4 and 3.2×10^2 s^{-1}, respectively. Correlation of log k_2 *vs.* Hammett σ_p results in ρ equal to −4.4 with a correlation coefficient of 0.998 based on this limited set of data. The activation energies for the cleavage of *1* (X=H and Br) were observed to be the same (3.4 kcal/mol) while the value observed in this study for X=CN was much greater (11.5 kcal/mol). The general trend appears to be that electron withdrawing groups stabilize the anion radicals. This trend is also found in substituted phenyl halides. The anion radicals of bromobenzene and chlorobenzene are very short lived[9] while those of 4-halobenzophenones[3,10] react at moderate rates and those derived from 4-halonitrobenzenes[2,11] react slowly. The very low reactivity of the latter can be rationalized in terms of the localization of charge on the nitro groups of the anion radicals which retards the rate of halide loss.[2] This same type of stabilization, *albeit* to a lesser extent, can be attributed to other electron withdrawing substituents.

The kinetics of the reactions of 9-cyano-10-

chloroanthracene anion radical. The most unusual feature of the kinetic data presented by Heinze and Schwart[4] is that relating to a smaller apparent activation energy for the cleavage of chloride ion than for the cleavage of bromide ion even though the chloro-substituted anion radical is much less reactive. This proposal is particularly perplexing when comparisons are made with the parameters for the cleavage of halide ions from 9-haloanthracene anion radicals. In that case the rate constants in DMF were observed to be 2.5×10^5 s^{-1} for the bromo-substituted anion radical and 117 s^{-1} for the loss of chloride from 9-chloroanthracene anion radical. The corresponding activation enthalpies were observed to be equal to 3.4 and 14.6 kcal/mol. The rate and activation parameters that we observed for the cleavage of bromide from *1* (X=Br) deviate from those reported but the deviations are not too serious in terms of the activation enthalpy, *i.e.* 11.5 kcal/mol in this study as compared to 7.3 kcal/mol reported previously.[4] On the basis of the relative activation enthalpies observed in the 9-haloanthracene anion radical series, a prediction of greater than 14.6 kcal/mol for $\Delta H^{\neq}$ for the cleavage of 9-cyano-10-chloroanthracene anion radical is required in order to be consistent. Thus, the observed value[4] of 3.4 kcal/mol is clearly inconsistent with the simple cleavage of chloride ion from the anion radical.

Activation energies were determined for the decomposition of 9-cyano-10-chloroanthracene anion radical in both acetonitrile and propionitrile (Table 2). The activation energies were determined by derivative cyclic voltammetry using relationship (5)[12] where $\upsilon_{0.85}$ refers to the voltage sweep rate necessary for the derivative peak ratio to equal 0.85. The high value of the

$$\ln \upsilon_{0.85}/T = E_a/RT + c \qquad (5)$$

Table 2. Activation energies for the reactions of 9-cyano-10-chloroanthracene anion radical.[a]

Solvent	$(\upsilon_{0.85})_{298}$[b]/V s^{-1}	E_a/kcal mol^{-1}
AN	1.55	5.4
Propionitrile	0.434	6.7

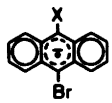
[a] In solvent containing Bu_4NBF_4 (0.1 M). [b] The voltage sweep rate necessary for the derivative peak ratio to equal 0.850. Obtained from the correlation line for measurements ranging from 17 to 60 °C.

Table 3. Reaction order analysis of the reaction of 9-cyano-10-chloroanthracene anion radical in acetonitrile.[a]

C_A[b]/mM	$\upsilon_{0.85}$/V s^{-1}	$\upsilon_{0.85}/C_A$	$\upsilon_{0.85}/C_A^{1.25}$
1.00	0.83	0.83	0.83
0.50	0.36	0.72	0.86
0.25	0.15	0.60	0.85

[a] In solvent containing Bu_4NBF_4 (0.1 M) at 22.1 °C.

derivative ratio was necessary because of the very low rate of decomposition of the anion radical. The E_a values in Table 2, 5.4 and 6.7 kcal/mol, are higher than those reported earlier[4] but confirm that the parameter is much lower for the reactions of the chloro-substituted anion radical than for *1* (X=Br).



A reason for the inconsistencies noted above is evident from the data in Table 3. According to the "reaction order approach",[13] the value of $\upsilon_{0.85}$ is predicted to be independent of the substrate concentration (C_A) for a first order cleavage of halide ion from the anion radical. The data indicate that the apparent rate constant, proportional to $\upsilon_{0.85}$, is strongly dependent upon C_A. The last column in Table 3 indicates that the sum of the reaction orders in substrate (A) and anion radical (B) is equal to 2.25. This means that the overall reaction order is greater than 2 under the conditions of the measurements. This suggests some sort of a dimer-forming mechanism rather than a simple halide cleavage reaction.

The products of the decomposition of 9-cyano-10-chloroanthracene anion radical. The products of the reactions of the anion radical were observed to be 9-cyanoanthracene and the dimer, 9,9′-bianthryl-10,10′-dicarbonitrile by preparative electrolysis in both acetonitrile and propionitrile. The dimer was identified by its voltammetric spectrum which consists of six reversible redox couples with oxidation states ranging from +2 to −4.[14] The voltammogram recorded in acetonitrile is illustrated in Fig. 3. The yields of isolated products were <10 and 65 %, for 9-cyanoanthracene and the dimer, respectively,

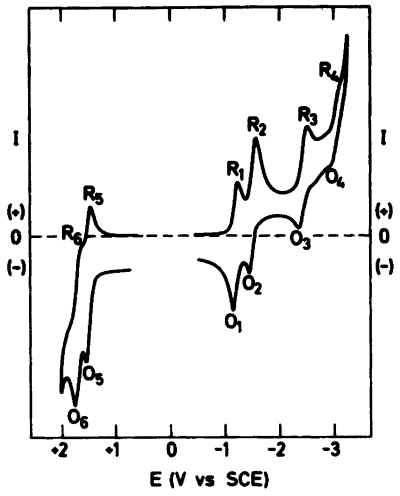


Fig. 3. Cyclic voltammogram of 9,9′-bianthryl-10,10′-dicarbonitrile in acetonitrile containing Bu_4NBF_4 (0.1 M). Sweep-rate: 400 mV s^{-1}, t=22 °C.

for the reaction carried out in acetonitrile.

Conclusions. In a number of aprotic solvents, the anion radical generated during reduction of 9-cyano-10-bromoanthracene undergoes unimolecular decomposition with the expulsion of bromide ion. With the exception of a smaller than expected entropy of activation in acetonitrile, the rate and activation parameters for the cleavage reaction are consistent with expectation based on other recent work.[1-3] Although some 9-cyanoanthracene is generated during preparative reduction of 9-cyano-10-chloroanthracene, the major product of the decomposition of the anion radical is 9,9′-bianthryl-10,10′-dicarbonitrile. In this case the kinetic studies indicate a higher order mechanism possibly involving a dimerization of the anion radical or the coupling between an anion radical and a neutral molecule. The mechanism of this reaction requires further study. In any event, the activation parameters observed in this case cannot be related to those for the cleavage of chloride ion from the anion radical.

EXPERIMENTAL

The instrumentation, electrodes, cells and data handling procedures as well as solvent and electrolyte purification were the same as recently described.[15] 9-Cyano-10-bromoanthracene[16] and 9-cyano-10-chloroanthracene[17] were prepared by standard procedures.

Preparative electrolysis of 9-cyano-10-chloroanthracene. 9-Cyano-10-chloroanthracene (1 mmol, 0.238 g) suspended in acetonitrile (50 ml) containing Bu_4NBF_4 (0.1 M) as supporting electrolyte was subjected to constant current reduction at 12.5 mA for 155 min (1.2 F/mol). The electrodes were platinum gauzes and the cell was divided by a horizontal sintered glass disk (G 3) and a 3 mm layer of neutral alumina at the anolyte side. During the electrolysis all the starting material went into solution and a new yellowish material precipitated out. The product was isolated by filtration followed by several washings with cold acetonitrile and drying. The isolated yield was 131 mg (65 %). The identity of the product was established through its voltammetric behavior as already described. GLC analysis (OV 17, 3 % 280 °C) of the filtrate demonstrated that only negligible amounts of starting material were left in solution. The amount of 9-cyanoanthracene formed during the electrolysis was estimated to be less than 10 %.

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Short Communications

The 2-Nitrophenylsulfenyl (Nps) Group for the Protection of Amino Functions of Cytidine, Adenosine, Guanosine and Their 2′-Deoxysugar Derivatives

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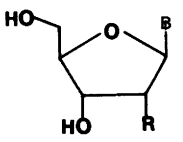
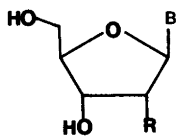
It is clear from the reports in the literature that the exocyclic amino groups of cytosine, adenine and guanine residues should be blocked for the chemical synthesis of DNA and RNA fragments.[1] It is a usual practice that such exocyclic amino groups are protected as amide derivatives[1,2]; the removal of these amides are time-consuming and is dependent upon the nature of the base residues.[1] Thus, the relative rates of the hydrolysis of a particular *N*-acyl group from the nucleoside base residues follow the order: cytosine>adenine>guanine. There are a few examples of carbamates[3–7] and one example each of (dimethylamino)methylene[8a] and amidine[8b] groups in the literature which have been employed for this purpose. Except for two examples,[6,7] all these blocking groups are removed under alkaline hydrolytic conditions. Recently we have reported on the 9-fluorenylmethoxycarbonyl (Fmoc) group[9] for the protection of amino functions of cytidine, adenosine, guanosine and their 2′-deoxysugar derivatives. The Fmoc groups are removable from these protected nucleosides by a tertiary, non-nucleophilic base like triethylamine as well as by alkaline hydrolytic conditions within 3 h at room temperature. Here we report on the preparation, properties and applications of 2-nitrophenylsulfenyl (Nps) as a new exocyclic amino protective group of cytosine, guanine and adenine residues in both the DNA and RNA series as shown in the derivatives, *2* to *7*, which have been prepared from common ribonucleosides and their 2′-deoxysugar derivatives of the general formula *1*. The application of the Nps group has its origin in the work of Goerdeler and Holst who synthesized the first Nps-amino acids.[10] Subsequently, several groups of workers[11] have employed the Nps-amino acids in the peptide synthesis. However, it was Fontana *et. al.*[12] and Tun-kyi[13] who first demonstrated that the Nps group could indeed be successfully cleaved from Nps-protected peptides using a thiolysis procedure. We reasoned that if the Nps group from Nps-protected nucleosides and nucleotides could be similarly removed under such mild conditions, one should be able to reduce the time of deprotection which is the most time-consuming part in the chemical synthesis of DNA fragments on solid support. It also occurred to us that such a method of removal of the Nps group from the Nps protected nucleic acid residues, prepared by the phosphite-triester approach,[14] should constitute an easy access to fully deprotected DNA fragments since the removal of the methyl groups from internucleotide phosphates and the Nps groups from the base residues might be accomplished in a single chemical operation. These considerations have led us to prepare the Nps derivatives of cytidine, adenosine and guanosine and their corresponding 2′-deoxyribose derivatives, as in *2* to *7*, from their respective parent nucleosides through a "one-





GENERAL FORMULA: (1)

R=H or OH	*2*; R=OH; B=(a)
B=1-Cytosinyl	*3*; R=OH; B=(b)
9-Adeninyl	*4*; R=OH; B=(c)
9-Guaninyl	*5*; R=H; B=(a)
	6; R=H; B=(b)
	7; R=H; B=(c)

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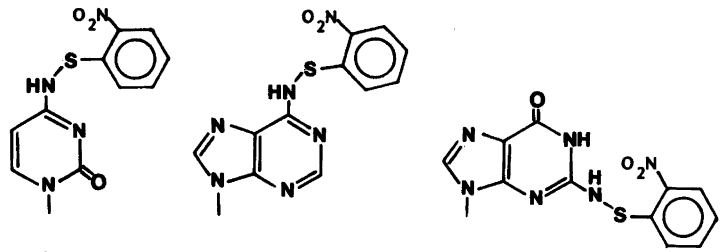
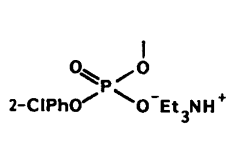
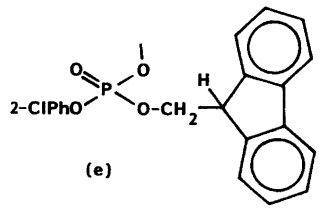
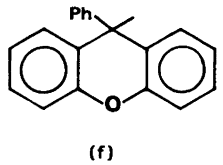
Table 1. The physical and the spectroscopic properties of Nps protected ribonucleosides and 2'-deoxyribonucleosides.

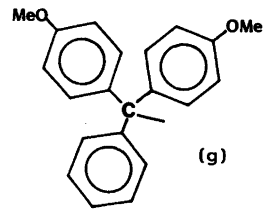
Compound	M.p.(°C) (crystallization media)	Yield %	R_f MeOH–$CHCl_3$(3:7v/v)	UV absorption properties in Methanol (nm) λ_{max}	^1H NMR absorptions in DMSO-d_6+D_{20} (δ scale)
2	156 (Toluene)	88	0.51	(pH 2):288 (pH 7):282 (pH 13)282(sh.),327	8.37–7.20(m,5H),6.16(d,7.2Hz,1H) 5.79(d,2.4Hz,1H);3.97(m,2H); 3.64(m,2H)
3	145 (EtOH–H_2O 1:1v/v)	84	0.63	(pH 2): 240(sh.),270; (pH 47):240(sh.),270; (pH 13):240(sh.),270	8.53(s,1H);8.32(s,1H);8.20–7.20(m,4H; 6.00(d,5.4Hz,1Hz);4.62(m,1H);4.13–366(m,4H)
4	190 (EtOH–H_2O 1:1v/v)	80	0.28	(pH 2):250,260,276(sh.) (pH 7):250,276(sh.) (pH 13):250(sh.),260(sh.)	8.39–7.44(m,5H);5.67(q,7.2Hz,1H); 4.44(m,1H);4.00–3.40(m,4H)
5	193 (EtOH–H_2O 1:4v/v)	82	0.56	(pH 2):284; (pH 7)282 (pH 13):284(sh.), 327	8.83–7.17(m,6H);6.16(t,1H); 4.32(m,1H);4.1–3.3(m,5H)
6	175 (EtOH–H_2O 1:1v/v)	90	0.65	(pH 2):242(sh.)270 (pH 7):242,272 (pH 13):242,270	8.55–7.40(m,6H);6.45(t,1H); 4.48(m,1H);3.93–3.40(m,5H)
7	216 (EtOH–H_2O 1:1v/v)	88	0.37	(pH 2):248,256,276(sh.) (pH 7):246,262(sh.),276(sh.) (pH 13):250(sh.),260(sh.),266(sh.)	8.73–7.20(m,5H);6.07(t,1H) 4.19(m,1H);3.83–3.19(m,5H)

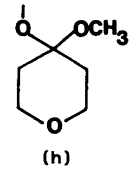












pot" synthesis in 88, 84, 80, 82, 90 and 88 % yields, respectively, as crystalline compounds. The general procedure for such a synthesis involves trimethylsilylation[15] of the nucleoside in dry pyridine solution which is followed by the addition of 2-nitrophenylsulfenyl chloride (Nps-Cl) (1.2 equiv., with respect to the nucleoside) and then hydrolysis. Table 1 records some physical and spectroscopic properties of compounds *2* to *7* in support of their structures. The Nps groups in *2* to *7* are completely stable under the following conditions which illustrates its compatibility with the commonly available protective groups that are employed in the phosphotriester[1] or the phosphitetriester[14] approaches in the chemical synthesis of DNA or RNA fragments: (1) stable for 12 h in the presence of tetrabutylammonium fluoride (10 equiv.) in dry tetrahydrofuran [$(F^-) \simeq 0.1$ *M*] at room temperature; thus it is compatible with t-butyldimethylsilyl-[16] and 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl-[17]; (2) stable for 24h in triethylamine (25 equiv.) in dry pyridine solution (10 ml/mmol); thus it is compatible with 2-phenylsulfonylethyl-[18], 2-phenylsulfonylethoxycarbonyl-[19], 2-(4-chlorophenyl)-sulfonylethoxycarbonyl-,[20] 9-fluorenylmethoxycarbonyl-[21] and 9-fluorenylmethyl-[22]; (3) stable in presence of 1*M* hydrazine hydrate in acetic acid: pyridine::3:4 (v/v) at room temperature for over 2 h; thus it is compatible with the levulinyl group[23]; (4) finally, the Nps group is also completely stable in presence of 5 % 4-toluenesulfonic acid. H_2O in 2 % ethanol-chloroform mixture for over 8 h. at room temperature, which makes it compatible with the acid-labile 5'-protective groups like 9-phenylxanthen-9-yl (pixyl)[24] and 4,4'-dimethoxytrityl (DMTr)[25] groups.

The Nps group may be smoothly cleaved, from *2* to *7* within an hour at room temperature, as shown in Table 2, using a dry pyridine solution (1 ml/mmol) of triethylammonium thiocresolate (3 equiv.) under an atmosphere of argon.

We then examined the effect of the Nps, as the 6-amino protective group of 2'-deoxyadenosine (*6*), on the cleavage of the glycosidic bond (depurination)[1] in view of the recent reports in the literature.[26] Thus, the half-lives of depurination reaction of 6-*N*-benzoyl-2'-deoxyadenosine,[26] 6-*N*-phthaloyl-2'-deoxyadenosine[26] and 6-*N*-Fmoc-2'-deoxyadenosine[9] are respectively 30, 120 and 180 min. in 80 % aqueous acetic acid at 30 °C. Under a similar acidic condition, the glycosidic bond on 6-*N*-Nps-2'-deoxyadenosine

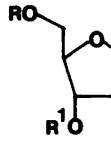
Table 2. Removal of the Nps group from Nps protected nucleoside derivatives, (2) to (7), using triethylammonium thiocresolate (3 equiv.) in dry pyridine (1 ml/mmol) under argon at 20 °C.

Substrates	$t_{\frac{1}{2}}$ (min)	t_{∞} (min)
2	—	10
3	6	60
4	—	20
5	—	10
6	6	60
7	—	20

(*6*) remained completely intact for 24 h. Thus it is clear that the employment of a building block like *6* should provide an acceptable solution to the problem of depurination of 2'-deoxyadenosine residues in the chemical synthesis of DNA segments.

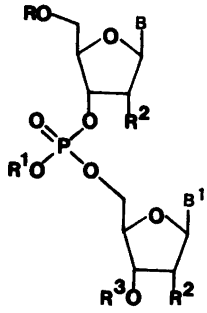
Finally the applicability of the Nps group in the chemical synthesis of DNA and RNA fragments have been demonstrated by the preparation of two dimeric RNA units: cytidylyl-(3'→5')-adenosine-3'-*O*-2-chlorophenylphosphate (*8*) and guanylyl-(3'→5')-cytidine-3'-*O*-2-chlorophenylphosphate (*9*) and a tetrameric DNA segment: $^{5'}$d(TpGpCpA)$^{3'}$ (*25*), starting with the corresponding Nps protected ribonucleosides and 2'-deoribonucleoside derivatives. The building blocks for the dimeric RNA units, *10* to *13*, have been prepared in high yields as powders using a procedure which has already been reported by us in the literature.[27] Two condensation reactions in dry pyridine solutions with the appropriate building blocks, *11*+*13* and *10*+*12* respectively in presence of an excess of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS-NT)[29], gave the fully protected derivatives *14* and *15* in 72 and 69 % yields respectively. For the synthesis of the fully protected tetrameric DNA segment, $^{5'}$d(TpGpCpA)$^{3'}$ (*24*), the building blocks *16* to *19* were also prepared in high overall yields using our literature procedure.[28] They were then coupled, in dry pyridine solutions using MS-NT, to give the fully protected dimers, *20* and *21* in 74 and 68 % yields respectively. The dimer blocks were then converted to 5'-hydroxy-(*22*) and 5'-protected-blocks (*23*) in the usual manner[28] in 80 and 91 % yields respectively. Finally the fully protected tetramer (24) was obtained, upon the condensation of *22* and *23* under an usual condition,[28] in 70 % yield as powder. The fully protected oligonucleotides, *14*, *15* and *24* were then subsequently deprotected with (1) 4-nitrobenzaldoximate ions[29]; (2) then with triethylammonium thiocresolate (3 equiv.) in dry pyridine (1 ml/mmol) under argon (compound *24* was then subjected to a treatment of aq. NH_3 (d 0.9) for 24 h. at 22 °C to remove the 3'-*O*-benzoyl group) and (3) with 80 % aq. acetic acid at 20 °C (incubation times for *14* and *15* were 6 h; and for *24*, it was 20 min). The reaction mixture was worked up using standard procedures[27,28] and purified by a DEAE sephadex A25 column ($Et_3NH^+HCO_3^-$ buffer; pH 7.4; linear gradient 0.001 *M* to 0.3 *M*).

The compounds 8, 9 and 25 were thus obtained in 60, 57 and 73 % yields, in terms of A_{260} o.d. units, respectively. They were completely



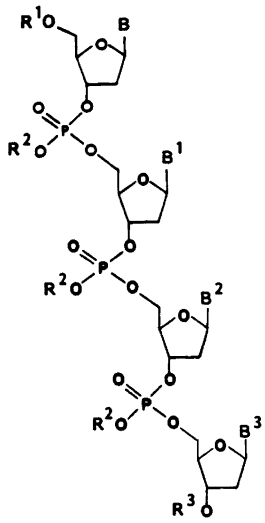
10; B=(c); R=(f); R^1=(d); R^2=(h);
11; B=(a); R=(f); R^1=(d); R^2=(h);
12; B=(a); R=H; R^1=(e); R^2=(h);
13; B=(b); R=H; R^1=(e); R^2=(h);

16; B=1-Thyminyl-; R=(g); R^1=(d); R^2=H;
17; B=(c); R=H; R^1=(e); R^2=H;
18; B=(a); R=(g); R^1=(d); R^2=H;
19; B=(b); R=H; R^1=Bz; R^2=H;



8; B=1-Cytosinyl-; B^1=9-Adeninyl-; R=R^1=H; R^2=OH; R^3=(d);
9; B=9-Guaninyl-; B^1=1-Cytosinyl-; R=R^1=H; R^2=OH; R^3=(d);
14; B=(a); B^1=(b); R=(f); R^1=2-ClPh; R^2=(h); R^3=(e);
15; B=(c); B^1=(a); R=(f); R^1=2-ClPh; R^2=(h); R^3=(e)

20; B=(a); B^1=(b); R=(g); R^1=2-ClPh; R^2=H, R^3= −OBz;
21; B=1-Thyminyl-; B^1=(c); R=(g); R^1=2-ClPh; R^2=H; R^3=(e);
22; B=(a); B^1=(b); R=H; R^1=2-ClPh; R^2=H; R^3= −OBz;
23; B=1-Thyminyl-; B^1=(c); R=H; R^1=2-ClPh; R^2=H; R^3=(d);



24; B=1-Thyminyl-; B^1=(c); B^2=(a); B^3=(b); R^1=(9); R^2=2-ClPh; R^3=Bz

25; B=1-Thyminyl-; B^1=9-Guaninyl-; B^2=1-Cytosinyl-; B^3=9-Adeninyl-; R^1=R^2=R^3=H;

digested to monomeric components by spleen phosphodiesterase and were subsequently quantitiated by HPLC to give the desired ratios of the monomeric components confirming the structure of the oligonucleotides. The chemical syntheses of these oligonucleotides have thus clearly established the stability of the Nps, as an exocyclic amino protective group both in DNA and RNA series, through the actual multistep chemical operations that are required in the synthesis nucleic acids using the phosphotriester approach. Further work is in progress in this laboratory to establish the application of the Nps group in the solid phase chemical synthesis of DNA and RNA fragments using the phosphite-triester approach.

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A Simple Mechanochemical Method for Studying Structure and Dynamics of Biopolymer Fibers in Various Media

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A great deal of interest has been devoted to the influence of various chemicals on the structure and helix-to-coil transition of DNA. Recent publications have shown that ethanol can induce a B-to-A transition in DNA.[1-3] Ethanol is also known to influence the helix-to-coil transition.[4]

In this communication a simple mechanochemical method is described which can be used for studying structural and dynamic transitions in biopolymer fibers immersed in various media. In this method the length, l, of a fiber bundle is measured as illustrated schematically in Fig. 1. It hangs down from a thin glass hook positioned at the upper index of the measuring cylinder scale. The fiber is held straight by a small V-shaped Pt weight which is also used as marker for reading off the position of the lower end of the bundle on the measuring cylinder scale (a simple device with two parallel hairs serves to reduce parallax).

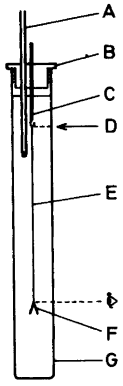

Fig. 1. The mechanochemical set-up. A, thermometer; B, Teflon-plug (cross-section); C, glass hook; D, upper index of the measuring cylinder scale; E, biopolymer fiber bundle; F, V-shaped Pt weight; G, 250 ml glass measuring cylinder with ethanol solution.

This set-up is very cheap and can easily be duplicated for simultaneous measurements.

The oriented biopolymer fibers are conveniently prepared with a wet spinning method.[5,6] The spinning of a nearly 20 m long DNA bundle laid out helically on a Teflon-coated cylinder has been described in detail previously.[7] A great number of "identical" fiber bundle samples, with length l_o typically 12–15 cm, can be taken from the cylinder[7] when immersed in 75 % ethanol (used as spinning bath for NaDNA) for successive or simultaneous measurements in the mechanochemical set-up.

The mechanochemical method will be illustrated with results from two different experiments on calf-thymus NaDNA fibers (Worthington) in ethanol solutions. In a first experiment the variation of relative length, l/l_o, with ethanol concentration was determined for bundles consisting of about 5000 DNA fibers. 10 mg Pt weights were used and the NaCl concentration was 0.01 M. As is seen in Fig. 2, the curve indicates a structural transition in the DNA fibers, occurring between 70 and 80 % concentration of ethanol. Since the A and B forms of DNA differ in axial translation per dinucleotide residue[8] (h_A=0.26 nm, h_B=0.34 nm) the decrease in fiber length can be taken as support for the ethanol-induced B-to-A transition suggested by various authors.[1-3] The clearest evidence comes from X-ray diffraction work.[2]

In a second type of experiment the temperature-induced helix-to-coil transition in DNA fibers in 75 % ethanol (0.15 M NaCl) was followed with the measuring cylinder immersed in a thermostated bath, the temperature of which was linearly increased with time (about 0.29 °C/min). Fig. 3 shows the relative change in length, $(l-l_o)/l_o$, of the DNA fiber bundle as function of ethanol temperature. The marked contraction at about 66.7 °C is taken as evidence for a helix-to-coil transition in the DNA fibers. After this contraction the fibers have lost most of their mechanical strength; cf. Ref. 7. This melting

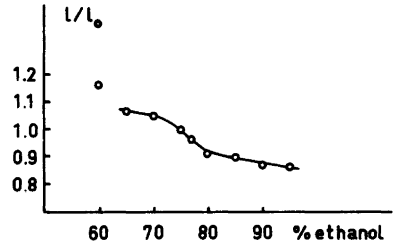

Fig. 2. Relative length, l/l_o, of NaDNA fibers in ethanol solutions containing 0.01 M NaCl.

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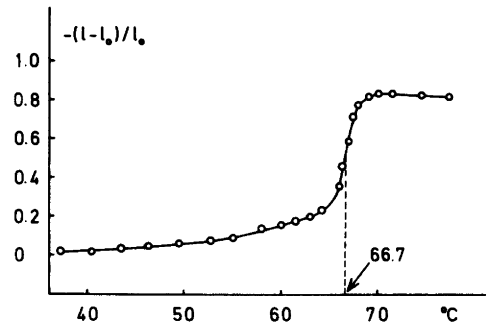

Fig. 3. Relative change in length , $(l-l_o)/l_o$, of NaDNA fibers in 75 % ethanol as function of temperature. Heating rate: 0.29 °C/min.

temperature in 75 % ethanol should be compared with a melting temperature of about 85 °C for DNA dissolved in 0.15 M NaCl(aq).[9]

The experiment illustrated in Fig. 3 was repeated with various concentrations of NaCl (0.00005–0.4 M NaCl) in the 75 % ethanol, but there was practically no salt effect on the melting temperature in this range. This differs considerably from measurements on DNA dissolved in water where a marked salt effect on the melting temperature is observed.[9]

Work is in progress to apply this mechanochemical method to DNA fibers with various counterions (Li^+, Na^+, K^+ and Cs^+), and a full report will be given later.

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The Nature of the Hydroxyl Group π-Electron Interaction. Repulsive Instead of Attractive?

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The ^{1}H NMR spectrum of 9-fluorenol at high dilution indicates that the molecule exists in one conformer only, with the OH proton pointing in between the two aryl rings. The IR spectrum shows no intramolecular hydrogen bond. The interaction is proposed to be due to the repulsion of the hydroxyl oxygen lone-pair electrons and the π-electrons of the aromatic rings. Earlier data on benzyl alcohols and open chain olefinic alcohols are reinterpreted on the basis of this model and it is not necessary to invoke intramolecular hydrogen bonding except in the case of 3-buten-1-ol.

Alcohols containing π-electron systems *e.g.* benzyl alcohol and allyl alcohol have been proposed to have weak, intramolecular hydrogen bonds between the OH proton and the π-electrons.[1–3] The proposals have mainly been based on IR spectroscopy in the OH stretching region and also on microwave spectroscopy[4,5] and electron diffraction.[6]

During our NMR study of benzyl alcohol and 1,2-diarylethanols we found no evidence of intramolecular hydrogen bonding to the π-electron system, despite the existence of OH bands in the IR spectra indicative of such bonds.[7,8] This has led us to reconsider the criteria of weak intramolecular hydrogen bonds in such systems. In the following, we will show that some of the earlier results are not consistent with the existence of such bonds, and we will also propose a new model for the intramolecular interactions.

During our study of 1,2-diarylethanols, only compounds substituted with hydrogen bond acceptors in the *ortho*-positions on both rings [*e.g.* 1,2-di(*o*-methoxyphenyl)ethanol] were found to have an appreciable proportion of an intramolecular hydrogen bonded conformer. The hydrogen bond was manifested both in IR (a band at 3560 cm^{-1}) and the NMR spectra (20 % of a conformer with the hydroxyl proton pointing in between the two *ortho*-groups).[8] From this it appeared to us that the possibility of an internal hydrogen bond to the π-electron system would increase if there were two aryl rings to accept the hydrogen bond. The obvious compound to investigate for this effect was 9-fluorenol. Indeed, its proton NMR spectrum at high dilution in CCl_4 showed a $^3J_{CHOH}$ of 10.75 Hz, indicating the molecule to be locked in one conformation with the hydroxyl proton pointing in between the two aryl rings (Fig. 1). This was, of course, highly indicative of a strong intramolecular hydrogen bond. We also observed the CHOH coupling at different temperatures and this gave information on the rotamer composition around the O–H bond. From these data, the equilibrium constants and thermodynamic parameters were obtained, $\Delta H° = -3.2$ kcal mol^{-1}, $\Delta S° = -3.5$ cal mol^{-1} K^{-1}.

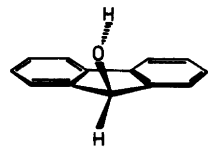

Fig. 1. Predominant conformer (from NMR) of 9-fluorenol.

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Table 1. Hydroxyl stretching absorbtion of alcohols.

Compound	$\nu_{max}(cm^{-1})$	$\Delta\nu_{1/2}(cm^{-1})$	Ref.
Methanol	3643.8	22.2	12
Ethanol	3637.3, 3627[a]	18.2, 19	12
2-Propanol	3627.1, 3617 (sh)	13.8, (2.6)	12
t-Butyl alcohol	3616.9	15.2	12
Benzyl alcohol	3636.3 (sh), 3617.1	(24.0), 17.0	12
1,2-Diphenylethanol	3620	26	18
1,2-Di(*o*-nitrophenyl)ethanol	3615, 3540	25, 49.5	18
1,2-Di-(*o*-methoxyphenyl)ethanol	3615, 3560	25, 80	18
9-Fluorenol	3602	16	This work
Diphenylmethanol	3633 (sh), 3615	26	This work
Allyl alcohol	3634.7 (sh), 3619.4	(23.4), 16.4	15
3-Buten-1-ol	3635.2, 3596.1	24.0, 32.3	15
4-Penten-1-ol	3638.3, 3624.5 (sh)	20.8, (24.0)	15
5-Hexen-1-ol	3639.2, 3626.0 (sh)	20.8, (17.2)	15

[a] Overlapping bands.

These data may have errors due to the uncertainties in J_t and J_g, but they certainly indicate a strong hydrogen bond to be present. However, the chemical shift of the hydroxyl proton (δ_∞=1.505 ppm) was not very different from those of alcohols without such strong bonds (*e.g.* benzyl alcohol, 1.09 ppm, diphenylmethanol, 1.62 ppm) but well below the ones we have found to have intramolecular hydrogen bonds (*e.g.* 1,2-di(*o*-methoxyphenyl)ethanol, δ=2.31 ppm).[8] The downfield shift of hydroxyl protons on hydrogen bond formation is well documented.[9]

Further, the IR spectrum at high dilution in CCl_4 did not give any indications of such a bond (ν_{max}=3602 cm^{-1}, $\Delta\nu_{1/2}$=16 cm^{-1}, Table 1). Two phenomena are manifested in the IR spectrum of the OH stretch region if a hydrogen bond is formed: a shift of the band to lower frequency and an increase in the intensity accompanied by an increase in the $\Delta\nu_{\frac{1}{2}}$ of the band. The magnitude of these changes is connected to the strength of the hydrogen bond, although no simple relationship exists.[10] In Table 1, the OH stretch frequency together with $\Delta\nu_{1/2}$ are given for a selection of alcohols. From that, it is clear that the frequency and particularly $\Delta\nu_{1/2}$ for 9-fluorenol are not very different from those of alcohols without internal hydrogen bonds, *e.g.* 1,2-diphenylethanol and diphenylmethanol. From its NMR spectrum (see experimental) diphenylmethanol has its hydroxyl proton pointing away from one of the phenyl rings and towards the edge of the other without possibility for an internal hydrogen bond (Fig. 2). On the other hand, for 1,2-di(*o*-methoxyphenyl)ethanol a band at 3560 cm^{-1}, $\Delta\nu_{1/2}$=70 cm^{-1} was assigned to the internal hydrogen bonded conformer.[8] These IR data indicate a stronger hydrogen bond than the one in 9-fluorenol. Nevertheless, in 1,2-di(*o*-methoxyphenyl)ethanol only 32 % of the rotamers around the OH bond participated in a hydrogen bond.[8] In 9-fluorenol on the other hand, nearly 100 % was in a position where hydrogen bonding was possible. Further, the thermodynamic data for 9-fluorenol corresponds to $\Delta G°$(298 K)=−2.2 kcal mol^{-1}. For intermolecular hydrogen bonds $\Delta G°$(298 K)=−2.7 kcal mol^{-1} corresponded to a shift of *ca.* 300 cm^{-1} of the OH stretching frequency,[19] much more than the shift observed *e.g.* in going from diphenylmethanol (3615 cm^{-1} without an internal hydrogen bond) to 9-fluorenol (3602 cm^{-1}).

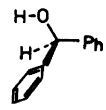

Fig. 2. Predominant rotamer around the C−OH bond (from NMR) of diphenylmethanol.

If the reason for this conformational homogeneity were indeed an intramolecular hydrogen bond, it must have been a stronger one than in 1,2-di(*o*-methoxyphenyl)ethanol. It does

not appear probable that such a bond should not be manifested in the IR and NMR spectra in a more obvious way than is evident in those of 9-fluorenol.

In our opinion, only two explanations of this paradox are possible, either that some hydrogen bonds do not result in the normal changes in the IR and NMR spectra or that the reason for the conformational homogeneity of 9-fluorenol is not an internal hydrogen bond. The first of these would be against all known evidence.[1,2,10] We therefore prefer the second explanation and propose the reason to be a *repulsion* between the π-electrons of the aryl rings and the lone-pair electrons of the hydroxyl oxygen. These are positioned approximately opposite to the hydroxyl proton. To minimize the repulsion the hydroxyl proton would be placed in between the two aryl rings. The effect of repulsion between the oxygen lone-pair electrons and double bond π-electrons has been observed for 3-methoxy-1-methylenecyclohexanes.[11] This explanation also applies to other benzyl alcohol systems and to olefinic alcohols. We will treat each of these separately.

Benzyl alcohols. The presence of two OH bands in the IR spectra of aliphatic alcohols has been convincingly explained as due to several conformers in the solution.[12,13] In molecules containing double bond systems, such as benzyl alcohols, the low frequency band is *ca.* 10 cm^{-1} below the corresponding band in the purely aliphatic alcohols. This has led several authors to propose the existence of weak intramolecular hydrogen bonds, the low frequency band resulting from the bonded conformer.[1,2,3]

Ōki has however explained the low frequency band as due to an interaction between the hydroxyl group and the π-electrons of the aromatic nucleus. The nature of the interaction was not discussed in any detail.[14] The IR spectra of substituted benzyl alcohols showed two bands, at *ca.* 3655 and 3615 cm^{-1}. The intensity of these bands was dependent on the substituent. With electron attracting substituents, *e.g.* *p*-nitro, the high frequency one was the most intense, but with electron releasing ones *e.g.* *p*-methoxy, the opposite was the case. The relationship was successfully correlated with the Hammett σ values.[14] This effect could be ascribed to the existence of a conformer with an internal hydrogen bond. With increasing electron density in the π-electron system, an increase in the population of the bonded conformer (the one with the low frequency absorption) would take place, as observed. However, if this were the case, the increase in the electron density would lead to a stronger hydrogen bond. This would not only give a higher population of the hydrogen bonded species, but also a shift to lower frequency in the OH absorption. This was, however, not observed. The bands of *p*-nitrobenzyl alcohol are at the same frequencies as those of *p*-methoxybenzyl alcohol (and of the other ones studied).

We see now that this apparent paradox is resolved if the interaction between the hydroxyl group and the π-electrons is a repulsion between the lone-pair electrons of the hydroxyl oxygen and the π-electrons. In *p*-nitrobenzyl alcohol, with low electron density in the π-electron system, the repulsion is low and we have an appreciable population of the conformer with the oxygen lone-pair electrons pointing towards the ring and the hydroxyl proton pointing away from it (the so-called free conformer). In *p*-methoxybenzyl alcohol on the other hand, with its relatively high electron density, the repulsion is increased and the conformer with the oxygen lone-pair electrons pointing away from the ring and the hydroxyl proton pointing towards it (the so-called hydrogen bonded conformer) becomes the most populated. One would not expect the frequencies of the OH bands of the two conformers to be greatly influenced by the electron density of the π-electron system as the OH bond is not directly influenced by the repulsion process (as it would have been by the formation of a hydrogen bond). On the other hand the observed correlation of the intensities with the Hammett σ values is in accordance with our hypothesis. Our own results from the NMR study of benzyl alcohols also support this, as we found the most abundant conformer to have the hydroxyl proton pointing towards the aromatic ring, but not in that orientation which would be thought to be the best for hydrogen bonding.[7] The same was the case for 1,2-diarylethanols, the hydroxyl proton pointed towards the 1-aryl ring and the oxygen lone-pair electrons away from it.[8]

Olefinic alcohols. The IR spectra of alcohols of this type have been studied by several authors.[3,15] Except for 3-buten-1-ol, the open chain ones show absorption above 3600 cm^{-1} only, and with $\Delta\nu_{1/2}$ from 15–25 cm^{-1} (Table 1). The spectra

usually have more than one band in the OH region, and this has again led to the suggestion of an internal hydrogen bond. Allyl alcohol has also been investigated by microwave[4] and NMR spectroscopy,[16] and 3-buten-1-ol by microwave spectroscopy[5] and electron diffraction.[6] In both cases, the authors agree that the molecules have a major conformer with the hydroxyl proton pointing towards the double bond. This has again been interpreted as a stabilisation of this conformer by an internal hydrogen bond.

Allyl alcohol has bands at 3634 (sh) and 3619 ($\Delta\nu_{1/2}$=23 cm^{-1}). From comparison with the other alcohols in Table 1, these bands cannot be taken as indicative of a hydrogen bond strong enough to make the observed conformer the major one. In analogy with the benzyl alcohols, we again propose that the reason for this conformational homogeneity is the repulsion between the lone-pair electrons on the hydroxyl oxygen and the π-electrons of the double bond.

For 3-buten-1-ol, this cannot be the explanation. Repulsive forces would of course tend to move the lone-pair electrons of the oxygen as far away from the double bond as possible, probably by extending the molecule instead of folding it. The IR spectrum of 3-buten-1-ol is, however, different from that of allyl alcohol (and the other open chain olefinic alcohols) in that it contains a band at significantly lower frequency and with greater bandwidth than observed for the other alcohols, 3596 cm^{-1}, $\Delta\nu_{1/2}$=32 cm^{-1}.[15] We interpret this to mean that in 3-buten-1-ol the internal hydrogen bond is strong enough to counteract the repulsion between the lone-pair electrons and the π-electrons. It is well known that the strength of a hydrogen bond is dependent on the O–H--- acceptor angle, 180° being close to the optimal in most cases.[17] With the π-electron system as acceptor, the precise point of acceptance is hard to define. It must, however, be more difficult to obtain an angle close to 180° in systems like allyl alcohol and benzyl alcohol than in the more flexible molecule 3-buten-1-ol. For 4-penten-1-ol and 5-hexen-1-ol, the IR spectra again show only narrow bands at 3638 and 3639 cm^{-1}, respectively.[15] For these alcohols, the energy loss in the formation of an internal hydrogen bond is larger than that gained and no such bond is formed.

Conclusion. The predominance of one conformer in benzyl alcohols and in allyl alcohol and the predominance of one particular band in the OH stretching region is not caused by the attractive forces of an intramolecular hydrogen bond. Instead, we propose the reason to be a repulsion between the π-electrons and the lone-pair electrons of the hydroxyl oxygen.

Table 2. Observed $^3J_{CHOH}$ and δ_{OH} of 9-fluorenol (5 mM) in CCl_4 at different temperatures.

Temperature (°C)	$^3J_{CHOH}$ (Hz)	δ_{OH}	$\Delta\nu_{1/2}$ (Hz)
-20.2	11.24	1.645	1.84
-8.9	11.23	1.584	1.03
2.6	10.99	1.548	0.98
26.0	10.75	1.505	0.78
36.4	10.74	1.498	0.90
48.6	10.50	1.487	0.97
59.2	10.50	1.477	0.98
66.2	10.50	1.472	1.12

EXPERIMENTAL

9-Fluorenol and diphenylmethanol were purchased from Aldrich-Europe and Koch-Light Laboratories, respectively. The NMR and IR spectra were run in CCl_4 dried over molecular sieves (3A, activated at 400 °C for 4 h). A capillary with $^2H_6$-benzene supplied the lock signal, and a sphere of molecular sieves (3A) in the sample removed any OH-exchange. The NMR spectra were run on a Bruker 400 MHz or a JEOL FX 100 MHz. The IR spectra were run in CCl_4 in a 1 cm Vitreosil cell on a Beckman IR 4250.

9-Fluorenol (5 mM) had δ_{OH_x}(26 °C)=1.505 ppm, $^3J_{CHOH}$=10.75 Hz, corresponding to 96 % of the conformer with hydroxyl proton *trans* to the C_9 proton ($^3J_{CHOH\text{-}trans}$ assumed to be 11.24 Hz and $^3J_{CHOH\text{-}gauche}$ 2.2 Hz[6]). Diphenylmethanol (4 mM) had δ_{OH_x}=1.619 ppm, $^3J_{CHOH}$=3.4 Hz corresponding to 14 % of the conformer with the hydroxyl proton *trans* and 86 % of the one with it *gauche* to the proton on the hydroxyl bearing carbon. 9-Fluorenol (5 mM) had IR ν_{max}=3602 cm^{-1}, $\Delta\nu_{1/2}$=16 cm^{-1}, diphenylmethanol (4 mM) had IR ν_{max}= 3633 cm^{-1} (sh.), 3618 cm^{-1}, $\Delta\nu_{1/2}$= 26 cm^{-1}.

The variable temperature experiments were run in CCl_4 with a sphere of molecular sieves (3A) and a capillary with $^2H_4$-methanol for lock signal. This also served as a temperature monitor as introductory experiments had shown the

methanol in the capillary to have the same shifts between the OH and CH_3 signals as that observed in a normal methanol sample. The aromatic protons were decoupled during the experiments. The observed $^3J_{CHOH}$ splittings from the CH and OH bands were within 0.01 Hz of each other. The percentage of the two rotamers was calculated from the $^3J_{CHOH}$ (see above), X=percentage of *trans* rotamer. The equilibrium constants, K, were calculated after taking into account that the *gauche* rotamer has a statistical weight of 2: $K=X/\frac{1}{2}(1-X)$. The data from t=2.6 °C to 66.2 °C was used in a van't Hoff plot to obtain $\Delta H°=-3.2\pm0.4$ kcal mol^{-1}, $\Delta S°=-3.5\pm1.4$ cal mol^{-1} K^{-1}, correlation coefficient $r^2=0.955$ for the plot.

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The Kinetics and Thermodynamics of Transient Equilibria of Reactive Intermediates. I. Theoretical Relationships for the Evaluation of Rate and Equilibrium Constants

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The double potential step chronoamperometry (DPSC) current ratio (R_I)-time(τ) curve due to the generation of an intermediate (B) which participates in a rapidly established equilibrium (i) is

$$2\,B \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} B_2 \qquad \text{(i)}$$

parabolic. The values of the current ratio at the minima, $(R_I)_{min}$, are a direct measure of the equilibrium constants (K_i) for reaction (i). The forward rate constants (k_i) are related to both $(R_I)_{min}$ and τ_{min}, the pulse widths at the minima. Results of theoretical calculations for reaction (i) are presented in the form of polynomial equations. The method of analysis is applicable for K_i ranging from about 10^2 to 10^6 M^{-1} and for k_i ranging from about 5×10^1 to 5×10^7 $M^{-1}s^{-1}$. The theoretical relationships were tested on the known reaction (ii) in DMF with 9-cyanoanthracene (ANCN) concentrations ranging from 0.25 to 2.00 mM. At 288 K,

$$2\,ANCN^{\cdot-} \underset{k_{-ii}}{\overset{k_{ii}}{\rightleftharpoons}} (ANCN)_2^{2-} \qquad \text{(ii)}$$

K_{ii} was found to be $3.83(\pm0.22)\times10^4$ M^{-1} and k_{ii} was equal to $1.51(\pm0.06)\times10^5$ $M^{-1}s^{-1}$.

A number of reactions of reactive intermediates (B) generated in charge transfer reactions (1) has been observed to follow mechanisms in which the primary step involves either reaction with substrate in a comproportionation equilibrium (2), complexing

$$A + ne^- \rightleftharpoons B \qquad (1)$$

$$B + A \rightleftharpoons 2C \qquad (2)$$

$$B + A \rightleftharpoons B(A) \qquad (3)$$

$$2B \rightleftharpoons B_2 \qquad (4)$$

reversibly with substrate (3) or a reversible monomer-dimer equilibrium (4).

Pertinent examples of reaction (2) are the $2e^-$ reduction of lucigenin dication to the neutral molecule which undergoes electron transfer with the dication to generate cation radical,[1,2] and the reduction of bianthrone to the dianion which reacts with substrate to produce the anion radical.[3,4] In these reactions, equilibrium (2) is a side reaction which must be taken into account in the study of the irreversible reactions of the primary intermediate (B). Equilibrium (3) has been established to take place upon reduction of SO_2 to the anion radical in aprotic solvents.[5–9] Anthracenes substituted with electron withdrawing groups in the 9 position form anion radicals which participate in equilibrium (4) and the dimer dianions, B_2, are stable in aprotic solvents.[10,11]

The equilibria and associated kinetics have been studied by double potential step chronoamperometry,[1,12] derivative cyclic voltammetry,[4,11] spectroelectrochemistry,[2,3] polarography[5–8] and e.s.r. spectroscopy.[5,6] It has recently been pointed out[9] that the slower techniques such as polarography are not suitable

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for the study of the primary steps in the equilibria since the reactions are generally very rapid. The most suitable transient techniques for the study of the equilibria are double potential step chronoamperometry (DPSC), derivative cyclic voltammetry (DCV) and spectroelectrochemistry. DPSC and spectroelectrochemistry are most readily treated theoretically since much less computer time is necessary to simulate step experiments than is required in the sweep methods such as DCV. In this paper we will only consider DPSC as the measurement technique and we will be concerned with the reversible dimerization equilibria (4).

The DPSC response for equilibria (4) was first treated by Bezilla and Maloy.[13] They were concerned with cases in which the dimer, B_2, undergoes further irreversible reaction to products. Digital simulation was used in a similar theoretical treatment of the DPSC response for reaction (2) by Ahlberg, Hammerich and Parker.[1] Amatore, Pinson and Savéant[12] have presented calculations for the DPSC response due to equilibrium (4) in the absence of further reactions.

The pertinent experimental observation which indicates that reaction (4) is reversible is that the DPSC or DCV current ratio decreases with the increasing time-gate of the experiment as expected for an irreversible reaction but goes through a minimum and then increases at longer times. The behaviour was reported during the reduction of 9-cyanoanthracene in DMF using DCV analysis by Hammerich and Parker.[11] Amatore, Pinson and Savéant[12] studied the same reaction by DPSC and observed similar behaviour. In the latter study, both k_5 and K_5 were evaluated by fitting

$$2\ \mathrm{ANCN}^{\cdot-} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} (\mathrm{ANCN})_2^{2-} \qquad (5)$$

experimental DPSC data to theoretical working curves.

It was the purpose of this study to examine theoretical data for the DPSC response to equilibria (4) to attempt to find general relationships for the evaluation of rate and equilibrium constants which avoid the necessity of calculating theoretical working curves for each case observed experimentally. In particular, it was hoped that the kinetic and thermodynamic parameters could be related to magnitudes and position (on the time scale) of the current ratio minima. Relationships which allow for the quantitative determinations of the parameters were found and are reported here.

RESULTS AND DISCUSSION

Theoretical DPSC data. Double potential step chronoamperometry involves stepping the electrode potential from a rest value where no faradaic processes occur to a value where the charge transfer of interest takes place at a diffusion controlled rate.[14] In practice it is convenient to set the potential limits by placing the cyclic voltammetry couple for the forward and back processes in the center of the potential interval. In order to ensure that the charge transfers are diffusion controlled, the limits are usually >200 mV from the respective cyclic voltammetry peaks. The quantity observed is the normalized current ratio R_I which is the current measured at 2τ divided by that at τ where τ is the pulse width of the experiment. Normalization of R_I is achieved by dividing by $1-2^{-1/2}$ which is the theoretical value for Nernstian charge transfer without kinetic complications.

The digital simulation method[15] used for theoretical calculations involved dividing the

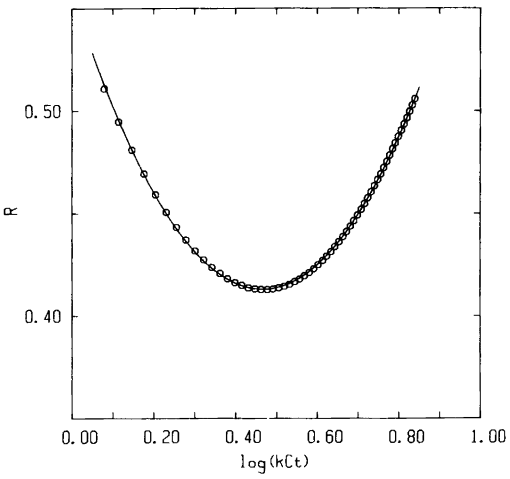

Fig. 1. Theoretical double potential step chronoamperometry data for reversible dimerization reactions. For this curve log $K_1C_A=1.50$ and log $k_1C_A=-1.00$.

diffusion layer into J space elements of total thickness limited to 6 $(Dt)^{1/2}$ where D is the model diffusion coefficient (0.45 in this case) and t is the number of time elements and is related to the pulse width. The input for the calculations consisted of τ, expressed as number of time elements, k_4 and k_{-4}. The kinetics were taken into account using finite difference forms of rate equations (6) and (7). For example if $\tau=100$, the

$$-d[B]/dt=2(k_4[B]^2-k_{-4}[B_2]) \quad (6)$$

$$d[B_2]/dt=k_4[B]^2-k_{-4}[B_2] \quad (7)$$

concentrations of A, B and B_2 in the diffusion layer were calculated for each of the 100 time iterations for the forward and the backward steps and R_I was printed. The simulations assume a dimensionless concentration of 1.

Relationships between DPSC parameters and kinetic and thermodynamic constants for the reversible dimerization equilibria. Theoretical working curve data were calculated corresponding to $\log(K_4C_A)$ ranging from 0.000 to 3.000 with $\log(k_4C_A)$ ranging from -1.000 to -3.500. An example of one of the working curves is shown in Fig. 1. The circles are the theoretical data and the solid line is for the best fit to the second order polynomial expression (8). The fit of the data to eqn. (8) is quite good but not exact.

$$R_I=a+b\log(k_4C_A\tau)+c\log(k_4C_A\tau)^2 \quad (8)$$

A much more precise fit is obtained by limiting the R_I values to those close to the minimum. Under the latter conditions, differentiating expression (8) and setting the first derivative equal to zero allows one to calculate both the magnitude of R_I at the minimum, $(R_I)_{min}$, and the value of $\log(k_4C_A\tau_{min})$. The analysis results in $(R_I)_{min}=0.4068$ and $\log(k_4C_A\tau_{min})=0.452$ for the working curve in Fig. 1.

Values of $(R_I)_{min}$ which resulted from the analysis of 25 working curves are summarized in Table 1. The columns marked b are the differences in values of $(R_I)_{min}$ between successive increments of $\log(k_4C_A)$ at a particular value of $\log(K_4C_A)$. What the data reveal is that for each $\log(K_4C_A)$, $(R_I)_{min}$ tend to converge to a single value at small $\log(k_4C_A)$. Furthermore, the convergence takes place in a predictable manner. Each successive b value is approximately 1/3 of the previous one in the horizontal rows. The most extensive calculations were for $\log(K_4C_A)$ equal to 1.000. In this case the successive values of b were; 0.0086, 0.0030, 0.0010 and 0.0003.

The data in Table 2 are values of $k_4C_A\tau_{min}$ obtained from the same calculations. The same trends are observed in this case with $k_1C_A\tau_{min}$ converging to a single value for each $\log(K_4C_A)$ as $\log(k_4C_A)$ was made smaller.

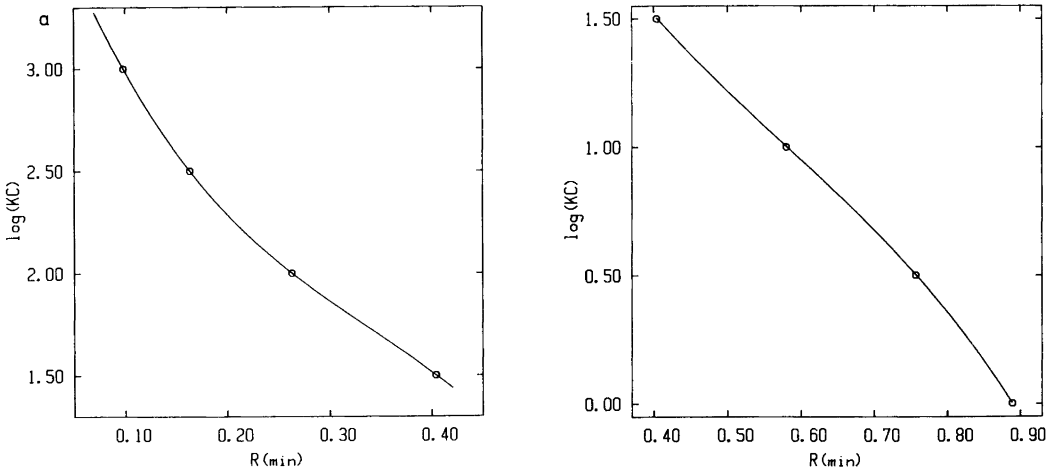

Fig. 2. Theoretical double potential step chronoamperometry equilibrium data for reversible dimerization. Third order polynomial fit to (a), the lower $(R_I)_{min}$ range and (b), the upper $(R_I)_{min}$ range.

Table 1. Theoretical double potential step chronoamperometry equilibrium data for reversible dimerization reactions.[a]

	R_{min} at $-\log(k_1C_A)$ equal to									
$\log(K_1C_A)$	1.00	[b]	1.50	[b]	2.00	[b]	2.50	[b]	3.00	3.50
3.00	0.0986	0.0001	0.0987		–		–		–	
2.50	0.1632	0.0001	0.1633	0.0001	0.1634		–		–	
2.00	0.2626	0.0001	0.2627	0.0000	0.2627		–		–	
1.50	0.4060	0.0014	0.4046	0.0005	0.4041	0.0002	0.4039		–	
1.00	0.5930	0.0086	0.5844	0.0030	0.5814	0.0010	0.5804	0.0003	0.5801	
0.50	–		0.7754	0.0121	0.7633	0.0040	0.7593	0.0014	0.7579	
0.00	–		–		0.9091	0.0134	0.8957	0.0046	0.8911[c]	0.8897[c]

[a] Data obtained by digital simulation as described in the text. [b] The differences between successive values. [c] The difference between the last two values was 0.0014.

The reason behind the convergence observed in $(R_I)_{min}$ and $\log(k_4C_A\tau_{min})$ in Tables 1 and 2 is that the resolution of the simulations increases in going from left to right and down to up in the tables. For example, taking the case where $\log(K_4C_A)$ is equal to 1.000 once more, the number of time elements corresponding to τ_{min} were from left to right, 15, 44, 135, 420 and 1325. Thus, in order to find the true $(R_I)_{min}$ and $\log(k_4C_A\tau_{min})$ it would be necessary to carry out calculations at even more negative values of $\log(k_4C_A)$. This was not done. Instead, calculations were carried out until successive b values were of the order expected for experimental error in the parameters. The true values were obtained by estimation of the values at convergence from the data in Tables 1 and 2. In all cases these differed negligibly from the observed values. Improvements in the computer time necessary for the calculated values to converge could have been made by the use of an implicit rather than explicit finite difference method.[16] Since this would not have affected the accuracy of the results it was deemed to be unnecessary.

The values of $(R_I)_{min}$ and $\log(k_4C_A\tau_{min})$ are summarized in Table 3. The numbers in parentheses after those listed for $(R_I)_{min}$ are the nearest values obtained from the calculations.

Attempts were made to relate $(R_I)_{min}$ to $\log(K_4C_A)$ and to $\log(k_4C_A\tau)$ by means of third order polynomial equations. In both cases the fit of the data to the curves generated was found to be unsatisfactory for kinetic and thermodynamic analyses. However, in both cases excellent fits of the data to third order polynomial equations were observed when the data were divided into

Table 2. Theoretical double potential step chronoamperometry kinetic data for reversible dimerization reactions.[a]

	$(k_1C_A\tau_{min})$ at $-\log(k_1C_A)$ equal to									
$\log(K_1C_A)$	1.00	[b]	1.50	[b]	2.00	[b]	2.50	[b]	3.00	3.50
3.00	20.9	0.0	20.9		–		–		–	
2.50	10.9	0.0	10.9	0.0	10.9		–		–	
2.00	5.60	0.00	5.60	0.00	5.60		–		–	
1.50	2.90	0.05	2.85	0.04	2.81	0.01	2.80			
1.00	1.48	0.11	1.38	0.035	1.34	0.01	1.33	0.00	1.33	
0.50	–		0.652	0.052	0.600	0.018	0.582	0.002	0.580	
0.00	–		–		0.263	0.021	0.242	0.010	0.232[c]	0.228[c]

[a] Data obtained by digital simulation as described in the text. [b] The differences between successive values. [c] The difference between the last two values was 0.004.

Table 3. Theoretical double potential step chronoamperometry data for the evaluation of equilibrium and rate constants of reversible dimerization reactions.[a]

$(R_I)_{min}$	$\log(K_1C_A)$	$\log(k_1C_A\tau_{min})$
0.0987 (0.0987)	3.000	1.320
0.1633 (0.1633)	2.500	1.037
0.263 (0.2627)	2.000	0.748
0.404 (0.4039)	1.500	0.446
0.580 (0.5801)	1.000	0.124
0.757 (0.7579)	0.500	−0.237
0.889 (0.8897)	0.000	−0.646

[a] The values at convergence estimated from the data in Tables 1 and 2.

two equal segments. The curves are shown in Fig. 2 (for $\log(KC_A)$ and Fig. 3 (for $\log(k_4C_A\tau_{min})$. The coefficients for the polynomial equations along with the appropriate ranges of $(R_I)_{min}$ are summarized in Table 4.

Application of the theoretical data to an experimental case. The reversible dimerization of 9-cyanoanthracene anion radical, equilibrium (5), has previously been studied in great detail.[10–12] DPSC data obtained from measurements in DMF at 14.8 °C at [ANCN] ranging from 0.25 to 2.00 mM are shown in Fig. 4. The solid lines are those for the corresponding second order polynomial equations best fitting the experimental data (circles). The equations were differentiated and the first derivatives set equal to zero to obtain $(R_I)_{min}$ and τ_{min}. The resulting values of k_4 and K_4 calculated using the relationships in Table 4 are listed in the last two columns of Table 5. The values obtained were

$k_4=1.51(\pm0.06)\times10^5$ $M^{-1}s^{-1}$ and

$K_4=3.83(\pm0.22)\times10^4$ M^{-1}

For both constants, the experimental value deviating furthest from the mean value was that obtained at the lowest substrate concentration, *i.e.* 0.25 mM. This is not surprising since the experimental error is the highest in this case. The results obtained are in accord with those expected from previous work.[11,12]

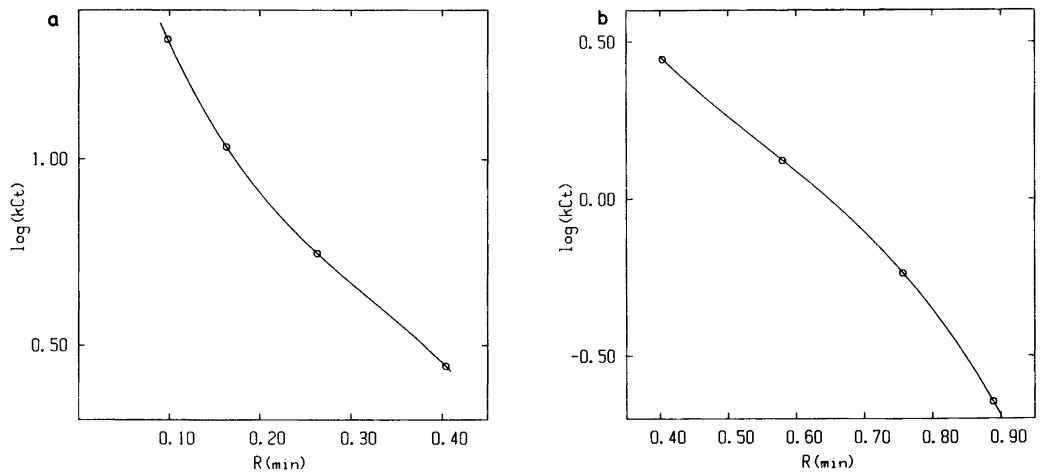

Fig. 3. Theoretical double potential step chronoamperometry kinetic data for reversible dimerization. Third order polynomial fit to (a), the lower $(R_I)_{min}$ range and (b), the upper $(R_I)_{min}$ range.

Table 4. Polynomial coefficients for the calculation of equilibrium and kinetic constants from experimental $(R_I)_{min}$ and τ_{min}.[a]

y^b	$(R_I)_{min}$ Range	a	b	c	d
$\log(K_1C_A)$	0.404–0.889	3.82	−9.29	11.42	−6.53
$\log(K_1C_A)$	0.0987–0.404	4.18	−15.00	34.6	−34.3
$\log(k_1C_A\tau_{min})$	0.404–0.889	2.09	−6.98	9.59	−5.85
$\log(k_1C_A\tau_{min}$	0.0987–0.404	1.98	−8.38	19.1	−19.2

[a] The coefficients for the equation $y=a+bx+cx^2+dx^3$, where x is $(R_I)_{min}$ and y is as designated. The lines are shown in Figs. 2 and 3. [b] Defined as in the eqn. above.

Table 5. Experimental double potential step chronoamperometry data for the reversible dimerization of 9-cyanoanthracene anion radical in *N,N*-dimethylformamide.[a]

10^3C_A/M	$(R_I)_{min}$	τ_{min}/s	$10^{-5}k_1$/M^{-1}s^{-1}	$10^{-4}K_1$/M^{-1}
2.00	0.302	0.01552	1.48	3.59
1.00	0.383	0.0200	1.56	3.79
0.50	0.477	0.0260	1.56	3.80
0.25	0.575	0.0378	1.44	4.12
			1.51(0.06)	3.83(0.22)

[a] Measurements in solvent containing Bu_4NBF_4 (0.1 M) at 14.8 °C.

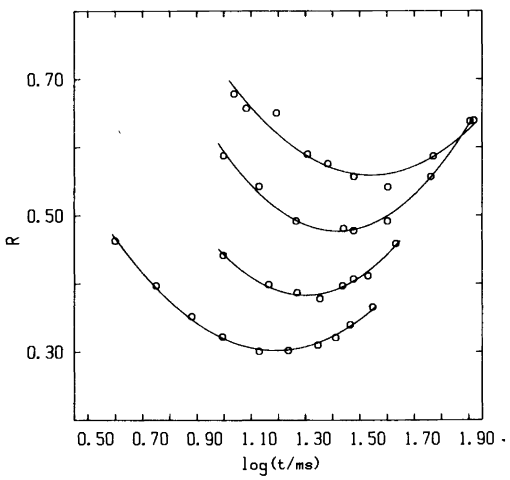

Fig. 4. Experimental double potential step chronoamperometry data for the reversible dimerization of 9-cyanoanthracene anion radical. From top to bottom, [AN−CN] equal to 0.25, 0.50, 1.00 and 2.00 mM in DMF at 14.8 °C.

CONCLUSIONS

Double potential step chronoamperometry data can be related by general equations to the rate and equilibrium constants for reversible dimerization reactions (4). This allows the rate and equilibrium constants to be determined without doing further theoretical calculations. With the minimum value of τ which is experimentally practical taken to be 1 ms and limiting τ to 100 ms with substrate concentrations ranging from 0.1 to 10 mM, the equations presented apply to k_4 ranging from 5×10^1 to 5×10^7 M^{-1}s^{-1} and K_4 ranging from 10^2 to 10^6 M^{-1}. The pertinent experimental parameters, τ_{min} and $(R_I)_{min}$, can be determined to a high degree of precision by taking advantage of the parabolic nature of the response curves and fitting the data to second order polynomial equations.

EXPERIMENTAL

Digital simulation was carried out using a Hewlett Packard 9825A desk computer. Curve fitting was accomplished with the computer

interfaced to a Hewlett Packard 9872 Plotter. DPSC experiments were carried out with 600 mV potential steps with the reversible couple for the reduction of ANCN situated near the center of the potential interval. Other experimental and data handling procedures were those previously described.[7]

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On the Formation of 2-Acylpyrroles and 3-Pyridinols in the Maillard Reaction through Strecker Degradation

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The effects of pH, reactant ratio and reaction time on the yields of 5-methylpyrrole-2-carboxaldehyde (*6*), 6-methyl-3-pyridinol (*7*), methyl 2-pyrrolyl ketone (*8*) and 2-methyl-3-pyridinol (*9*) in the reaction of D-glucose with glycine at 100 °C in aqueous solution have been studied. The use of [1-^{13}C]-D-glucose showed that the methyl group in each of *6*–*9* is derived from C-6 of the glucose. The formation of *6*–*9* from some potential intermediates in the glucose–glycine reaction has also been investigated. The results support the previously proposed routes to *6* and *7* but disqualify those to *8* and *9*. Based on the smooth formation of *8* and *9* from 2-deoxy-D-*arabino*-hexose (*12*) and ammonia, a new route to *8* and *9*, through an enamine derived from *12*, is proposed. This route involves a modified Strecker degradation, which was supported by the formation of 2,3-dideoxy-D-*erythro*-hexose from 3-deoxy-D-*ribo*-hexose and glycine.

In the Strecker degradation,[1,2] an amino group is transferred from an α-amino acid *via* Schiff base intermediates to a carbonyl compound. In the process, the amino acid is oxidatively degraded to carbon dioxide and an aldehyde, while the carbonyl compound is reduced. It is generally believed that the latter reactant may be an α-dicarbonyl compound (being reduced to an α-aminocarbonyl compound, Scheme 1a) or one of its vinylogues but not, for example, an α-hydroxycarbonyl compound (Scheme 1c).

The occurrence of Strecker degradations in Maillard reactions[2,3] is shown by the formation of aldehydes, expected from naturally occurring α-amino acids according to Scheme 1, and heterocyclic nitrogen compounds, formally derived from reduced sugars and ammonia. The α-dicarbonyl compounds *1*–*3* ** or their enol forms

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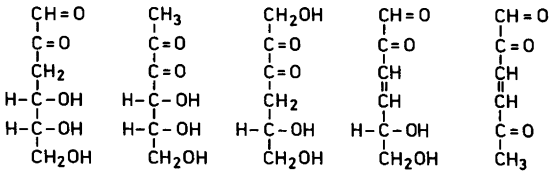
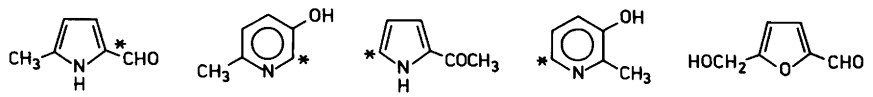
** Sugars and related compounds will here be formulated as acyclic species, even though their less reactive cyclic forms generally predominate. Geometric isomerism due to unsaturation will be neglected.

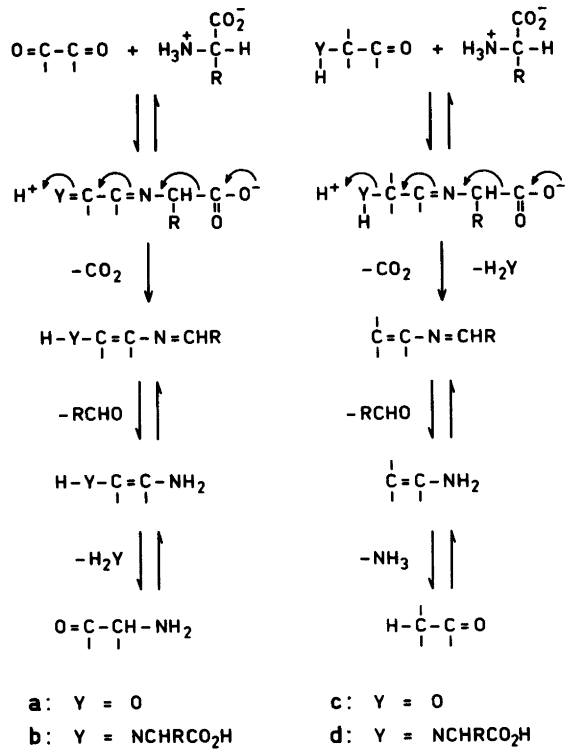


1 *2* *3* *4* *5*



6 *7* *8* *9* *10*

0302-4369/83 $2.50



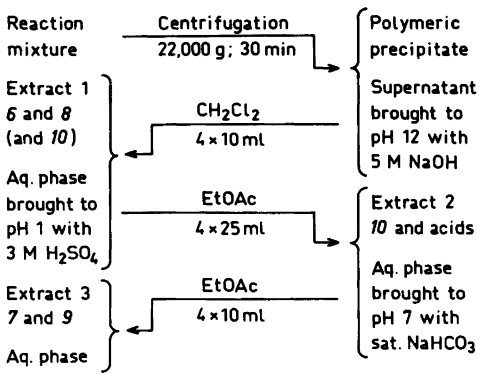
Scheme 1. Strecker degradation induced by an α-dicarbonyl (*a* and *b*), α-hydroxycarbonyl (*c*) or α-aminocarbonyl compound (*d*). In the present paper, R=H.

are important intermediates in the dehydration of D-glucose or D-fructose.[4,5] In Maillard reactions of these sugars, any of *1–3* or their dehydration products (*e.g.*, *4* or *5*) might therefore induce a Strecker degradation.

The Strecker degradation products *6–9* were identified in an investigation of the reaction between D-glucose and glycine at this Department.[6] In each product the carbon chain of the glucose is apparently retained, extending from the methyl group to the carbon atom marked with an asterisk (*) in the formula. Routes were proposed to *6* and *7 via 1*, and to *8* and *9 via 2* or *3*, implying that C-1 of the glucose appears as C* in *6* and *7* but as methyl in *8* and *9*. The pyrroles *6* and *8* have also been obtained from D-fructose and L-alanine.[7] Routes to both products *via 1* were proposed, implying that C-1 of the fructose appears as C* in *6* and *8*. Because of these largely speculative and partly conflicting views, the glucose–glycine reaction has now been reinvestigated, in particular with 1-^{13}C-labelled glucose. Based on the results and on the behaviour of some potential intermediates, a new route to *8* and *9* will be proposed, extending the scope of the Strecker degradation. A brief account of the present work was given at a recent meeting.[8]

RESULTS

In order to optimize the yields of *6–9*, calculated on the expensive [1-^{13}C]-glucose, aqueous solutions of unlabelled glucose and glycine in a molar ratio ranging from 1:2 to 1:20 were refluxed for 72 h. The initial pH of the solutions, which were 0.17 M in glucose, was varied from 2 to 8. Samples were withdrawn at suitable intervals and processed according to Scheme 2. The resulting extracts 1–3 were analyzed by GLC. All of the relatively lipophilic *6* and *8* was found in extract 1, and all of the weakly basic *7* and *9* in extract 3. Other products, including any 5-(hydroxymethyl)-2-furaldehyde (*10*), were found



Scheme 2. Processing of the Maillard reaction mixtures.

mainly in extract 2. A large excess of glycine was required for reasonable yields of *6–9* and for suppressing the formation of *10*. Accordingly, only results obtained with glucose and glycine in the molar ratio 1:20 will be reported. Those obtained at initial pH 2, 3 or 6 are summarized in Fig. 1. In these experiments, pH remained practically constant at 2 or 3 owing to the buffer effect of the glycine but decreased gradually from 6 to about 4.5.

The experiments at pH 2, 3, and 6 were repeated with [1-^{13}C])-glucose (90 atom % ^{13}C). The entire reaction mixtures were processed according to Scheme 2 after refluxing for 48, 13 and 24 h, respectively. The resulting extracts 1 and 3 were analyzed by ^{13}C NMR spectrometry and GLC–MS, using electron impact (EI). The spectra obtained were compared with those of authentic unlabelled *6–9*, recorded under the same conditions. Unless fragmentation of the glucose is involved, the labelled atom will appear in *6–9* as C* and/or methyl. To distinguish between these alternatives, unambiguous assignment of relevant NMR signals and fragment ions from the unlabelled compounds is essential.

Table 1. ^{13}C NMR chemical shifts (δ) for Strecker degradation products in CD_3OD. Shifts in italics refer to C*.

	6	*7*	*8*	*9*
CH_3	12.9	22.5	25.5	18.4
C-2	140.6[a]	*137.0*	133.2	147.7
C-3	124.2	153.6	118.8	153.7
C-4	111.3	125.3	111.2	123.2[a]
C-5	135.5[a]	125.3	*126.7*	123.5[a]
C-6/C=O	*179.4*	149.4	189.9	*139.4*

[a] Mutual assignment uncertain.

The ^{13}C NMR chemical shifts for these compounds are collected in Table 1, where the assignments were made as follows. Each of *6–9*

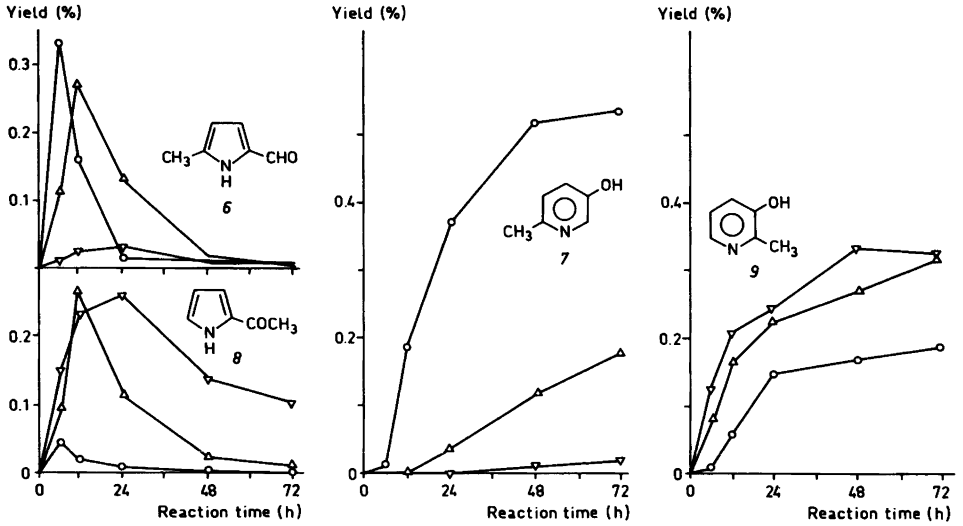


Fig. 1. GLC yields (calc. on glucose) of Strecker degradation products (*6–9*) from D-glucose and glycine (molar ratio 1:20) in aq. solution at 100 °C and initial pH 2.0 (○), 3.0 (△) or 6.0 (▽).

Table 2. Relative intensities (I for unlabelled and *I* for ^{13}C-labelled compounds) in the EI mass spectra of Strecker degradation products obtained from D-glucose and glycine at pH 2 (*7*) or 3 (*6*, *8* and *9*).

Compound *6*			Compound *7*			Compound *8*			Compound *9*		
m/z	I	*I*	*m/z*	I	*I*	*m/z*	I	*I*	*m/z*	I	*I*
50	6	6	50	6	6	66	54	9	50	2	1
51	11	11	51	8	9	67	5	56	51	3	3
52	13	14	52	8	8	68	0	5	52	4	4
53	40	43	53	16	16	94	100	11	53	12	13
54	3	4	54	11	14	95	5	100	54	7	9
55	0	0	55	6	10	96	0	4	55	4	6
78	5	6	56	0	5	109	77	7	56	0	1
79	3	3	80	53	8	110	5	79	80	76	8
80	52	55	81	15	58	111	0	4	81	14	73
81	3	3	82	5	11				82	2	13
82	0	0	83	1	0				83	0	1
108	85	9	108	13	0				108	3	0
109	100	91	109	100	27				109	100	14
110	6	100	110	7	100				110	5	100
111	0	5	111	0	7				111	0	5

shows three doublets in the "off-resonance" spectrum. Being linked to oxygen or nitrogen, C* is responsible for the doublet at lowest field. The methyl group, of course, corresponds to the signal at highest field. Table 1 shows that the C* and methyl signals from *6*–*9* may be clearly distinguished from each other and from the other signals without separation of *6* from *8* or *7* from *9*. After changing the solvent to methanol-d_4, the proton-decoupled ^{13}C NMR spectra of extracts 1 and 3, obtained from labelled glucose, showed only the solvent signal and the C* signals from *6*–*9*. From the signal-to-noise ratios in these spectra, from the intensity ratios of the C* and methyl signals in the spectra of unlabelled *6*–*9*, and from the ^{13}C content (90 atom %) at C-1 of the glucose, the following maximum values were estimated for the percentage of the label located at the methyl group in the labelled *6*–*9*.

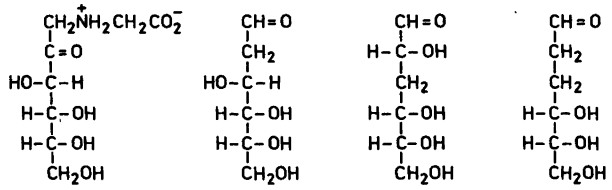
Compound	*6*	*7*	*8*	*9*
pH 2	–	2	–	6
pH 3	5	20	2	2
pH 6	25	–	5	4

Similar values were estimated in the same way for the internal carbon atoms of the C_6 chain present in each of *6*–*9*. The values 20 and 25 % simply reflect poor yields with consequent low signal-to-noise ratios and neither rule out nor imply minor labelling at sites other than C*. However, the other values show clearly that the ^{13}C label appears almost exclusively at C* in *6*–*9*, when each is formed at optimum pH.

Although a confirmation of this conclusion

Table 3. Extent (*p*) of labelling in ions formed on electron impact from ^{13}C-labelled *6*–*9*. The *p* values were calculated from the data in Table 2.

m/z	Main ion	*p*, %
5-Methylpyrrole-2-carboxaldehyde (*6*)		
50–55	M–C*HO·–HCN	2
78–82	M–C*HO·	0
108–111	M	102
6-Methyl-3-pyridinol (*7*)		
50–56	M–CHO·–HC*N	37
80–83	M–CHO·	100
108–111	M	98
Methyl 2-pyrrolyl ketone (*8*)		
66–68	M–CH_3CO·	96
94–96	M–CH_3·	100
109–111	M	102
2-Methyl-3-pyridinol (*9*)		
50–56	M–CHO·–HC*N	21
80–83	M–CHO·	99
108–111	M	100



11 *12* *13* *14*

appeared superfluous, we wished to establish whether it was possible to arrive at the same conclusion from the mass spectra. If so, future tracer experiments might be performed on a much smaller scale. Relevant mass spectral data for unlabelled *6*–*9* and for the labelled compounds obtained at pH 2 (*7*) or 3 (*6*, *8* and *9*) are collected in Table 2. The extent (*p*) of labelling in each molecular ion (M) and diagnostic fragment from the labelled compounds was calculated from these data and from the ^{13}C content (90 atom %) at C-1 of the labelled glucose, as outlined in the experimental part. The results are listed in Table 3, where the fragment symbols are based on previous investigations of 2-acylpyrroles[9] and 3-pyridinols.[10]

As seen from Table 2, the mass spectra showed clusters of peaks, including those of interest, at consecutive *m/z* values, resulting in extensive overlap. To simplify the calculation of *p*, we had therefore to assume that the ^{13}C label was located at *one* carbon atom, and that the ions forming each cluster differed only in hydrogen content and/or isotopic composition and not in the origin of their carbon atoms. The first assumption appears reasonable in view of the ^{13}C NMR results, but the second one is more doubtful.

Thus, a minor part of the cluster at *m/z* 80–83 in the spectra of *7* is due to M–HC*N.[10] Although this has been neglected in Table 3, *p* is close to 100 %. At lower *m/z*, however, such neglected fragments may be more abundant and lead to meaningless *p* values. This is exemplified by the high *p* values for the clusters at *m/z* 50–56 in the spectra of *7* and *9*. As seen from Table 2, the clusters shown by corresponding labelled and unlabelled compounds differed mainly at *m/z* 54–56, indicating the presence of ions which had lost unlabelled C_2H_2 instead of HC*N. Accordingly, a high resolution mass spectrum of unlabelled *7* showed five peaks at *m/z* 54. The major peaks were due to $C_4H_6^{\cdot+}$ and $C_3H_4N^+$. Their intensity ratio was about 2:1. At *m/z* 55, $C_3H_3O^+$ and $C_3H_5N^{\cdot+}$ predominated. Such ions may also

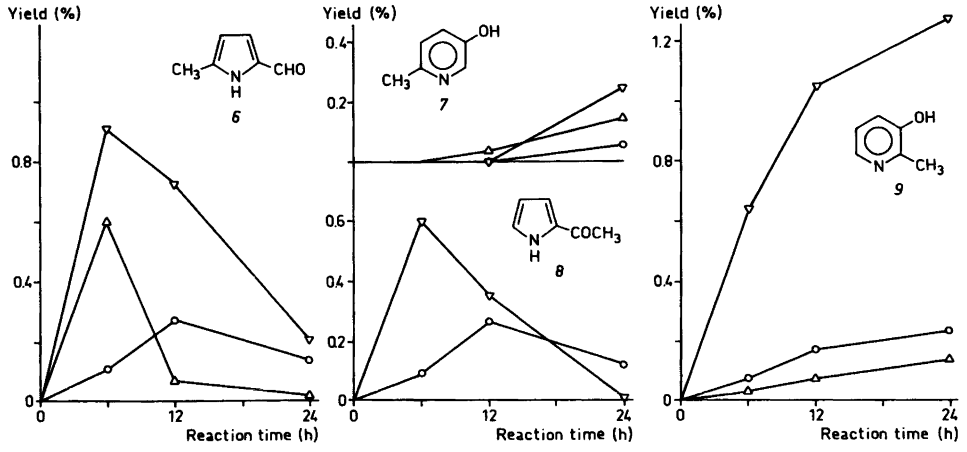


Fig. 2. GLC yields (calc. on glucose, *1* or *11*) of Strecker degradation products (*6*–*9*) in the reaction of D-glucose (○), *1* (△) or *11* (▽) with glycine (molar ratio 1:20) in aq. solution at 100 °C and initial pH 3.0. The yield of *8* from *1* and glycine was negligible.

contribute to the cluster at *m/z* 50–55 shown by *6*, but since C* has already been lost from all ions in the cluster, the *p* value is not affected.

For the pyrroles *6* and *8*, the clean loss of the acyl group from M led to *p* values close to 0 or 100 %, confirming the ^{13}C NMR results. As exemplified by the pyridinols *7* and *9*, however, detailed knowledge of the fragmentation is imperative in more complex cases. Even so, it may be hard to obtain more than qualitative results.

Some (unlabelled) potential intermediates in the glucose–glycine reaction, including 1-deoxy-1-glycino-D-fructose (*11*), 2-deoxy-D-*arabino*-hexose (*12*), and 3-deoxy-D-*ribo*-hexose (*13*), were treated for 24 h at 100 °C and initial pH 3 as already described for the glucose–glycine experiments, see Table 4. Thus, *1, 6, 8, 10* or *11* was treated with glycine in the molar ratio 1:20. The experiment with *11* was repeated without glycine. Compound *6, 8* or *12* was treated with ammonium acetate (molar ratio 1:20), and *13* with glycine (molar ratio 1:5). The experiments with *12* and *13* were repeated at initial pH 6.

The yields of *6–9* from *1* or *11* after reaction with glycine are compared in Fig. 2 with those obtained from glucose (Fig. 1, pH 3). When treated with glycine or ammonium acetate, the pyrroles *6* and *8* disappeared gradually without forming any pyridinol *7* or *9*. Compound *10* also disappeared, when treated with glycine, yielding only traces of *6* and *7*. From *11* alone, no *6–9* was obtained. The maximum yields 0.9 % of *8* and 28 % of *9* from *12* and ammonium acetate were obtained after only about 3 h reaction at initial pH 6. In the experiments with *13*, the sugars in the final aqueous phase (Scheme 2) were analyzed by GLC–MS as their per-*O*-acetylaldononitriles.[11] Among the several GLC peaks, two were due to the nitrile derivatives of *13* and 2,3-dideoxy-D-*erythro*-hexose (*14*). The latter derivative was identified by prominent $M+H^+$ and $M+H^++NH_3$ peaks in the chemical ionization (CI) spectrum, recorded with ammonia as reaction gas. The maximum yield of *14* was obtained after about 6 h reaction at initial pH 6 and was 5 %, if the difference in GLC response factor between the nitrile derivatives of *13* and *14* is neglected.

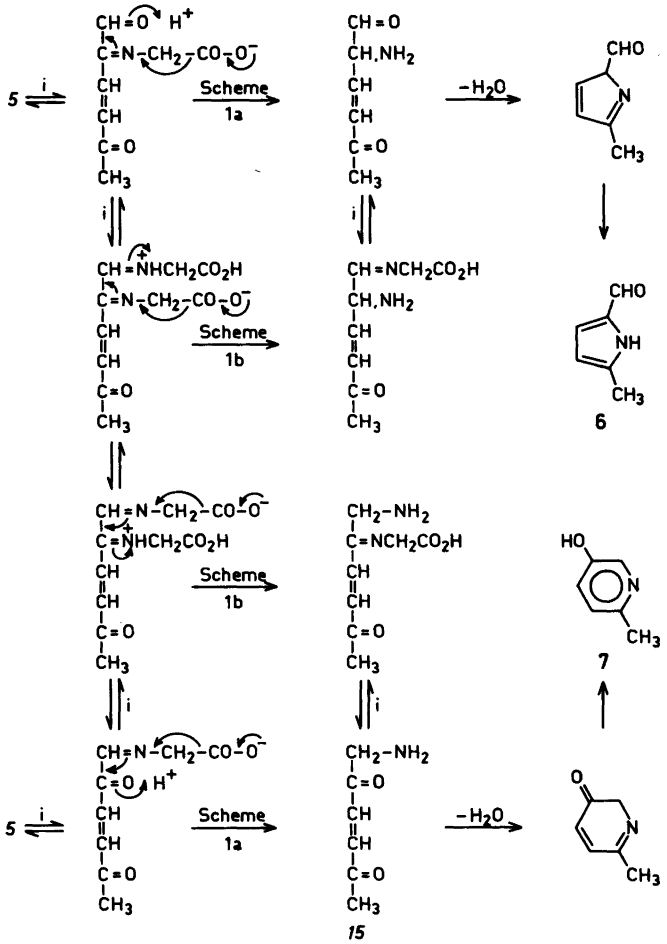
DISCUSSION

As seen from Fig. 1, the yields of *7* and *9* rose steadily with increasing reaction time up to a certain limit, whereas those of *6* and *8* reached a maximum after 6–24 h and then declined. This was not due to the conversions *6*→*7* and *8*→*9*, as shown by the experiments where *6* or *8* was treated with glycine or ammonium acetate. Higher acidity seemed to favour the further reactions of *6* and *8*, since the maximum yields were obtained earlier. Higher acidity also favoured the formation of *6* and *7* but disfavoured that of *9*, and perhaps also that of *8*. These results seemed to support the routes to *6–9* proposed[6] for the glucose–glycine reaction, since *1* is formed through the 1,2-enol but *2* and *3* through the 2,3-enol of fructose; the relative importance of the 2,3-enol is believed to increase with pH.[5] However, this interpretation is incorrect, since the routes to *8* and *9* are incompatible with the ^{13}C-tracer results (see below). These results also indicate that little or no *6–9* is formed through fragmentation and recombination of the C_6 chain.

All the present results support the previously proposed routes to *6*[6,7] and *7*[6] *via 1*. The routes to *6* differ only as to the stage at which the Strecker degradation takes place. We now prefer the route through *4* and *5*,[7] since the necessary dehydration steps are more readily rationalized before than after the Strecker degradation. However, the results are equally compatible with several closely related routes, some of which are more likely than that through *1, 4* and *5*.

In the first place, *6* and *7* formed faster from *1* than from glucose (Fig. 2), but this does not necessarily imply that *1* is an intermediate in the latter reaction. An obvious alternative is that *1* is converted faster than glucose to the true intermediate. Indeed, this is probably not *1* but rather its enol.[4,5] Similarly, this enol is probably not formed through *11*[2] or fructose[5] but rather through their 1,2-enols, despite the smooth formation of *6* and *7* from *11* and glycine (Fig. 2) and the reported[7] formation of *6* from fructose and alanine.

Secondly, the well-known amine catalysis of sugar dehydration in Maillard reactions is due to partial conversion of the various carbonyl intermediates into Schiff bases and enamines. In a nearly neutral medium, these largely take over



Scheme 3. Alternative routes to products *6* and *7* from Schiff bases derived from compound *5*. i=+glycine−H_2O.

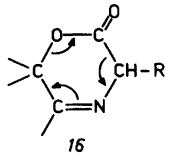
the parts played by the less reactive keto and enol forms in the absence of amines. In our experiments, *1*, *4*, *5* and their enols are therefore less probable intermediates than the corresponding Schiff bases and enamines, particularly in view of the large excess of glycine generally employed.

It is even possible that the Strecker degradation proceeds *via* a diimine ("double" Schiff base) according to Scheme 1b rather than by the accepted route in Scheme 1a. These alternative routes to *6* and *7* from Schiff bases derived from *5* are shown in Scheme 3. In a weakly acidic medium, where the carboxyl group of the amino acid being degraded may ionize sufficiently, a carbonyl oxygen is protonated (if at all) to a much smaller extent that a Schiff base nitrogen. The electrophilic centre is therefore expected to be more powerful in Scheme 1b than in Scheme 1a. Until experimental evidence for the existence and equilibrium concentration of the diimines has been obtained, it is however impossible to choose between the alternative routes. It may be noted here that metabolic transaminations, racemizations and decarboxylations of α-amino acids take place through a vinylogous diimine derived from the B_6 vitamin pyridoxal, but in this case one imino group forms part of an aromatic ring.

The negligible formation of *6* and *7* from *10* and glycine supports the view that the dehydration *4*⟶*5* does not proceed *via* *10*.[4] It also shows

that the cyclization *4*→*10* is essentially irreversible under the reaction conditions and that *10*, being a vinylogous α-hydroxycarbonyl compound, cannot induce a Strecker degradation. (According to Scheme 1c, such a degradation, followed by hydrolytic ring opening, might yield *15* in Scheme 3.)

Since C* in each of *6*–*9* has now been shown to originate mainly or exclusively from C-1 of the glucose, the proposed[6] routes to *8* and *9* *via 2* or *3* are unimportant or incorrect. The negligible formation of *8* from *1* and glycine (Fig. 2) disqualifies the route proposed[7] to *8* *via 1*. A difficulty with all of *1*–*5*, their enols and their



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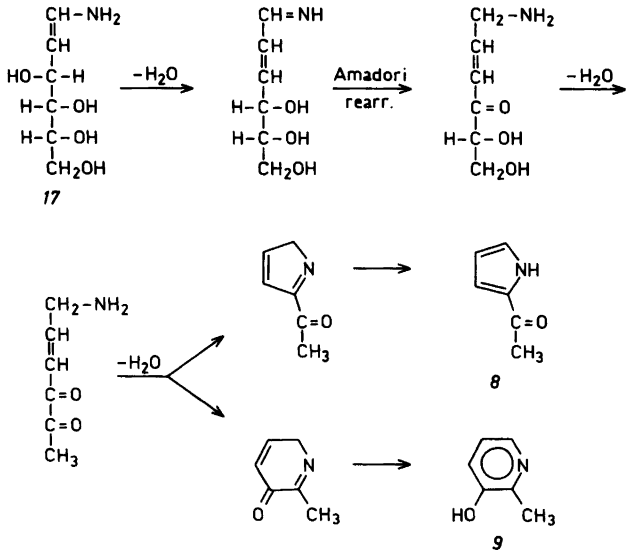
amine derivatives as intermediates in the formation of *8* or *9* is to account for the necessary elimination of the hetero atom at the bifunctional C-2 atom.

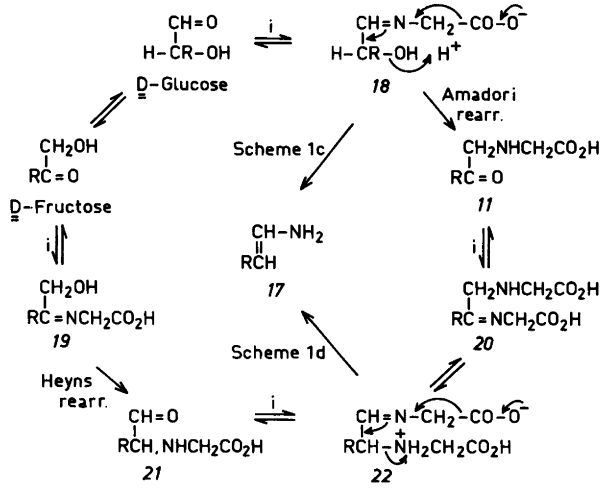
This difficulty is avoided, if elimination of the hydroxyl group at C-2 of the glucose according to Scheme 1c is assumed. Such elimination might be promoted by initial lactonization, as indicated in formula *16*. Several early samples from glucose–glycine reaction mixtures were analyzed for the 2-deoxy sugar *12*, expected according to Scheme 1c. Thus, the final aqueous phase obtained by processing each sample according to Scheme 2 was analyzed for sugars by three different methods,[11,12] but in no case was any *12* detected. However, this might be because dehydration of the enamine *17* through the β-elimination shown in Scheme 4 is much faster than its hydrolysis to *12* according to the last step in Scheme 1c.

That this may be the case was indicated by the high yields of *8* and *9* readily obtained from *12* and ammonium acetate – presumably *via 17* – compared with the optimum yields of 0.3 % obtained from glucose and glycine after 12–48 h (Fig. 1). Further support was offered by the formation of the dideoxy sugar *14* in about 5 % yield from *13* and glycine within 6 h. In this case, Scheme 1c may be followed all the way, since there is no β-elimination competing with the last step. Possible routes to *8* and *9* from *17* are suggested in Scheme 4, where the "vinylogous" Amadori rearrangement following the dehydration of *17* may be noted.

Although the evidence in favour of *17* as an intermediate in the formation of *8* and *9* from glucose and glycine seems convincing, there are several objections against the simple route to *17* according to Scheme 1c. A related but less direct route to *17* involving Scheme 1d will therefore



Scheme 4. Routes to products *8* and *9* from the precursor *17*.



Scheme 5. Alternative routes to the postulated precursor *17* of products *8* and *9*. i=+glycine−H_2O, R=*arabino*-H(CHOH)$_4$.

also be discussed. The alternative routes are outlined in Scheme 5.

In the first place, α-hydroxycarbonyl compounds like glucose and other sugars are not expected to induce Strecker degradations. This may be due to insufficient proton assistance to the hydroxyl group in the Schiff base *18* (this may be less critical for the more electrophilic carbonyl group in Scheme 1a). This problem does not occur in the corresponding Scheme 1d intermediate *22*.

Secondly, Scheme 1c does not explain why *8* and *9* are formed faster from *11* than from glucose in the presence of glycine (Fig. 2), unless the improbable reversal of the Amadori rearrangement *18*→*11* is postulated. On the other hand, the sequence *11*→*20*→*22* has already been carried out with *p*-chloroaniline as the amine,[13] and the cyclic *N*-glycoside forms of products analogous to *20* and *22* have been isolated. The formation of *8* from fructose and alanine[7] is also easier to rationalize by means of the well-known[14] Heyns rearrangement *19*→*21* than by invoking glucose, as required by Scheme 1c. Some aliphatic amines may even convert fructose to analogues of *22*.[15] In this connection, the catalyzing effect of glycine on the dehydration of 2-amino-2-deoxy-D-glucose[5] may be recalled (*cf. 21*).

Elimination of the 1-glycino group from *20* according to Scheme 1d might also occur but should be less favourable than elimination from a secondary atom like C-2 of *22*. No attempt was made to identify the products of any such elimination from C-1 of *20*. In these products, C-1 of glucose should appear as a methyl group. In view of the ^{13}C-tracer results, *6*–*9* are therefore probably not among the products.

For the reasons given above, and because no *8* or *9* was obtained from *11* without glycine, we tend to prefer Scheme 1d to Scheme 1c. However, before making a final choice, we wish to complete a study on reactions of *11* and amino sugars related to *20*–*22*.

EXPERIMENTAL

Chromatography

Separate GLC analyses were performed at 30 ml N_2/min with a Varian 1840-1 instrument, fitted with dual flame-ionization detectors and 1.8 m × 2 mm i.d. glass columns. Sugars were analyzed as described in Ref. 11 or 12, and *6*–*9* on 100–120 mesh Varaport 30 coated with 3 % NPGS ("neopentyl glycol succinate"). The pyrroles (*6* and *8*, extract 1) were analyzed at 165 °C with biphenyl as internal standard, and the pyridinols (*7* and *9*, extract 3) at 200 °C with fluorene as internal standard. Peak areas were measured with a Varian CDS 111C instrument.

Spectrometry

^{13}C NMR spectra (Table 1) were recorded at 22.53 MHz and *ca.* 35 °C with a Jeol FX-90Q instrument, using 5 mm o.d. NMR-tubes. The lock signal was provided by deuterium of the solvent (CD_3OD). The chemical shifts (δ) were related to internal tetramethylsilane.

The high resolution EI mass spectrum of unlabelled 7 was recorded at 100 eV with a V. G. ZAB instrument at the Institute of Medical Chemistry, University of Gothenburg. Low resolution EI mass spectra (Table 2) were recorded at 70 eV with a Finnigan 4021 GC/MS/Data System. The samples were introduced through a 20 m×0.25 mm i.d. capillary GLC column coated with OV-225. The helium flow rate was 25 cm/s (*ca.* 0.7 ml/min) and the column temperature was programmed from 80 to 150 °C at 6 °C/min. The background was subtracted from all spectra, which were recorded under as similar conditions as possible. Ammonia CI mass spectra were recorded under the same conditions, but the capillary column was coated with CP Sil 5 and its temperature programmed from 100 to 250 °C at 10 °C/min.

The extent (p, Table 3) of labelling in the molecular ion or in a diagnostic fragment ion from any of the ^{13}C-labelled Strecker degradation products (*6*–*9*) was calculated by comparing the spectrum with that of the respective unlabelled reference sample. This was done as follows, assuming any label to be located at *one* carbon atom. Each ion belonged to a series of n ions, CEH_k^+, where C is the potentially labelled atom, E the other atoms common to the ions, H hydrogen and $k=0, 1, \ldots, n-1$. In the mass spectrum, these ions form a cluster of r peaks, where $r > n$. The average ^{13}C content (c) of C was assumed to be the same for all the ions CEH_k^+. The possible contribution of other ions to the cluster was neglected. Since the mass distribution of EH_k due to its natural isotopic composition has been tabulated,[16] the relative intensity of each peak (j) in the cluster is given by

$$I_j=\sum_k \lambda_{j,k} A_k$$

where each coefficient $\lambda_{j,k}$ is a function of c only, and A_k is the relative abundance of CEH_k^+. The spectrum of the unlabelled compound yielded r such equations, and that of the labelled compound r more equations. In the former equations, $\lambda_{j,k}$ may, of course, be evaluated, since $c=1$ %. In the latter equations, c was varied. For each c value, $\lambda_{j,k}$ were evaluated; the remaining unknowns, A_k, were then calculated from all the $2r$ equations by the method of least squares. By minimization of the resulting deviation, the preferred c value was obtained. The extent of labelling is then given by

$$p=100(c-1)/(90-1)=1.124(c-1)$$

if c and p are expressed in percent. The calculations were performed with a Commodore PET computer, using a program written in BASIC. The program is available on request.

Materials

Compounds *1*,[17] *6*,[18] *9*,[19] *11*[20] and *13*[21] were prepared according to the literature for use as starting materials or reference samples. Other reagents were commercial samples, including [1-^{13}C]-D-glucose (Prochem, London; 90 atom % ^{13}C). Solvents were freshly distilled before use.

Maillard reaction procedure

The experiments with unlabelled materials are listed in Table 4. Reactant 1 (15.0 mmol) and the appropriate amount of reactant 2 were dissolved in water (60 ml) by gentle heating. The generally supersaturated solution was brought to the desired pH at about 25 °C by addition of conc. hydrochloric acid or 2 M sodium hydroxide, diluted to 90 ml and refluxed for 24–72 h. At

Table 4. Experiments with unlabelled materials at 100 °C in aq. solution 0.17 M in reactant 1. Glu=D-glucose.

Reactants		Molar ratio	Initial pH
1	2		
Glu	Glycine	1:20	2.0
Glu	Glycine	1:20	3.0
Glu	Glycine	1:20	6.0
1	Glycine	1:20	3.0
6	Glycine	1:20	3.0
6	NH_4OAc	1:20	3.0
8	Glycine	1:20	3.0
8	NH_4OAc	1:20	3.0
10	Glycine	1:20	3.0
11	Glycine	1:20	3.0
11	—	—	3.0
12	NH_4OAc	1:20	3.0
12	NH_4OAc	1:20	6.0
13	Glycine	1:5	3.0
13	Glycine	1:5	6.0

suitable intervals, 15 ml aliquots were withdrawn and processed according to Scheme 2. The resulting extracts 1 and 3 were analyzed by GLC. The final aqueous phase was somtimes analyzed for sugars according to Ref. 11 and/or 12.

[1-^{13}C]-D-Glucose was treated as unlabelled glucose but on a 10 times smaller scale. The entire reaction mixture was processed after 48, 13 or 24 h according to whether the initial pH was 2.0, 3.0 or 6.0. The resulting Extracts 1 and 3 were analyzed by GLC–MS, dried with sodium sulfate and evaporated at reduced pressure below 40 °C. Each residue was dissolved in methanol-d_4 and the ^{13}C NMR spectrum of the solution recorded.

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Oxidation-reduction Potential of Soluble and Membrane-bound Rabbit Liver Microsomal Cytochrome P-450 LM2

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The redox midpoint potentials of rabbit liver microsomal cytochromes P-450 and of soluble and membrane-bound rabbit liver microsomal cytochrome P-450 LM2 were determined using EPR-spectroscopy and absorption difference spectrometry with NADPH or dithionite as reductants. Using EPR, a redox midpoint potential of −0.36 V was obtained both for the low spin and the high spin components of microsomal cytochrome P-450. Spectrophotometrical determinations yielded very similar values: −0.37 V and −0.34 V for the low and high spin signals, respectively. Soluble cytochrome P-450 LM2 had a midpoint potential of −0.32 V. This redox potential was not significantly affected by incorporation of the protein into an artificial membrane structure or, furthermore, by the presence of cytochrome b_5 the same membrane.

The redox midpoint potentials of cytochromes P-450 are surprisingly low. Both the bacterial cytochrome P-450cam,[1] the hepatic cytochromes P-450[2,3] and the mitochondrial cytochrome P-450scc[4] have midpoint redox potentials around −0.30 V. In the liver microsomal hydroxylase system cytochrome P-450 receives electrons either from NADPH-cytochrome P-450 reductase, having midpoint potentials related to the various reduced forms between −0.11 and −0.36 V[5] or from cytochrome b_5[6,7] with a midpoint potential of about 0 V.[8] As a consequence of the non-functionally related redox potentials between the different proteins in the hydroxylase system, suggestions have been made that cytochrome P-450 exerts altered redox properties when it interacts with cytochrome b_5 in the native membrane.[8] In view of our results indicating altered properties of cytochrome P-450-catalyzed hydroxylation reactions upon incorporation of purified components of the hydroxylase system into a membrane structure,[9] it was considered of interest to evaluate whether the redox properties of cytochrome P-450 LM2 were affected by the presence of a neighbouring membrane or by cytochrome b_5 incorporated into the membrane.

The results indicate that the redox midpoint potential of cytochrome P-450 LM2 is unaffected by the membrane or by cytochrome b_5, when measurements are performed either by EPR-spectroscopy or absorption difference spectrometry using NADPH or dithionite as reductants.

MATERIALS AND METHODS

Microsomal phospholipids were prepared according to Bligh and Dyer.[10] Egg yolk phosphatidylcholine, type III E, was purchased from Sigma. Electrophoretically homogeneous cytochrome P-450 LM2 was prepared from phenobarbital-treated rabbits as described previously[11]. The method, based on that described by Haugen and Coon,[12] yielded preparations with specific contents of 12.5–14.2 nmol/mg protein. Cytochrome b_5 was purified as described elsewhere.[7]

Unilamellar phospholipid vesicles containing cytochrome P-450 LM2 were prepared in the following way. Ten mg of egg yolk phosphatidylcholine or microsomal phospholipids in chlo-

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roform was taken to absolute dryness under nitrogen. The residue was suspended in 0.5 ml of 50 mM potassium phosphate buffer, pH 7.4, containing 20 % glycerol and sonicated at 22 °C under nitrogen until clarity, using an MSE sonifier. One ml of cytochrome P-450 LM2 (13–15 μM) was then added and the solution was incubated for 20 min at 37 °C. This procedure resulted in complete incorporation of the cytochrome into the vesicles as was evident from Sepharose 4 B chromatography of the preparations (*cf.* Ingelman-Sundberg *et al.*[11]).

Oxidation-reduction potential measurements were carried out with EPR at low temperature, essentially as described previously.[13] The following modifications were made. Antraquinone 2-sulfonate was added to the gas scrubbing bottles and the second bottle contained 0.2 g sodium dithionite per 100 ml of 10 mM potassium hydroxide. The samples were withdrawn by overpressure through the gas outlet by a bent glass tube leading into a quartz EPR tube. The following redox mediators in a final concentration of 50 mM were used: phenosafranine T, benzyl viologen and methylviologen. In negative direction the potentials were adjusted by a solution containing 0.1 M sodium dithionite and 20 mM potassium hydroxide. The EPR spectra were recorded with a Varian V-5402 spectrometer as described before.[14]

Oxidation-reduction potential measurements were also carried out at 20 °C using a dye equilibrium technique. The samples in butyl rubber sealed glass spectrophotometer cuvettes were reduced anaerobically under stirring by a flow of argon for 1 h. The argon was cleaned by passage over heated BASF catalyst R 3–11 followed by passage over a gas scrubbing bottle containing water to restore the moisture to the gas. Each sample contained one of the dyestuffs phenosafranine, safranine T or benzylviologen. To vary the degree of oxidation, small amounts of sodium dithionite, NADPH or potassium ferricyanide were added to the sample. The difference light absorption spectra were recorded by an Aminco DW-2 UV-VIS spectrophotometer. The potentials of the solutions were then calculted by the observed light absorption using

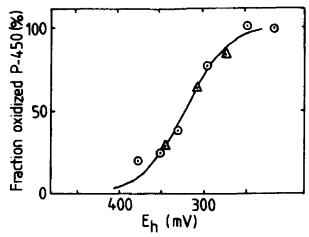

Fig. 1. Potentiometric titration of soluble cytochrome P-450 LM2. To samples with 11 μM cytochrome P-450 LM2 in 0.1 M potassium phosphate buffer, pH 7,0, containing mediators (*cf.* Methods) and 20 % glycerol, 0.1 M sodium dithionite in 20 mM potassium hydroxide was added to adjust the potential. The samples were subsequently frozen and analyzed by EPR as described under Methods. ▽ indicates the results obtained during reoxidation of the hemoprotein.

Table 1. Redox midpoint potentials of cytochrome P-450. Midpoint potentials were determined as described under Methods in liver microsomes isolated from phenobarbital-treated rabbits or in purified cytochrome P-450 LM2-preparations. When indicated, an equimolar amount of purified rabbit liver cytochrome b_5 was introduced into the vesicles together with P-450 LM2.

Sample	Assay Method	Reductant	$E_{m,7}$ (V)
Microsomes	EPR g=2.4	dithionite	–0.36
Microsomes	EPR g=8	dithionite	–0.36
P-450 LM2	EPR g=2.25	dithionite	–0.32
P-450 LM2 in vesicles	EPR g=2.25	dithionite	–0.32
P-450 LM2 in vesicles containing cytochrome b_5	EPR g=2.25	dithionite	–0.31
Microsomes	ΔA 465 nm	dithionite	–0.37
Microsomes	ΔA 648 nm	dithionite	–0.34
P-450 LM2 in vesicles	ΔA 455 nm	dithionite	–0.33
Microsomes	ΔA 465 nm	NADPH	–0.36
Microsomes	ΔA 648 nm	NADPH	–0.34

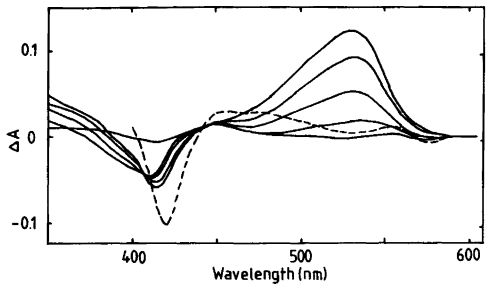

Fig. 2. Redox titration of membrane-bound cytochrome P-450 LM2 in the presence of safranine T. The cytochrome was incorporated into vesicles prepared from microsomal phospholipids as described under Methods. Safranine T was added to a final concentration of 2.7 μM and the solution was diluted with 50 mM potassium phosphate buffer, pH 7.4, containing 20 % glycerol to give a final concentration of the cytochrome of 3 μM. The potential was adjusted in negative and positive directions by small additions of 50 mM sodium dithionite in 10 mM potassium hydroxide and 25 mM potassium ferricyanide, respectively. A fully reduced sample is shown by the dotted line. The system potentials were measured at 530 nm from the fraction of safranine T oxidized, whereas the degree of cytochrome P-450 reduction was calculated farom the absorbance difference 455−419 nm.

the Nernst equation. At pH 7.4, the midpoint potentials of −265 mV and −298 mV were used for phenosafranine and safranine T, respectively.[15]

RESULTS

Redoxmidpoint potentials detected by EPR. The midpoint potentials of cytochrome P-450 were determined by EPR-spectroscopy at low temperatures in frozen anaerobic samples of rabbit liver microsomes or of purified cytochrome P-450 LM2 either in soluble (Fig. 1) or membrane-bound state. In microsomes, also the high spin EPR-signal at g=8.0 was detected, whereas with the purified protein, only the low spin signal was taken into consideration.

From the results presented in Table 1, it is evident that the midpoint potentials obtained were relatively similar and not dependent upon the presence of a surrounding membrane. A value of about − 0.34 V was obtained both for the high and low spin forms of cytochrome P-450 in microsomes and for the purified P-450 LM2 preparations in both the soluble and membrane bound state. The presence of an equimolar amount of cytochrome b_5 in the membranes did not affect the redox potential of cytochrome P-450 LM2.

Redoxmidpoint potentials detected by adsorption spectra. Midpoint potentials of cytochrome P-450 were also determined by a dye equilibrium technique at 20 °C. Difference absorption spectra of cytochrome P-450 LM2 in the presence of safranine T at different oxidation-reduction states are shown in Fig. 2. Even with this technique low midpoint potentials around −0.34 V were obtained for all cytochrome P-450 preparations and, furthermore, also for both the low and high spin forms of the protein, as detected by absorption difference at 465 and 648 nm, respectively, using sodium dithionite as reductant (*cf.* Table 1).

In microsomal samples NADPH was also used as reductant. However, in these cases the redox midpoint potentials of microsomal P-450 were not significantly different from those obtained when using dithionite either (*cf.* Table 1).

DISCUSSION

The results presented indicate that the membrane has little influence upon the redox properties of cytochrome P-450 LM2, when measurements were performed either by EPR at low temperatures or by absorption difference spectra at room temperature using phenosafranine, safranine T or benzylviologen as mediators. The results obtained using microsomal samples agree well with previous redox midpoint potentials of hepatic cytochrome P-450 determined by Waterman and Mason [16], and soluble cytochrome P-450 LM2 presented by Guengerich *et al.*[3] In experiments where we used NADPH as reductant, still a very low midpoint potential was obtained indicating that in this way an indirect reduction of P-450 *via* NADPH-cytochrome P-450 reductase, does not affect the midpoint potential obtained when measuring the formation of ferrous cytochrome P-450.

Recent results have more or less established the participation of cytochrome b_5 in the liver

microsomal hydroxylase system, where it most probably donates the second electron to cytochrome P-450.[6–8,18,19] The presence of cytochrome b_5 in the membrane did not affect the redox properties of P-450 LM2. A midpoint potential of cytochrome b_5 in these reconstituted membranes of about 0 V confirms the marked difference in redox properties between the two microsomal hemoproteins. It is therefore tempting to speculate about altered and more similar values of these midpoint potentials in the functionally working microsomal hydroxylase system. The results obtained by Werringloer and Kawano,[8] using cytochrome b-5 as an endogenous microsomal mediator, suggest that this is the case although an overestimation of the midpoint potential of P-450 seems plausible due to the measurement of the ferrous-carbonyl complex (including measurement of two equilibria) instead of ferrous P-450. Our recent results [19] indicate that cytochrome b_5, NADPH-cytochrome P-450 reductase and cytochrome P-450 form a functionally active ternary complex in the membrane. It thus seems likely that the formation of this complex in an intact membrane structure is a prerequisite for obtaining the native redox properties of the enzymes.

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Rates of the Ring Opening of Some Bicyclic 3-Thienyllithium Derivatives

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The rates of ring opening of some cycloalkane [*b*]-annellated thienyllithium derivatives have been determined. The cyclopenta[*b*] system appeared to be several thousand times more reactive than the cyclohepta[*b*] and cycloocta[*b*] systems. The rate difference is explained by a considerable ring strain in the five-membered case.

We recently reported that the ring opening of bicyclic 3-thienyllithium derivatives gave, after *S*-alkylation, cyclic acetylenic vinlyl sulfides.[1] Even though a variety of cycloalkyl-annellated 3-thienyllithium derivatives ring-opened easily, some others showed great resistance towards this reaction. This made us investigate, a little more in detail, how the rate of ring opening was affected by the size of the annellated rings.

RESULTS AND DISCUSSION

Compounds *1a-f* (Scheme 1) were all treated with phenyllithium in ether at +2 °C in a thermostated reaction vessel. For comparison the ring-opening rate of the fully substituted lithium derivative of *1g* was determined, since it possesses no ring strain due to annellation. The reaction mixtures were quenched with dimethyl sulfate at different intervals and the methylated products were analyzed with GLC/MS. The mass spectra of the two types of reaction products *4* and *5* were consistently and sufficiently different to permit correlation of GLC peak and structure. In all cases the ring-opened products *5* had longer retention times than *4*. The molecular ion peak was quite abundant (57–100 %) in the mass spectra of all reaction products except for *4e* (13%) and *4f* (19 %). However, the M−1 ion fragment was weak in the spectra of the ring-opened products *5*, while the methylated thiophenes, except for *4e* and *4f* had more abundant M−1 ions than *5*. Compounds *4e* and *4f* had instead a predominant M−15 fragment. Additional structural evidence was obtained from ^{13}C NMR data. Thus, all compounds *5* had their acetylenic carbon resonances at 75.8–81.2 ppm and 90.6–92.4 ppm, in agreement with our previously reported data[1]. Moreover, compound *5b* was synthesized by another route[1] and its structure was therefore rigorously determined. This fact, together with the spectral data, certainly provides good evidence for the proposed structures of compounds *4* and *5*. The *S*-benzyl and *S*-acetic acid derivates of the ring-opened products have previously been fully characterized.[1]

In Table 1 the rate constants and half-lives are shown, measured as the time necessary for the 3-methylthiophene derivatives *4* to decrease to 50 % of the amount of *5* (quenching with Me_2SO_4). This approximation was justified since the only products were the ring-opened substances, together with 10–15 % of the corresponding hydrolyzed 3-thienyllithium derivatives. It is impossible to judge whether these originate from the thienyllithium *2* or the lithium thiolate *3* and were not considered further. The halogen–metal exchange was shown to be complete within less than 10 s at +2 °C, and we assume that the

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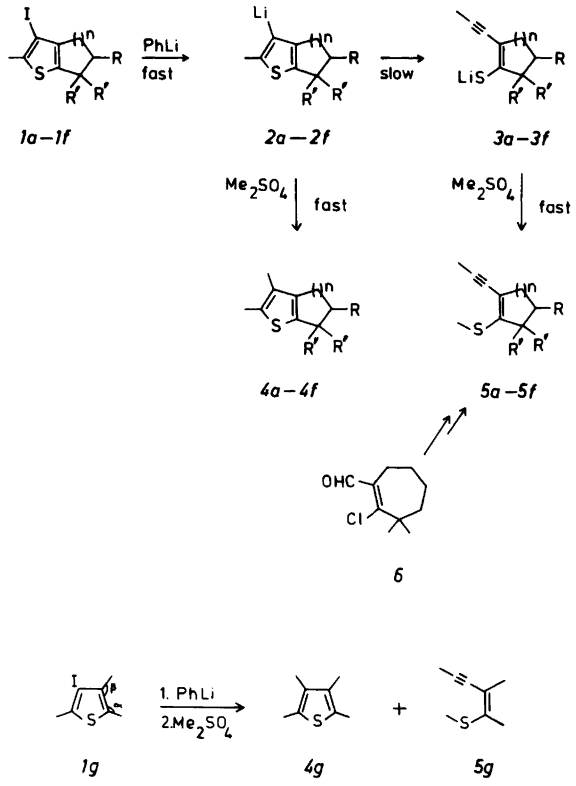


Scheme 1. *a*, n=1, R=CH_3, R′=H; *b*, n=2, R=R′=H; *c*, n=3, R=R′=H; *d*, n=4, R=R′=H; *e*, n=2, R=H, R′=CH_3; *f*, n=3, R=H, R′=CH_3.

quenching with DMS does not significantly influence the ratio of *2* and *3*, *i.e.* that the half-lives of the 3-thienyllithium derivatives *2* are reflected by the time at which the ratio of *4*:*5* is 1:1.

Table 1. Kinetic data for the ring opening of *2a–f* at +2 °C. 2,3,5-Trimethyl-4-thienyllithium is included for comparison.

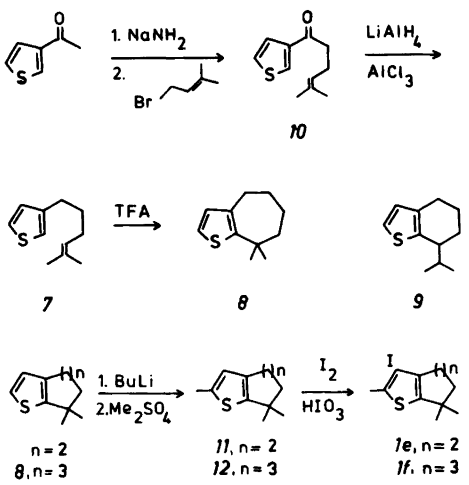
Substance	$t_{½}$ (min)	k 10^3 (min^{-1})
2a	<0.08	>8.10^3
2b	35	20
2c	510	1.4
2d	138	5.0
2e	3120	0.22
2f	–[a]	–[a]
2,3,5-trimethyl-4-thienyllithium	198	3.5

[a] Ring opening could not be observed.

The ring-opening of some non-annellated 3-thienyllithium derivatives has been shown to obey first-order kinetics,[2] and there is no reason to believe that the annellation should change this matter. The rate constants were thus calculated accordingly ($\ln 2/t_{½}=k$).

As expected, the five-membered derivative *2a* had the highest rate of reaction, which we believe is due to a considerable ring strain. Compounds *2b–2d* and 2,3,5-trimethyl-4-thienyllithium were more similar, although the differences are significant. It is possible that *2b* has a higher ring strain than *2c* and *2d*, which would explain part of the 15-fold increase in rate as compared to *2c*.

An indication of the ring strain in the tetrahydrobenzo[b]thiophene system could be sensed by its synthesis. Cyclization of *7* to give *8* could conceivably give *9* as a by-product (Scheme 2), as was the case with the corresponding benzene compounds, which gave the cycloheptane and



Scheme 2.

cyclohexane systems in a 1:2 ratio.[3] The absence of *9* in the crude product could indicate that the ring strain in *9* is not negligible. Inspection of molecular models gives an additional explanation for the difference in ring-opening rate between *2b* and *2c*. It appeared that the hydrogens of the α-thenylic position 7 in *2b* (and even more those of 6 in *2a*) are in a staggered position in relation to the sulfur atom, while in *2c* and *2d* they are almost eclipsed in the reasonable low-energy conformations.[4] Thus, the α-thenylic hydrogens in *2c* and *2d* would impose more steric hindrance than those in *2a* and *2b* when the negative charge on the sulfur increases as the ring opening proceeds, resulting in an increase of the radius of the sulfur. Thus, ring strain and steric hindrance from the α-thenylic hydrogens seem to operate in the same direction.

The effect of a *gem*-dimethyl functionality at the α-thenylic position demonstrates the steric hindrance more clearly, which is shown in the cases of *2e* and *2f*. While there is a 100-fold decrease in reaction rate on going from *2b* to *2e*,*2f* did not ring-open at all. This parallels the case previously reported, in which a *t*-butyl group decreased the ring-opening rate by a factor of 82 as compared to a methyl group in a similar situation.[2] Not even at room temperature after 24 h could any ring-opening products be observed. A 100-fold decrease in rate would have given 5–10 % ring-opening product after this time. From a synthetic view point, the resistance of *2f* to give *5f* is not serious, since it can almost certainly be obtained *via* the β-chlorovinyl aldehyde *6*, as outlined previously.[1]

The relatively slow ring opening of the trimethylthiophene system (as compared to 2,5-dimethyl-3-thienyllithium, $t_{1/2}$=4.5 min at +2 °C)[2] could be due to an increasing strain resulting from the approach of the 4- and 5-methyl groups owing to the decrease of the outer angles of thiophene (α and β) from 128 and 125° to approximately 120°.These values represent the "normal" thiophene sp^2-hybridized carbon angles and do not refer to experimentally determined values for these specific cases.

Explanations for why the 3-lithium derivatives of the 5-membered heterocyclic aromatics ring-open at all have never been discussed. The 3-thienyllithium derivatives seem to be less "aromatic" than expected and the following suggestion may account for this. Several aromatic lithium derivatives have been shown to exist as aggregates of dimeric or tetrameric nature with the anionic carbon coordinating up to three different lithium atoms.[5] This would imply that this carbon must rehybridize its orbitals to some extent as compared to the parent or halogen-substituted derivative in order to distribute its electrons to the lithium atoms. It is conceivable that the resonance energy of the aromatic system is reduced in this way. Thus, the ring-opening process would be more like the β-elimination of an aliphatic system.

EXPERIMENTAL

GLC analyses were performed on a Perkin-Elmer 900 gas chromatograph, GLC/MS analyses were performed on a Finnegan 4021 mass spectrometer. NMR spectra were recorded with a Jeol MH 100 or a Varian XL 200 NMR spectrometer. All reactions with organometallic reagents were performed in ether freshly distilled over sodium wire under a nitrogen atmosphere. The iodothiophenes *1a–1d* were prepared as previously described.[1] The syntheses of *1e* and *1f* are described below. 3-Iodo-2,4,5-trimethylthiophene *1g* was prepared according to Ref. 6.

RATE DETERMINATION

A 1.5-fold excess of phenyllithium was added to 0.1 g samples of the iodothiophenes *1a–1g*[1]

and 4-iodo-2,3,5-trimethylthiophene[6] in 1 ml of dry ether at +2 °C under a nitrogen atmosphere. The temperature was controlled with a cryostat and kept within ±0.5 °C. After appropriate times, a large excess of freshly distilled dimethyl sulfate was added with a syringe. After 30 min the mixture was treated with aqueous ammonium hydroxide and the ethereal layer analyzed by GLC/MS.

The ratio of *4* and *5* was taken as the degree of ring opening, although in all cases 10–15 % of dehalogenated starting material was present. In some cases small amounts of dimethylated compounds were found, probably originating from metallation of the propargylic position of *3*, followed by methylation by dimethyl sulfate. The dimethylated compounds were structure determined from their mass spectra. Similar compounds have been isolated and characterized previously.[6] The amount of these dimethylated products was added to that of compounds *5* in order to estimate the half-lives of *2*. The ring-opened products were isolated (preparative TLC, hexane) and characterized by ^{13}C NMR spectroscopy. The thioenol ethers had satisfactory analytical data (±0.4 % for C and H).

1-(3'-Thienyl)-5-methyl-4-hexen-1-one 10. To a suspension of 3.4 g (0.086 mol) of sodium amide in 25 ml of dry ether under a nitrogen atmosphere, 10.8 g (0.086 mol) of 3-acetylthiophene in 50 ml of dry ether was added. The mixture was refluxed for 3 h, whereupon 12.8 g (0.086 mol) of prenyl bromide in 50 ml of dry ether was added. Reflux was continued for another 3 h. Aqueous work-up and distillation afforded 7.0 g (42 %) of the title compound, b.p. 116–121 °C (1.3 mm). IR(film): 1675 cm^{-1} (C=O). NMR ($CDCl_3$): δ 1.62 (bs, 3H, Z-CH_3), 1.67 (d, 3H, J=1.2 Hz, E-CH_3), 2.38 (q, 2 H, 3-CH_2), 2.89 (m, 2 H, 2-CH_2), 5.15 (m, 1 H, 4-H), 7.28 (q, 1 H, J =3.0 Hz, 5.2 Hz, 5'-H), 7.53 (q, 1 H, J=1.3 Hz, 5.2 Hz, 4'-H), 8.02 (q, 1 H, J=1.3 Hz, 3.0 Hz, 2'-H). Anal. $C_{11}H_{14}OS$: C,H.

2-Methyl-6-(3'-thienyl)-2-hexene 7. Compound *10* (13.6 g, 0.070 mol) was reduced with $LiAlH_4$/$AlCl_3$ according to Brown and White.[7] The crude product was distilled: yield 8.0 g (63 %), b.p. 109–113 °C (8 mm). NMR ($CDCl_3$): δ 1.58 (bs, 3 H, Z-CH_3), 1.69 (d, 3 H, J=1.2 Hz, E-CH_3), 1.6–1.8 (m, 2 H, 5-CH_2), 2.00 (q, 2 H, 4-CH_2), 2.62 (t, 2 H, J=8 Hz, 6-CH_2), 5.14 (m, 1 H, 3-H), 6.90 (m, 2 H, 2'-H and 4'-H), 7.19 (q, 1 H, J=3.0 Hz, 4.8 Hz, 5'-H). Anal. $C_{11}H_{16}S$: C,H.

8,8-Dimethyl-4,5,6,7-tetrahydro-8H-cyclohepta-[b]thiophene 8. Compound 7 (7.5 g, 0.042 mol) was dissolved in 50 ml of dry, methylene chloride, and 18 ml of trifluoroacetic acid in 150 ml of dry methylene chloride was added at room temperature during 30 min.[8] The mixture was stirred for 24 h. After aqueous work-up the crude product was flash chromatographed on silica gel using light petroleum (40:60) as eluent. Distillation gave 4.6 g (62 %) of the title compound, b.p. 110–115 °C (9 mm). NMR ($CDCl_3$): δ 1.35 (s, 6 H, 2 CH_3), 1.4–2.0 (m, 6 H, aliphatic), 2.6–2.9 (m, 2 H, thenylic), 6.66 (d, 1 H, J=5.3 Hz, 3-H), 6.85 (d, 1 H, J=5.3 Hz, 2-H). Anal. $C_{11}H_{16}S$: C,H.

4,5,6,7-Tetrahydro-2,7,7-trimethylbenzo[b]-thiophene 11. 7,7-Dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene[8] (4.4 g, 0.028 mol) was dissolved in 30 ml of dry ether under a nitrogen atmosphere and 28 ml (0.042 mol) of 1.5 M butyllithium was added. The mixture was refluxed for 1 h and then cooled to −70 °C. Dimethyl sulfate (5.3 g, 0.042 mol) dissolved in 25 ml of dry ether was added dropwise. After 2 h, the cooling bath was removed and after 1 h at room temperature the reaction mixture was poured into aqueous ammonium hydroxide and worked up. Distillation gave 3.4 g (68 %) of the title compound, b.p. 110–112 °C (12 mm). NMR ($CDCl_3$): δ 1.28 (s, 6 H, 2 CH_3), 1.4–1.9 (m, 4 H, aliphatic), 2.3–2.7 (m, 2 H, thenylic), 2.38 (d, 3 H, J=1.2 Hz, CH_3), 6.33 (m, 1 H, 3-H). Anal. $C_{11}H_{16}S$: C,H.

4,5,6,7-Tetrahydro-2,8,8-trimethyl-8H-cyclohepta[b]thiophene 12 was prepared in the same way as *11*, starting from 2.5 g (0.014 mol) of *8*. Yield: 1.5 g (56 %), b.p. 128–131 °C (11 mm). NMR ($CDCl_3$): δ 1.32 (s, 6 H, 2 CH_3), 1.5–2.0 (m, 6H, aliphatic), 2.33 (d, 3 H, J=1.2 Hz, CH_3), 2.5–2.8 (m, 2 H, thenylic), 6.35 (m, 1 H, 3-H). Anal. $C_{12}H_{18}S$: C,H.

General procedure for the iodination of 11 *and* 12. The methylthiophenes *11* and *12* were iodinated by the iodine-iodic acid method.[9]

3-Iodo-4,5,6,7-tetrahydro-2,7,7-trimethyl-benzo[b]thiophene 1e. Yield: 2.2 g (62 %) from 2.1 g (0.012 mol) of *11*. B.p. 113–116 °C (1.5 mm), m.p. 52–53 °C (ethanol). NMR ($CDCl_3$): δ 1.27 (s, 6 H, 2 CH_3), 1.5–1.9 (m, 4 H, aliphatic), 2.3–2.6 (m, 2 H, thenylic), 2.37 (s, 3 H, CH_3). Anal. $C_{11}H_{15}IS$: C,H.

3-Iodo-4,5,6,7-tetrahydro-2,8,8-trimethyl-8H-cyclohepta[b]thiophene 1f. Yield: 1.4 g (71 %) from 1.2 g (6.2 mmol) of *12*. M.p. 43–44 °C (ethanol). NMR ($CDCl_3$): δ 1.30 (s, 6H, 2 CH_3), 1.5–2.1 (m, 6 H, aliphatic), 2.34 (s, 3 H, CH_3), 2.7–2.9 (m, 2 H, thenylic), Anal. $C_{12}H_{17}IS$: C,H.

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Structural Studies of Curcuminoids. III. Crystal Structure of 1,7-Diphenyl-1,5-heptadiene-3,5-dione

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The crystal and molecular structure of the curcumin derivative 1,7-diphenyl-1,6-heptadiene-3,5-dione has been determined at 121 K by X-ray crystallographic methods using 1553 reflections observed by counter methods. The crystals are orthorhombic, space group *P*bca with unit cell dimensions a=36.903(25) Å, b=13.998(8) Å and c=5.686(3) Å.

The structure was refined to a conventional R-factor of 0.068. Estimated standard deviations are 6×10^{-3} Å and 0.4° in interatomic distances and angles when hydrogen atoms are not involved. The enol-ring is found to be symmetric with complete delocalization of the double bonds.

Previous studies of the structure of the enol-ring in curcuminoids [1,2] indicate that the structure of these pseudoaromatic moieties may be very sensitive to intermolecular interactions such as hydrogen bonding.[2] In order to study this effect further the crystal structure determination of 1,7-diphenyl-1,6-heptadiene-3,5-dione (BPHDD)*, where the possibilites of intermolecular hydrogen bond formation are restricted as far a possible, has been carried out.

EXPERIMENTAL

The synthetic and crystallization procedures[3] resulted in a sample of strongly yellow coloured needle-formed crystals with melting point 425 K.

One of these crystals was used for the X-ray crystallographic experiments, details of which are described under experimental conditions. Cell parameters were determined by least squares fit to the diffractometer settings for 15 general reflections. The standard deviation in the measured intensities were calculated as $\sigma(I)=|C_T+(0.02C_N)^2|^{\frac{1}{2}}$ where C_T is the total number of counts and C_N is the scan count minus the background count. The intensity data were corrected for Lorentz and polarization effects. No corrections were made on the basis of test reflection variation which was less than 2 %. Scattering factors were those of Doyle and Turner[4] for oxygen and carbon atoms, and of Stewart, Davidson and Simpson[5] for hydrogen atoms.

EXPERIMENTAL CONDITIONS

Instrument	SYNTEX PĪ
Radiation	Graphite Crystal Monochromated Mo$K\alpha$, λ=0.71069 Å
Crystal dimensions (mm)	0.4×0.2×0.1
Scanning mode	ω
Scan speed (° min^{-1})	3.0
Scan range (°)	1.5
Background counts	For 0.35 of scan time at scan limits
Temperature (K)	121
2θ range (°)	3.0–50.0
Number of reflections measured	1968

* For simplicity this name is used even if the title compound does exist in the enol form in the crystals.

0302-4369/83 $2.50

Number of reflections with $I>2.5\sigma(I)$	1553
Number of standard reflections	3
Number of reflections between standard reflections	57

CRYSTAL DATA

1,7-Diphenyl-1,6-heptadien-3,5-dione, $C_{19}O_2H_{16}$, orthorhombic, $a=36.903(25)$ Å, $b=13.998(8)$ Å, $c=5.686(3)$ Å, $V=2937$ Å^3, $M=276.19$, $Z=8$, $F_{000}=1168$, space group *Pbca*.

Table 1. Fractional atomic coordinates. Estimated standard deviations in parentheses.

ATOM	X	Y	Z
O1	.3550(0)	.3809(1)	.0561(5)
O2	.4220(0)	.3803(1)	.0119(5)
C1	.1750(1)	.3833(2)	-.3769(7)
C2	.2015(1)	.3419(2)	-.5209(8)
C3	.2372(1)	.3414(2)	-.4499(7)
C4	.2480(1)	.3816(2)	-.2360(6)
C5	.2210(1)	.4226(2)	-.0926(7)
C6	.1851(1)	.4247(2)	-.1655(7)
C7	.2857(1)	.3815(2)	-.1492(7)
C8	.3155(1)	.3566(2)	-.2705(8)
C9	.3520(1)	.3616(2)	-.1671(7)
C10	.3830(1)	.3498(2)	-.3075(7)
C11	.4177(1)	.3620(2)	-.2094(7)
C12	.4505(1)	.3587(2)	-.3582(8)
C13	.4834(1)	.3864(2)	-.2779(7)
C14	.5179(1)	.3875(2)	-.4059(6)
C15	.5482(1)	.4312(2)	-.2983(7)
C16	.5816(1)	.4318(2)	-.4085(7)
C17	.5865(1)	.3860(2)	-.6218(7)
C18	.5566(1)	.3426(2)	-.7320(7)
C19	.5231(1)	.3435(2)	-.6233(7)
H1X	.388(1)	.383(3)	.098(10)
H1	.150(0)	.384(2)	-.438(6)
H2	.193(0)	.311(2)	-.669(6)
H3	.256(0)	.313(2)	-.555(6)
H5	.229(0)	.454(2)	.062(6)
H6	.166(0)	.456(2)	-.055(6)
H7	.289(0)	.407(2)	.015(7)
H8	.314(0)	.337(2)	-.427(7)
H10	.381(0)	.333(2)	-.470(7)
H12	.447(1)	.337(2)	-.523(7)
H13	.484(0)	.410(2)	-.107(6)
H15	.545(0)	.462(2)	-.140(6)
H16	.604(0)	.465(2)	-.319(6)
H17	.611(1)	.385(2)	-.705(7)
H18	.561(1)	.309(2)	-.888(7)
H19	.502(0)	.313(2)	-.707(6)

STRUCTURE DETERMINATION

The structure was solved by direct methods using the program assembly MULTAN.[6] Successive Fourier syntheses indicated the positions of

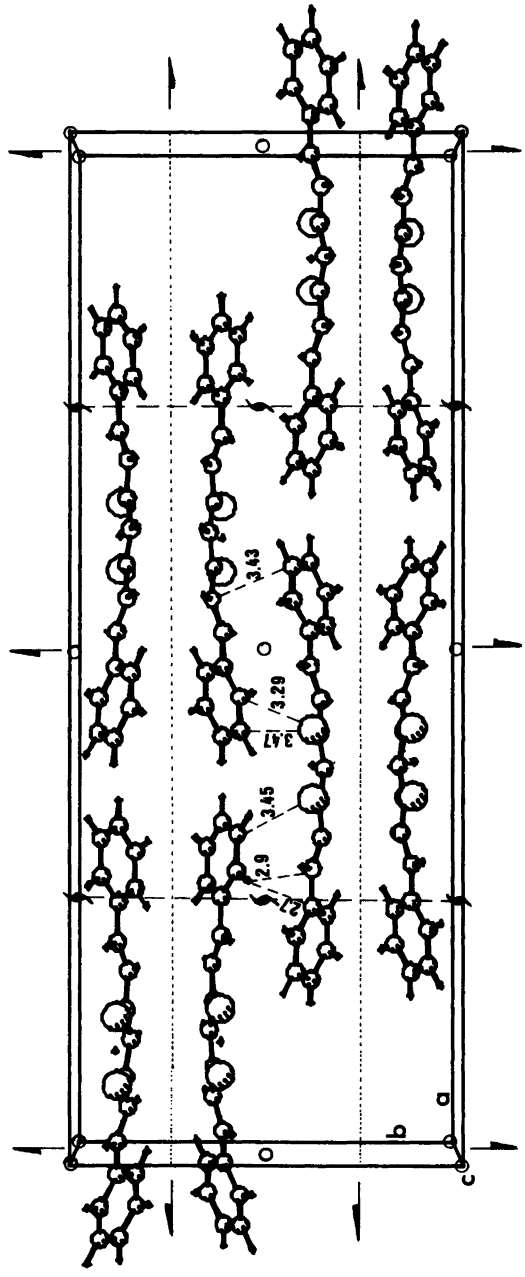

Fig. 1. Molecular packing in the BPHDD crystals as seen down the *c*-axis.

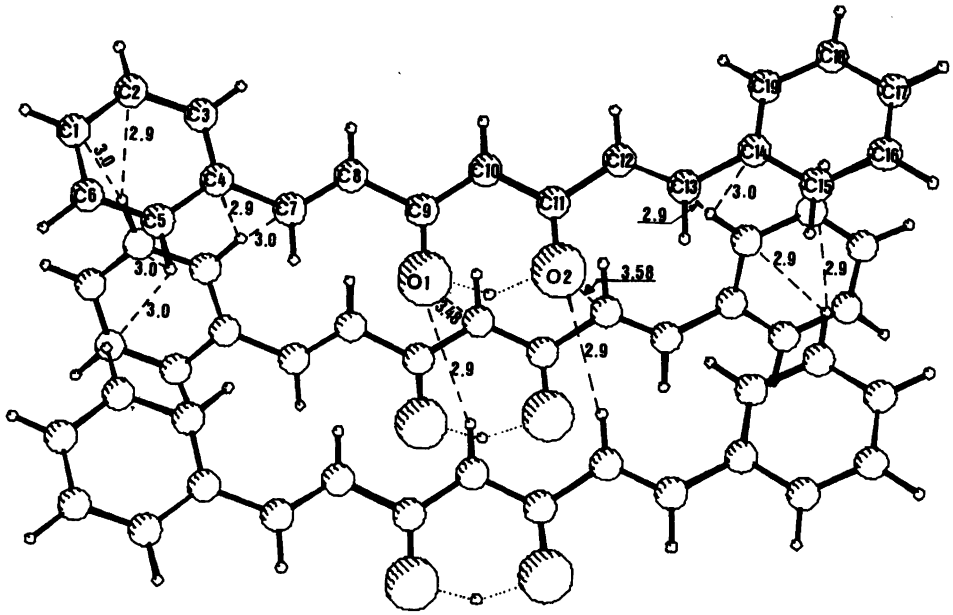

Fig. 2. Stacking of molecules in the BPHDD crystals.

Table 2. Bond lengths and angles in the BPHDD molecules.

DISTANCE	(Å)	DISTANCE	(Å)
C1 – C2	1.401(6)	C2 – C3	1.379(6)
C3 – C4	1.398(5)	C4 – C5	1.410(5)
C5 – C6	1.388(5)	C6 – C1	1.385(6)
C4 – C7	1.478(5)	C7 – C8	1.345(6)
C8 – C9	1.469(6)	C9 – O1	1.302(5)
C9 – C10	1.406(6)	C10 – C11	1.407(6)
C11 – O2	1.294(5)	C11 – C12	1.477(6)
C12 – C13	1.353(6)	C13 – C14	1.466(5)
C14 – C15	1.413(5)	C15 – C16	1.385(6)
C16 – C17	1.385(6)	C17 – C18	1.406(6)
C18 – C19	1.382(6)	C19 – C14	1.395(5)
C – H	1.01(4)		

ANGLE	(°)	ANGLE	(°)
C1 – C2 – C3	120.0(4)	C2 – C3 – C4	121.5(4)
C3 – C4 – C5	117.9(4)	C3 – C4 – C7	123.8(4)
C7 – C4 – C5	118.3(4)	C4 – C5 – C6	120.7(4)
C5 – C6 – C1	120.4(4)	C6 – C1 – C2	119.5(4)
C4 – C7 – C8	127.0(4)	C7 – C8 – C9	122.1(4)
C8 – C9 – O1	118.5(4)	C8 – C9 – C10	120.9(4)
O1 – C9 – C10	120.6(4)	C9 – C10 – C11	120.2(4)
C10 – C11 – O2	121.3(4)	O2 – C11 – C12	117.6(4)
C10 – C11 – C12	121.0(4)	C11 – C12 – C13	122.2(4)
C12 – C13 – C14	127.8(4)	C13 – C14 – C15	118.4(4)
C13 – C14 – C19	123.7(4)	C14 – C15 – C16	120.8(4)
C15 – C16 – C17	120.6(4)	C16 – C17 – C18	119.3(4)
C17 – C18 – C19	119.9(4)	C18 – C19 – C14	121.6(4)
C19 – C14 – C15	117.7(4)		

all the non-hydrogen atoms, and a difference synthesis after least squares refinement of the positions of the non-hydrogen atoms indicated the positions of 15 hydrogen atoms. All positional parameters, anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms were refined in the final least squares calculations giving a conventional R-factor of 0.068 and a goodness of fit $S=(\Sigma w\Delta^2/(m-n)^{\frac{1}{2}}=3.5$.

The final coordinates are given in Table 1. Temperature factors as well as tables of observed and calculated structure factors are available from the authors.

DESCRIPTION AND DISCUSSION

The packing of the molecules in the crystals of the present compound as seen down the c-axis is illustrated in Fig. 1. There are no intermolecular hydrogen bonds in these crystals and the molecular packing is thus governed by van der Waals' interactions. The glide planes in (0,1/4,0) and (0, 3/4, 0) give rise to molecular stacks as illustrated in Figs. 1 and 2 where the shortest intermolecular contacts are also given. The angle between the enol ring planes within these stacks is about 25° whereas the angles between the two pairs of phenyl ring planes are both close to 60°.

The numbering of the atoms is indicated in Fig. 2 whereas the bond lengths and angles are given in Table 2 and the values found to be in good agreement with those found in similar compounds.[1,2] This is also true for the molecular conformation as may be seen from the torsional angles given in Table 3.

Table 3. Some torsional angles in the BPHDD molecules in the crystal state.

DIHEDRAL ANGLE				(°)	
C3	C4	C7	C8	10.2(	6)
C5	C4	C7	C8	-171.4(	4)
C4	C7	C8	C9	178.4(	4)
C7	C8	C9	C10	169.8(	4)
O1	C9	C10	C11	-2.1(	6)
O1	C9	C10	O2	2.5(	5)
C10	C11	C12	C13	168.2(	4)
C9	C10	C11	O2	2.7(	6)
O2	C11	C12	C13	-9.0(	6)
C11	C12	C13	C14	-179.5(	5)
C12	C13	C14	C15	171.6(	4)
C12	C13	C14	C19	-11.9(	6)

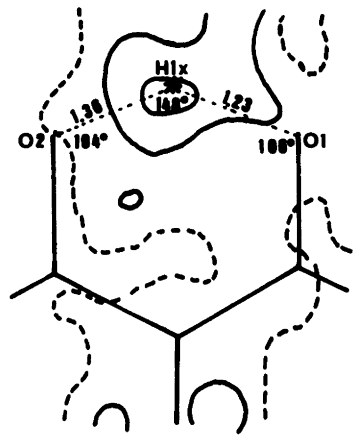

Fig. 3. Difference map in the enol-ring plane in the crystal structure of BPHDD. The solid lines represent the electron densities of 0.25 and 0.50, respectively. The position of the hydrogen atom is indicated.

The only hydrogen bond in the crystal structure of BPHDD is the intramolecular one between the two oxygen atoms in the enol-ring. In the two earlier structures in this series,[1,2] the hydrogen atom was found in one case to be statistically distributed between the two oxygen atoms and in the other to be bonded to one particular oxygen atom. In the present case the model giving the best fit to the data includes one hydrogen atom (H1X) about midway between the two oxygen atoms as illustrated in Fig. 3. However, an anisotropic refinement of the thermal vibrations of the H1X atom indicates a B-factor of about 18.0 in the O1–O2 direction and about 5.0 in the two other directions. Thus the data is also consistent with a statistical distribution of the H1X atom between two closely spaced positions.

The distance between the two oxygen atoms is 2.486 Å in the present structure. This is significantly longer than the respective distance found in the structure of curcumin[1] (2.446 Å) but shorter than that found in the B4HPDD-methanol complex.[2]

The geometry of the enol-ring in the present compound, however, is more like that found in curcumin; both the C–O distances being equal as are the two involved C–C distances.

The atoms C20 and C11 are found to be about 0.01 Å on each side of a least squares plane

through the 5 heavy atoms of the enol-ring. The hydrogen atom H1X is situated less than 0.05 Å out of that plane.

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Oxazoles in Diels-Alder Reactions. Novel Transformation of the Adducts to 6-Substituted Pyridines

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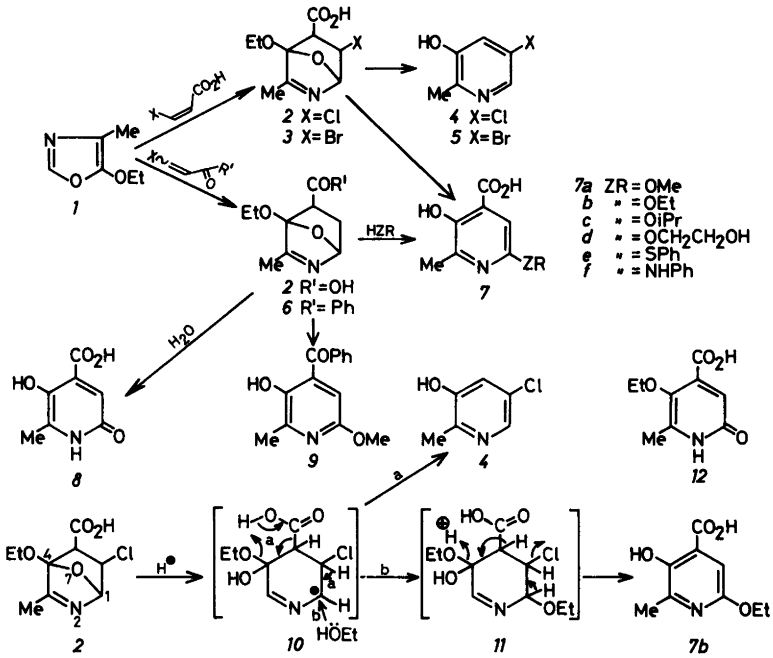
The cycloadducts from (*Z*)-β-halopropenoic acids and 4-methyl-5-ethoxyoxazole in the presence of hydroxy, mercapto or amino derivatives are converted as formed to 6-substituted 3-hydroxypyridines with concurrent elimination of hydrogen halide. In the absence of added nucleophile, a 3-hydroxy-5-halopyridine is formed in a competitive breakdown of the adduct involving decarboxylation.

A preparatively important route to substituted β-hydroxypyridines consists of an initial Diels-Alder reaction between an ethylenic dienophile and a 5-ethoxyoxazole which is followed by an acid catalyzed rearrangement.[1] For α,β-unsaturated carbonyl dienophiles the regioselectivity in the adduct formation is such that the carbonyl function becomes a 4-substituent in the pyridine.[2] Recently we have reported adduct formations from an α,β-unsaturated acid, ester, nitrile and ketones.[3] We herein report on the behaviour of (*Z*)-β-halo-α,β-unsaturated carbonyl dienophiles towards 4-methyl-5-ethoxyoxazole. Our new reaction leads to a novel introduction of a 6-heterosubstituent into the pyridine ring.



Scheme 1.

0302-4369/83 $2.50

The Diels-Alder adduct which is slowly formed from a mixture of 4-methyl-5-ethoxyoxazole and (*Z*)-3-bromo- or 3-chloropropenoic acid without a solvent, is unstable under the acid conditions of the reaction and is transformed as formed to a mixture of two β-hydroxypyridines. The 3-hydroxy-5-halopyridine *4* (*5*) has the expected substitution pattern,[3] except for the 4-carboxy group which has been lost. The other product has been identified as the 6-ethoxy derivative *7b*, which involves the loss of HCl (or HBr) from the intermediate adduct *2* (*3*). As shown in the formation of the 4-benzoyl derivative *9*, the latter type of reaction can also be effected from a β-chlorovinyl ketone.

The formation of the two types of products can be rationalized by a common cationic intermediate *10* which is formed by acid catalyzed cleavage of the *C*(1)−*O*(7) bond in the Diels-Alder adduct *2*. The cleavage may initially be catalyzed by the acidity of the carboxy function in the adduct, or more likely by the acidity of the 3-halopropenoic acid (pK_a 3.32 for 3-chloropropenoic acid).[4] Decarboxylation of the intermediate *10* leads to the 3-hydroxy-5-chloropyridine *4*. In a competitive reaction, however, the ethanol liberated can add to the cationic carbon giving the adduct *11;* the latter on HCl and ethanol elimination yields the 4-carboxypyridine *7b*. In the presence of added ethanol *7b* was obtained, which supports the postulated reaction paths. In the presence of methanol or 2-propanol the corresponding 6-methoxy *7a* and 6-propyloxy *7c* derivatives were formed. This novel way of introducing 6-substituents was further explored in the reaction of *2* with ethane-1,2-diol which gave the monosubstituted diol *7d*. In thiophenol the corresponding 6-thioether *7e*, and in aniline the corresponding 6-amine *7f*, were formed. By analogy, the reaction in aqueous ethanol led to the introduction of a hydroxy group and hence to the formation of the 2-pyridinone *8*.

The assignment of structure *7b*, rather than the isomeric 3-alkoxy-2-pyridinone structure *12* to the degradation product from the adduct *2*, is based on spectroscopy and chemical properties; *12* could be visualized as formed by initial cleavage of the *C*(4)−*O*(7) bond in *2*. Thus the product was not decarboxylated on heating in anisole, it was sublimed unchanged on heating at 200 °C at reduced pressure, and it was not decarboxylated when its vapour was passed through a small quartz tube (length 20 cm) kept in an oven at 400 °C. The resistance towards decarboxylation is best rationalized by intramolecular hydrogen bonding between vicinal hydroxy and carboxy groups as in *7b*, but not in *12*.

Potentiometric titration[5] gave pK_a 3.9 for *7b*, which is *ca.* 1 unit lower than the value (pK_a 4.86) reported [6] for 4-picolinic acid. The pK_a 4.20 for benzoic acid is reduced by *ca.* 1.3 units in 2-hydroxybenzoic acid; a 2- or 3-ethoxy group has little effect on the pK_a of benzoic acid.[7] The pK_a values therefore support the assignment of structure *7b*, which is further supported by the pK_a 3.8 for 4-carboxy-5-hydroxy-6-methyl-2-pyridinone *8*, which was determined by the same technique.

In ^{13}C NMR the chemical shifts for the pyridine carbons in 3-hydroxy- and 3-ethoxypyridine pairs are very similar; the shifts in 2-hydroxy and 2-ethoxy pairs, however, differ significantly.[8] The ^{13}C NMR shifts for *7b* were: δ 144.4 (C−2), 144.7 (C−3), 122.2 (C−4), 105.3 (C−5) and 154.9 (C−6), which are to be compared with the corresponding shifts for *8:* δ 147.7 (C−2), 140.9 (C−3), 126.1 (C−4), 107.3 (C−5) and 155.7 (C−6). The significant differences in the chemical shifts for the same positions in the two molecules are again interpreted in favour of the assigned structure *7b*.

EXPERIMENTAL

Mass spectra are presented as [70 eV; *m/z* (% rel.int.)].

2-Methyl-3-hydroxy-5-chloropyridine 4 *and 2-methyl-3-hydroxy-4-carboxy-6-ethoxypyridine* 7b. 4-Methyl-5-ethoxyoxazole[3] (1.27 g, 0.01 mol) and (*Z*)-3-chloropropenoic acid[4] (1.06 g; 0.01 mol) were stirred together at room temperature for 1 week. The reaction mixture was then dissolved in acetone and dry HCl was bubbled through the solution. The precipitated salt was dissolved in water and the solution neutralized with NaOH when the title compound *4* was precipitated; yield 0.11 g (8 %), m.p. 174 °C (H_2O). Anal. C_6H_6ClNO: C, H. ^1H NMR (NaOD/D_2O): δ 2.30 (2−Me), 6.90 (H−4, *J* 2 Hz), 7.50 (H−6). MS: 145/143 (33/100, *M*), 116 (7), 114 (18), 108 (9), 80 (18).

The acetone filtrate above was evaporated and the residue triturated with dichloromethane and recrystallized from water; yield of *7b* (13 %),

m.p. 212 °C. Analytical data are given below.

2-Methyl-3-hydroxy-5-bromopyridine 5 was prepared as above from (*Z*)-3-bromopropenoic acid[9] in 12 % yield, m.p. 221 °C (decomp.). Anal. C_6H_6BrNO: C, H. ^1H NMR (NaOD/D_2O): δ 2.27 (2−Me), 7.07 (H−4, *J* 2 Hz), 7.62 (H−6). MS: 189 (98, M), 187 (100, M), 160 (21), 158 (21), 119 (10), 117 (11), 108 (14), 81 (20), 80 (32).

2-Methyl-3-hydroxy-4-carboxy-6-ethoxypyridine *7b* was isolated from the acetone filtrate as above in 12 % yield.

2-Methyl-3-hydroxy-4-carboxy-6-methoxypyridine 7a. 4-Methyl-5-ethoxyoxazole[3] (1.27 g, 0.01 mol) and (*Z*)-3-chloropropenoic acid (1.06 g, 0.01 mol) were added to methanol (1 ml) and the solution left at room temperature for 1 week. The solvent was then distilled off, the residue triturated with dichloromethane and recrystallized from water; yield 0.61 g (33 %), m.p. 228 °C. Anal. $C_8H_9NO_4$: ^1H NMR (NaOD/D_2O): δ 2.29 (2−Me), 3.77 (6−OMe), 6.52 (H−5). IR(KBr): 2550 and 1660 cm^{-1} (CO_2H). MS: 183 (37, M), 165 (100), 137 (20), 136 (28), 109 (16), 94 (27).

When (*Z*)-3-bromopropenoic acid was used instead of the chloro analogue, the yield of *6a* was about the same.

2-Methyl-3-hydroxy-4-carboxy-6-ethoxypyridine 7b was prepared as above in ethanol solution: yield 29 %, m.p. 212 °C (H_2O). Anal. $C_9H_{11}NO_4$: C, H. ^1H NMR (NaOD/D_2O): δ 1.30 and 4.10 (OEt), 2.32 (2−Me), 6.63 (H−5). IR (KBr): 2500 and 1665 cm^{-1} (CO_2H). MS: 197 (63, M) 182 (17), 179 (100), 164 (22), 151 (74), 123 (58), 95 (30).

2-Methyl-3-hydroxy-4-carboxy-6-(2-propyloxy) pyridine 7c was prepared as above in 2-propanol solution; yield 24 %, m.p. 198−200 °C (H_2O): MS molecular ion: *m/z* 211.0839; calc. for $C_{10}H_{13}NO_4$: 211.0845. ^1H NMR (NaOD/D_2O): δ 1.30 and 4.12 (*O*-2-Pr), 2.27 (2−Me), 6.59 (H−5). IR (KBr): 2600 and 1665 cm^{-1} (CO_2H). MS: 211 (42, M), 193 (100), 165 (33), 137 (23), 95 (14), 67 (19).

2-Methyl-3-hydroxy-4-carboxy-6-(2-hydroxyethyloxy)pyridine 7d was prepared from 4-methyl-5-ethoxyoxazole (1.56 g, 0.012 mol), (*Z*)-3-chloropropenoic acid (1.32 g, 0.012 mol) and ethane-1,2-diol (1.5 g, 0.025 mol); the mixture was stirred for 2 weeks at room temperature when it was almost solidified. This mixture was triturated with chloroform and the residual material recrystallized from water; yield 0.58 g (25 %), m.p. 218 °C. Anal. $C_9H_{11}NO_5$: C, H. ^1H NMR (NaOD/D_2O): δ 2.25 (2−Me), 6.47 (5−H), 3.95 (CH_2CH_2). IR (KBr): 3470 (OH), 2500 and 1670 cm^{-1} (CO_2H). MS: 213 (22, M), 195 (25), 169 (28), 151 (100), 123 (58), 95 (21).

2-Methyl-3-hydroxy-4-carboxy-6-phenylthiopyridine 7e. 4-Methyl-5-ethoxyoxazole (0.63 g, 0.005 mol), (*Z*)-3-chloropropenoic acid (0.53 g, 0.005 mol) and thiophenol (1 g, 0.01 mol) were mixed and stirred together for 1 week at room temperature. The semisolid reaction mixture was then triturated with chloroform and the residue recrystallized from water; yield 0.32 g (25 %), m.p. >340 °C. Anal. $C_{13}H_{11}NO_3S$: C, H. ^1H NMR (NaOD/D_2O): δ 2.33 (2−Me), 7.27 (Ph), 7.30 (H−5). IR (KBr): 2500 and 1685 cm^{-1} (CO_2H). MS: 261 (61, M), 244 (19), 243 (100), 215 (14), 214 (49), 186 (26), 146 (13), 145 (13).

2-Methyl-3-hydroxy-4-carboxy-6-phenylaminopyridine 7f. 4-Methyl-5-ethoxyoxazole (0.63 g, 0.005 mol), (*Z*)-3-chloropropenoic acid (0.53 g, 0.005 mol) and anilin (0.92 g, 0.01 mol) were mixed and stirred together for 2 weeks at room temperature. The precipitate formed was collected by filtration and washed with chloroform and crystallized from water; yield 0.13 g (11 %), m.p. >340 °C. MS: molecular ion *m/z* 244.0848; calc. for $C_{13}H_{12}N_2O$: 244.0846. ^1H NMR (NaOD/D_2O): δ 2.28 (2−Me), 6.06 (H−5), 6.8−7.4 (Ph). IR (KBr): 3140 (NH), 2800 and 1670 cm^{-1} (CO_2H). MS: 244 (86, M), 227 (13), 226 (100), 198 (20), 170 (61), 169 (17), 129 (53).

4-Carboxy-5-hydroxy-6-methylpyridin-2-one 8. 4-Methyl-5-ethoxyoxazole[3] (1.27 g, 0.01 mol) and (*Z*)-3-chloropropenoic acid[4] (1.06 g, 0.01 mol) were dissolved in ethanol (4 ml) and water (1 ml) and the solution left at room temperature for 1 week. Evaporation of the solution left a solid which was triturated with dichloromethane and recrystallized from water; yield 0.22 g (13 %), m.p. 300 °C (decomp.). Anal. $C_7H_7NO_4$: C, H. ^1H NMR (NaOD/D_2O): δ 2.17 (6−Me), 6.12 (H−3). IR (KBr): 2550 and 1685 cm^{-1} (CO_2H). MS: 169 (44, M) 151 (100), 123 (67), 95 (43), 82 (11), 67 (44).

2-Methyl-3-hydroxy-4-benzoyl-6-methoxypyridine 9. (*E*)-1-Phenyl-3-chloro-2-propen-1-one[10] (1.66 g, 0.01 mol) and 4-methyl-5-ethoxyoxazole (1.27 g, 0.01 mol) were dissolved in methanol (15 ml) and the solution left at room temperature for 3 d before the solvent was distilled off. The residue was subjected to preparative TLC on silica gel (Merck PF 254) using dichloromethane as developer. The appropriate chromatographic band was scraped off from the plates and the title compound extracted into dichloromethane and recovered by evaporation of the solvent; yield 0.14 g (6 %), m.p. 106−107 °C. Anal. $C_{14}H_{13}NO_3$: C, H. ^1H NMR ($CDCl_3$): 2.49 (2−Me), 3.85 (OMe), 6.63 (H−5), 7.7 (Ph). IR (KBr): 1670 cm^{-1} (CO). MS: 243 (20, M), 242 (11), 148 (8), 147 (8), 105 (100), 77 (59).

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Synthesis of Strombine. A New Method for Monocarboxymethylation of Primary Amines

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Many common methods for monocarboxymethylation of primary amines give various amounts of dialkylated by-products. In this work the reaction of two equivalents of glyoxylic acid with representative primary aliphatic and aromatic amines, as well as with amino acids and a dipeptide, is shown to give only the *N*-(carboxymethyl)-*N*-formyl derivative of the amine under mild conditions in carboxylic acid solvents. Hydrolysis then produces the monocarboxymethylated primary amine in good to excellent overall yield. Proof that the intermediate product is not obtained *via* the Leuckart reaction is given.

Some monocarboxymethylated amino acids and dipeptides have interesting biological functions. In 1975, strombine *3b* was isolated from the clam *Strombus gigas* by Sangster *et al.* and was found to induce small fish to display so-called "exploratory feeding behaviour" in dilutions down to 10^{-8} g/l.[1,2] Recently, a series of substituted *N*-(carboxymethyl)dipeptides has been prepared and demonstrated to be active as inhibitors of angiotensin converting enzyme, which is involved in the control of blood pressure.[3]

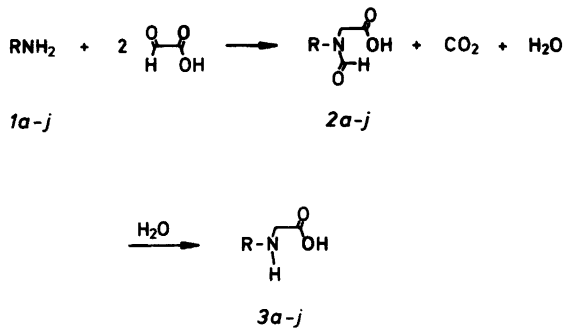
In an attempt to synthesize strombine, we found that alkylation of L-alanine with chloroacetic acid according to Sangster *et al.*[1] produced a very poor yield. This route to *N*-(carboxymethyl)amino acids, as well as their properties and their incorporation into peptides, has recently been extensively studied by Miyazawa.[4]

Seeking an alternative route, we considered a reductive amination of alanine with glyoxylic acid by conventional methods. In an attempted Leuckart reaction with alanine *1b* and glyoxylic acid in 98–100 % formic acid we were surprised to observe evolution of carbon dioxide even at room temperature, since the Leuckart reaction usually requires heating at above 150 °C.[5] The reaction product was *N*-formylstrombine *2b*, which was readily hydrolyzed to give strombine *3b* in a good overall yield. (*cf.* Scheme 1).

In the beginning of this century, Erlenmeyer and co-workers[6] reported a similar reaction between ammonia or $(NH_4)_2CO_3$ and α-oxoacids such as glyoxylic, pyruvic and phenylpyruvic acid, which afforded the corresponding *N*-acylated amino acids, in analogy with the first step of Scheme 1. It thus appeared that our case might represent an extension of the Erlenmeyer procedure, and we therefore set out to study its scope and limitations as a method for monocarboxymethylation of primary amines.

Normal Leuckart conditions usually afford a mixture of mono- and dialkylated products. The monoalkylated products become partly formylated by reaction with excess formic acid. On the other hand, the glyoxylic acid reaction studied here consistently yielded the monoalkylated formamide derivatives *2* as the first isolable products. Furthermore, at the relatively low temperatures employed in the present procedure no formylation of Leuckart monoalkylation products *3* with formic acid was found to occur. It is also significant that the reaction took the same general course in a variety of solvents, such as formic, acetic and trifluoroacetic acid, as well as in *N*,*N*-dimethylformamide. This suggests that the carbon dioxide evolved (and the formyl

0302-4369/83 $2.50

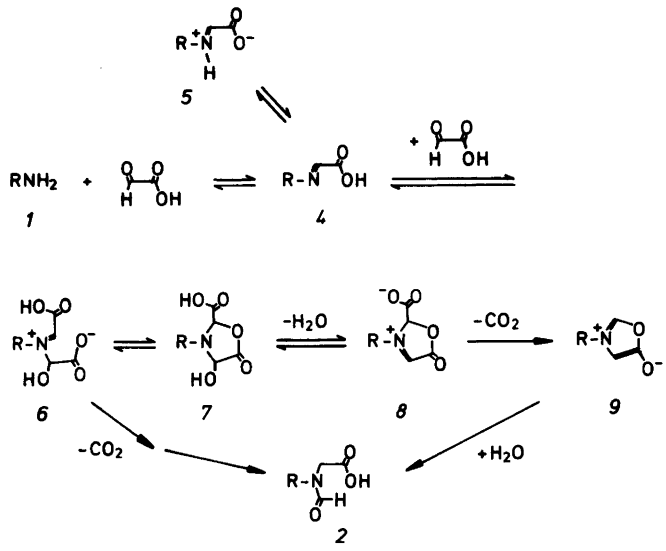


Scheme 1. R= *a*: CH_2CO_2H, *b*: CH_3CHCO_2H, *c*: $PhCHCO_2H$, *d*: $H_2N(CH_2)_4CHCO_2H$, *e*: $CH_3CH_2CH_2$, *f*: $(CH_3)_2CH$, *g*: $(CH_3)_3C$, *h*: *p*-$PhCO_2H$, *i*: *p*-$PhNO_2$, *j*: $-CH(CH_3)CONHCH(CH_3)CO_2H$.

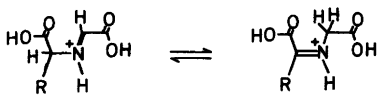
residue on the amine) originates from glyoxylic acid, and not from the formic acid solvent as in the Leuckart reaction. A mechanism that accommodates our observations is proposed in Scheme 2.

Increasing the water content of the carboxylic acid solvent to 20 % strongly retards the reaction, in accord with the first steps of the mechanism leading to the Schiff's base *4*. That a more acidic solvent decreases the rate of reaction by protonating the amine, is demonstrated by the primary aliphatic amines. Reaction in acetic acid at 40 °C is complete within a few hours, whereas use of formic acid requires more drastic conditions (*cf.* Table 1). Unfortunately, decarboxylation of glyoxylic acid giving formaldehyde (proposed to occur through intermediates *4* and/or *5* [7]) occurs to an extent of approximately 50 % in acetic acid, but is suppressed in formic acid. This dependence of decarboxylation on acidity has been described by von Euler *et al.*[7] Analogously, trifluoroacetic acid has to be used instead of formic acid in conjunction with the less basic aromatic amines. Nonacidic solvents seem to enhance by-product formation even more.

Racemization of an optically active amino acid might occur *via* the equilibrium in Scheme 3. However, when products *3* from DL- and L-



Scheme 2.



Scheme 3.

alanine were derivatized with L-proline and analyzed by GLC[8], neither racemization (optical purity >97 %) nor transamination, or aldol condensation with glyoxylic acid was observed.

The reaction of the Schiff's base *4* with a second molecule of glyoxylic acid may conceivably occur *via* alternative routes, *e.g.* by direct attack of the imino nitrogen of *4* at the formyl group of the acid as indicated in Scheme 2, or by a preceding addition of the carboxyl group over the C=N double bond of *4* followed by a ring closure to *7*. The end product *2* could be formed by decarboxylation of the open chain isomer *6* but the latter would be expected to cyclize very readily to the hydroxyoxazolidone *7*, which in turn might be in equilibrium with the dehydration product *8*. This should be prone to decarboxylate, forming the mesoionic oxazolone derivative *9*. Intermediates of this type have been invoked in the Dakin-West reaction[9] and the racemization of *N*-acylamino acids. Spontaneous hydrolysis of the oxazolone would then yield the end product *2*.

The derivatives *2* were obtained in better than 80 % yield (NMR), but did not crystallize,[4b] with the exception of the *p*-aminobenzoic acid derivative. The split formyl proton signals of these products arise from amide rotamers[10] (*cf.* Table 1).

While no problems were encountered in the acid hydrolysis of the formamides derived from the amino acids *2a–d*, the aliphatic amines *2e–g* or the dipeptide *2j*, hydrolysis of the aromatic formamides *2h,i* required more care. For example, hydrolysis of the *p*-aminobenzoic acid derivative under acidic catalysis led to decarboxylation of the aromatic carboxyl group. Consequently, alkaline hydrolysis was used for these two derivatives.[11]

That the difference in reactivity between primary aliphatic amines and α-amino acids (*cf.* Table 1) allows selectivity is demonstrated by the case of lysine monohydrochloride, which gave N^2-(carboxymethyl)lysine in 62 % overall yield under α-amino acid conditions. The position of reaction was assigned from the ^{1}H NMR spectra of N^2-(carboxymethyl)-N^2-formyllysine *2d*, where the C^2-hydrogen is shifted 0.65 ppm downfield relative to lysine whereas the C^6 hydrogens overlap for the two compounds. The assignment was corroborated by ^{13}C NMR (see Experimental part).

In conclusion, the glyoxylic acid reaction appears to be a generally useful method for monocarboxymethylation of primary amines, amino acids and peptides.

EXPERIMENTAL

^{1}H NMR spectra were run on either a Jeol JNM-PMX60 instrument at 60 MHz or on a Nicolet WB360 instrument at 360 MHz. TMS or 3-trimethylsilylpropanesulfonic acid (TriMS) were used as internal references. Mass spectra were recorded on a single-focusing, non-commercial instrument at 70 eV ionization potential. Melting points were determined with a Mettler FP1 instrument (temperature increase 2 °/min until melting begins), and are uncorrected.

Reactions were followed with TLC and/or HPLC. The following TLC-systems were used;

TLC 1: EtOAc–HOAc–H_2O= 2:2:1 on silica gel

TLC 2: EtOAc–HOAc–H_2O= 2:2:1 on cellulose

TLC 3: EtOAc–HOAc–HCO_2H–H_2O= 18:3:1:4 on silica gel

TLC 4: EtOAc–HOAc= 19:1 on silica gel

HPLC was run on a C-18 column (Merck LiChrosorb 5 μm silanized with octadecyldimethylchlorosilane) with UV-detection at 205 nm. A 20 mM phosphate buffer (pH=5.5) made 20 mM with $(C_4H_9)_4N^+HSO_4^-$ mixed with various amounts of methanol was used as eluent (flow rate: 1 ml/min). The following methanol concentrations were employed: HPLC 1: 5 %, HPLC 2: 25 %, HPLC 3: 32.5 %, HPLC 4: 40 %. The systems used for each amine are specified under the corresponding product in this section.

Amberlite CG-120 (200–400 mesh, H-form) cation exchanger was used for separations.

General synthetic procedure. (Detailed experimental conditions with the specific starting amines *1a–j* are given in Table 1.) To the

Table 1. Experimental conditions, yields and NMR shifts of formyl protons in *2a–j*.

Starting amine	Step 1 Solvent[a]	Temp./ °C	Time/ h	Step 2 Method of hydrolysis[b]	Time/ h	Product (% isolated yield)	NMR shifts of formyl proton[c]
Glycine (*1a*)	A	40	2.5	C	5	*3a*[12] (64)	8.17[d]
L-Alanine (*1b*)	A	40	4	C	5	*3b*[1,4a] (69)	8.27, 8.13[d]
D-Phenylglycine (*1c*)	A	40	4	C	5	*3c*[4a] (77)	8.18, 8.08[d]
L-Lysine HCl (*1d*)	A	40	4	C	5	*3d*[13] (62)	8.05, 8.00[d]
Propylamine (*1e*)	A	70	14	C	5	*3e*[14] (93)	8.18, 8.12[e]
Isopropylamine (1f)	A	70	14	C	5	*3f*[14] (90)	8.22, 8.13[e]
tert-Butylamine (*1g*)	A	70	14	C	5	*3g*[14] (77)	8.53[e]
p-Aminobenzoic acid (*1h*)	B	25	2.5	D	4	*3h*[15] (65)	8.80, 8.43[f]
p-Nitroaniline (1i)	B	25	1.5	D	0.5	*3i*[16] (92)	9.97[f]
L-Alanyl-L-alanine (*1j*)	A	40	4	E	1.5	*3j* (51)	8.30, 8.10[d]

[a] A:98–100 % HCO_2H; B:98–100 % CF_3CO_2H. [b] C:20 ml 1M HCl; D:20 ml 2M NaOH; E:30 ml 0.5 M HCl. [c] When two signals appear the largest is given first. [d] D_2O, TriMS as reference. [e] Acetone-d_6, TMS as reference. [f] DMSO-d_6, TMS as reference.

appropriate solvent (100 ml) at the specified temperature the amine (10 mmol) was added first, and then glyoxylic acid monohydrate (21 mmol, 1.93 g). The reaction was allowed to proceed with magnetic stirring and a reflux condenser was used when necessary. The reactions of the aromatic amines *1h* and *1i* were carried out under an inert nitrogen atmosphere. Evaporation of the solvent after the time specified gave the formyl derivative *2a–j*. Formyl derivatives *2d* and *2h* were purified before the hydrolysis step. The formyl derivative was then hydrolyzed at 100 °C, with the exception of *2i*, which underwent hydrolysis at 25 °C. The hydrolysate was then evaporated to dryness, with the exception of *3h* and *3i* which were precipitated with 6 M aqueous HCl. Portions of water were added and evaporated to remove excess hydrochloric acid before the final purification of the product, as specified for each compound below.

N-*(Carboxymethyl)glycine* 3a. The free amino acid was obtained by elution from a cation exchanger with 0.5 M aqueous NH_3 and purified by recrystallization from water–acetone. *3a* was analyzed by systems HPLC 1 and TLC 1; m.p. 229–230 °C(dec.). Anal. $C_4H_7NO_4$: C, H, N, O. ^{1}H NMR [60 MHz, TriMS, D_2O]: δ 3.88 (4 H, s).

N-*(Carboxymethyl)*-L-*alanine hydrochloride (strombine) 3b* was obtained by recrystallization from ethanol–ether and was analyzed by systems HPLC 1 and TLC 1; m.p. 168–169 °C(dec.), $[\alpha]_D^{20}$ +6.81° (*c* 5, 6 M HCl). Found: C 32.25; H 5.37; N 7.75; Cl 19.25; O 34.7. Calc. for $C_5H_{10}NClO_4$: C 32.71; H 5.49; N 7.63; Cl 19.31; O 34.86. ^{1}H NMR (60 MHz, TriMS, D_2O): δ 1.63 (3 H, d, *J* 7.0 Hz), 4.07 (2 H, s), 4.23 (1 H, q, *J* 7.0 Hz). The optical purity of synthetic *3b* was determined to be better than 97 % by convertion of the amino acid to its dimethyl ester followed by derivatization with *N*-(trifluoroacetyl)-L-prolyl chloride.[8] The diastereomeric derivatives were then analysed by GLC on a 47 m SE-52 glass capillary column, temperature programmed as follows: initial 105 °C (3 min), linear increase from 105 to 300 °C by 10 °C/min.

N-*(Carboxymethyl)*-D-*phenylglycine* 3c. The free amino acid was obtained by elution from a cation exchanger with 0.5 M aqueous NH_3 and was purified by recrystallization from water–ethanol–ether. *3c* was analyzed by system HPLC 2; m.p. 209–210 °C(dec.), $[\alpha]_D^{20}$ −139.4° (*c* 5, 6 M HCl). Anal. $C_{10}H_{11}NO_4$: C, H, N. ^{1}H NMR (60 MHz, TriMS, DMSO-d_6): δ 3.30 (2 H, s), 4.60 (1 H, s), 7.43 (5 H, s).

N^2-*(Carboxymethyl)*-L-*lysine monohydrate* 3d. *2d* was separated from unreacted lysine on a cation exchanger. After hydrolysis *3d* was converted to the free amino acid by elution from a cation exchanger with 0.5 M aqueous NH_3 and purified by recrystallization from water–ethanol. *3d* was analyzed by systems HPLC 2 and TLC 2; m.p. 248–250 °C(dec.), $[\alpha]_D^{20}$ +21.0° (*c* 5, 6 M HCl). Anal. $C_8H_{18}N_2O_5$: C, H, N. ^{1}H NMR (60 MHz, TriMS, D_2O): δ 1.63 (6 H, m), 3.05 (2 H,

t, *J* 6.5 Hz), 3.65 (2 H, s), 3.72 (1 H, t, *J* 5.0 Hz). ^{13}C NMR (100 MHz, TriMS, D_2O): δ 41.2 (C6), 64.1 (C2), [values for lysine: δ 40.9 (C6), 56.6 (C2)].

N-*Propylglycine hydrochloride 3e* was purified by recrystallization from ethanol–ether and analyzed by systems HPLC 2 and TLC 3; m.p. 172–173 °C(dec.). Anal. $C_5H_{12}NClO_2$: C, H, N. ^{1}H NMR (60 MHz, TriMS, D_2O): δ 0.98 (3 H, t, *J* 7.0 Hz), 1.67 (2 H. m), 3.12 (2 H, t, *J* 7.0 Hz), 3.98 (2 H, s).

N-*Isopropylglycine hydrochloride 3f* was purified and analyzed in the same way as *3e*; m.p. 182–183 °C(dec.). Anal. $C_5H_{12}NClO_2$: C, H, N. ^{1}H NMR (60 MHz, TriMS, D_2O): δ 1.33 (6 H, d, *J* 6.0 Hz), 3.52 (1 H, m), 3.95 (2 H, s).

N-tert-*Butylglycine hydrochloride 3g* was purified and analyzed in the same way as *3e*; m.p. 223–224 °C(dec.). Anal. $C_6H_{14}NClO_2$: C, H, N. ^{1}H NMR (60 MHz, TriMS, D_2O): δ 1.40 (9 H, s), 3.93 (2 H, s).

N-*(p-Carboxyphenyl)-N-formylglycine* 2h. After evaporation of the CF_3CO_2H solvent, unreacted *p*-aminobenzoic acid was removed by washing with 1 M aqueous HCl. *2h* was analyzed with system HPLC 3; m.p. 226–228 °C(dec.). Anal. $C_{10}H_9O_5N$: C, H, N. ^{1}H NMR (60 MHz, TriMS, DMSO-d_6) δ 4.53 (2 H, s), 7.47 (2 H, d, *J* 8.4 Hz), 8.03 (2 H, d, *J* 8.4 Hz), 8.80 (1 H, s). The dimethyl ester of *2h* was obtained by treatment of the amino acid with diazomethane. MS [*m/e* (% rel. int.)]: 251 (10, M), 223 (31), 220 (6.6), 192 (12), 164 (100), 132 (11), 104 (7.0), 77 (6.9). ^{1}H NMR (360 MHz, $CDCl_3$): δ 3.78 (3 H, s), 3.94 (3 H, s), 4.55 (2 H, s), 7.26 (2 H, d, *J* 8.6 Hz), 8.10 (2 H, d, *J* 8.6 Hz), 8.62 (1 H, s).

N-*(p-Carboxyphenyl)glycine 3h* was recrystallized by dissolving in aqueous Na_2CO_3 followed by precipitation with 2 M aqueous HCl. *3h* was analyzed by system HPLC 3; m.p. 233–234 °C(dec.). ^{1}H NMR (60 MHz, TriMS, DMSO-d_6): δ 3.97 (2 H, s), 6.67 (2 H, d, *J* 8.4 Hz), 7.80 (2 H, d, *J* 8.4 Hz). The dimethyl ester of *3h* was obtained by treatment of the amino acid with diazomethane. MS [*m/e* (% rel. int.)]: 223 (33, M), 192 (12), 178 (5.1), 164 (100), 132 (10), 120 (6.0), 105 (10), 104 (6.7). ^{1}H NMR (360 MHz, $CDCl_3$): δ 3.81 (3 H, s), 3.85 (3 H, s), 3.97 (2 H, s), 6.57 (2 H, d, *J* 8.6 Hz), 7.89 (2 H, d, *J* 8.6 Hz).

N-*(p-Nitrophenyl)glycine 3i* was recrystallized in the same way as *3h* and analyzed by systems HPLC 4 and TLC 4; m.p. 218–219 °C(dec.). ^{1}H NMR (60 MHz, TriMS, DMSO-d_6): δ 4.04 (2 H, s), 6.72 (2 H, d, *J* 9.0 Hz), 8.08 (2 H, d, *J* 9.0 Hz). The methyl ester of *3i* was obtained by esterification with diazomethane. MS [*m/e*, (% rel. int.)]: 210 (26, M), 151 (100), 135 (3.4), 121 (3.4), 105 (44), 76 (6.5). ^{1}H NMR (360 MHz, $CDCl_3$): δ 3.84 (3 H, s), 4.00 (2 H, s), 6.56 (2 H, d, *J* 8.6 Hz), 8.12 (2 H, d, *J* 8.6 Hz).

(N-(Carboxymethyl)-L-alanyl)-L-alanine hydrochloride 3j was purified by recrystallization from ethanol–ether and analyzed by system TLC 1; m.p. 166–168 °C(dec.). ^{1}H NMR (60 MHz, TriMS, D_2O): δ 1.47 (3 H, d, *J* 11.2 Hz), 1.60 (3 H, d, *J* 8.0 Hz), 3.97 (2 H, s), 4.18 (1 H, q, *J* 11.2 Hz), 4.43 (1 H, q, *J* 8.0 Hz). The dimethyl ester of *3j* was obtained by esterification with diazomethane. MS [*M/e* (% rel. int.)]: 246 (2.3, M), 187 (22), 130 (60), 117 (10), 116 (100), 70 (9.6), 59 (10), 57 (7.9), 56 (79). ^{1}H NMR (60 MHz, acetone-d_6): δ 3.73 (6 H, s, methyl esters).

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The Reduction Potential of Lactoperoxidase

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The reduction potential of Fe(III)/Fe(II) lactoperoxidase has been determined. Optical determinations of equilibria with 2-methyl-3-hydroxy-1,4-naphthoquinone as indicator and prereduced 9,10-anthraquinone-2-sulfonate as reducing agent gave $E_{m,7.0}$= −191±2 mV. Potentiometric determinations with 9,10-anthraquinone-2-sulfonate as mediator and, in the reduced form as reducing agent, gave $E_{m,7.0}$= −188±1 mV.

Addition of 0.5 % *N*-cetyl-*N,N,N*-trimethylammonium bromide, and using dithionite as reducing agent, gave $E_{m,7.0}$= −183 mV and −179 mV with 9,10-anthraquinone-2-sulfonate and 9,10-anthraquinone-2,6-disulfonate, respectively, as mediators.

Lactoperoxidase* consists of a single peptide chain,[1,2] carrying 10 % carbohydrates and one heme group[1] to give M_r= 78,500. The tertiary structure is maintained by eight disulfide bridges.[1,2] Acidic butanone will split LP into protoheme and protein only after digestion of the enzyme with pronase[3,4] or reduction of -S–S- bridges with dithiotreitol and rupture of H-bonds with 5 M guanidine (unpublished results). The spectrum of LP Fe(III) shows four absorption maxima within the range 450–650 nm, and its Soret maximum, at 412 nm, is shifted 10 nm bathochromically from that of HRP. If protoheme in HRP C2 is replaced by 2,4-diacetyldeuteroheme the spectrum becomes similar to that of LP and the redox potential at pH 7 increases from −246 mV to −109 mV.[5,6] A mixed type of spectrum is also found in turnip peroxidase P_7 with $E_{m,7.0}$−120 mV.[7] Thus in two other peroxidases a mixed type spectrum, such as that found in LP, is associated with a redox potential, rather positive for a peroxidase. The stability of the heme-protein bonds in LP and its peculiar spectrum have been attributed to an unusually narrow heme-accomodating cleft.[8] This would reduce the exposure of the heme to the solvent and increase the redox potential.[9] The properties of the heme surroundings as well as the spectrum focus interest on the redox potential of LP, hitherto unknown.

MATERIALS AND METHODS

LP was prepared from cow's milk.[10] The first main fraction to emerge from DEAE-Sephadex® (Pharmacia, Uppsala) in 10 mM TRIS-HCl buffer (pH 9.0 at 25 °C) at 5 °C was used. It showed A_{Soret}/A_{280}=0.96. On the basis of dry weight determinations after dialysis against 5 mM acetic acid, ε_{412}=112.3±0.9 (n=5) $mM^{-1} \cdot cm^{-1}$ was obtained, in agreement with ε_{mM}=112.2 for Carlström's corresponding fraction, B1.[3]

A detailed description of the redox cell used in this study is given elsewhere.[11] The bright Pt electrode surfaces were purified by cyclic voltammetry and submersed into an 0.1 KI solution for 3 min. The resulting absorbed layer of iodine prevented uncontrolled adsorption of dyes, enzyme, detergents and grease, but gave an active surface for potentiometry.[12] The high tendency of LP to precipitate could finally be overcome by using He for deaeration and a magnetic bar in KOH-treated glass for intermittent stirring. The turbidity caused by stirring, and its dependence

* Abbreviations: Lactoperoxidase, LP; horseradish peroxidase, HRP (E.C.1.11.1.7); 2-methyl-3-hydroxy-1,4-naphthoquionone, phthiocol; 9,10-anthraquinone-2-sulfonate, AQS; 9,10-anthraquinone-2,6-disulfonate, AQS_2; sodium dodecylsulfate, SDS; *N*-cetyl-*N,N,N*-trimethylammonium bromide, CETAB.

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upon the type of gas used during deaeration,[11] became more pronounced in the presence of non-ionic detergents like Triton® X-100 (0.1 %) and Tween 80 (0.5 %). 0.5 % SDS gave an instantaneous, complete precipitation of LP followed by a slow, but complete dissolution. 0.5 % CETAB, however, allowed stirring and flushing with Ar without any formation of turbidity, CETAB (0.5 %) in 100 mM sodium phosphate buffer pH 6.0 successfully eluted LP from an octyl-Sepharose® column whereas benzene, sodium octanoate, and several other additives did not.[10] Trials pointed at AQS, $E_{m,7.0}$= −225 mV, AQS_2, $E_{m,7.0}$= −184 mV[13,14], and phthiocol, $E_{m,7.0}$= −168 mV[11] as suitable mediators. Mediators and solutions were protected against light.

Commercially available He, Ar and H_2 (AGA, Stockholm) were purified by passage through an Oxy-Trap® system (Alltech. Ass., Ill.) to give O_2 <0.1 ppm. Fresh sodium dithionite (Merck, Darmstadt) was divided under argon into 2 ml tubes, which were sealed with paraffin wax and stored dark and dry at −20 °C. AQS and AQS_2 (BDH, England) were recrystallized from hot water and air-dried at room temperature. Other chemicals were of analytical grade. Water was bidistilled in glass vessels. All experiments were made in 100 mM sodium phosphate pH 7.0, 25 °C. Literature quotations refer to this pH and temperature. Potentials were registered with a battery powered potentiometer (Radiometer PHM 4, Copenhagen) and spectra with a Beckman ACTA®III or a Beckman DU 7® spectrophotometer. These were calibrated against a holmiumoxide filter and potassium dichromate solutions in 0.005 M sulphuric acid for wavelength accuracy and absorbance linearity, respectively.

Spectra were scanned over the range 450−700 nm, and the ratios $[mediator]_{ox}/[mediator]_{red}$ and $[LP]_{ox}/[LP]_{red}$ for the Nernst formula were determined from absorbance spectra at suitable wavelengths.

RESULTS AND DISCUSSION

The dithionite ion, $S_2O_4^{2-}$, rapidly reduces LP to give LP Fe(II)-1 that spontaneously shifts to a second stable form, LP Fe(II)-2 (Fig. 1, Table 1). Usually heme proteins are reduced by the dithionite monomer at a rate of 10^4 to 10^5 M^{-1} s^{-1}.[15–17] The first order transformation of LP Fe(II)-1 to LP Fe(II)-2 is pH-dependent[3] with $k_{obs}=3.0\times10^{-3}$ s^{-1} at pH 7.0 (Fig. 1). Since $S_2O_4^{2-}$ is catalytically decomposed by LP[18] an alternative reductant was desirable, and AQS, prereduced with H_2 and platinum black, was chosen. AQS must be kept at a concentration about three times that of LP to mediate reduction (Table 2). Spectrophotometric titration of LP with AQS, monotoring the change in absorptivity, showed a change in slope when 2.9 AQS/LP had been added. The mode of binding is not known but LP may bind AQS and other mediators not only in the vicinity of the heme but also at other areas of the molecule.[10] Unlike HRP, LP does not give a hyperbolic (1:1) spectral change when aromatic donors are added,[10] but from affinity chromatography we estimated that LP can bind aromatic donors with K_d about one fifth of that for HRP C, found as 0.5−10 mM.[10] Neither phthiocol nor AQS in their oxidized or reduced forms interfered with

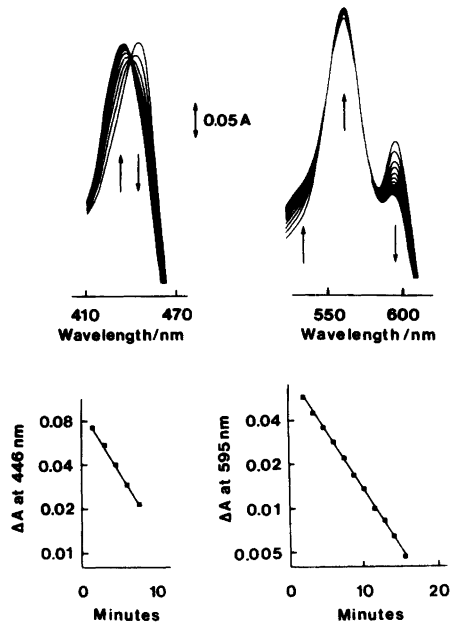

Fig. 1. Transformation of LP Fe(II)-1 to LP Fe(II)-2. In the Soret region (left) 5.3 and in the visible region 39.2 μM. 100 mM sodium phosphate, pH 7.0, 25 °C. First order plots of ΔA at 446 nm and 596 nm. 100 mM sodium phosphate pH 7.0, 25 °C.

Table 1. Absorption maxima LP Fe(III), LP Fe(II)-1 and LP Fe(II)-2. Isosbestic points for the two reduced forms and absorptivity in mM^{-1} cm^{-1} for some wavelengths are given (within parentheses). (s) Shoulder. 100 mM sodium phosphate pH 7.0, 25 °C.

Compound	Wavelength (nm)				
LP Fe(III)	412 (112.3)	501 (8.60)	542	585	630
LP Fe(II)-1	446 (77.8)		562 (13.6)	595	
Isosbestic points	408	440	502	552	573
LP Fe(II)-2	434 (94.6)		537(s)	565 (14.2)	595

the visible spectrum of oxidized or reduced LP. No precipitation occurred and the absorbances were additive.

Optical determinations of $E_{m,7.0}$. Phthiocol, with an absorptivity of the same magnitude as that of LP at about 500 nm, is only slowly reduced by H_2/Pt black. AQS was added to act as the mediator's mediator. AQS has too low absorptivity to be used alone. Typically, 29.9 μM LP was deaerated by bubbling He for eight hours without spectral changes. Deaerated solutions of phthiocol and AQS to give cuvette concentrations of 69.8 and 23.5 μM, respectively, were then added *via* the gas outlet from a gas-tight microsyringe. Then the gas was changed to H_2 and reduction *via* the Pt black electrode began. The spectrum of the system was scanned every 20 min during the first 2 h and then every 30 min. Data from this experiment are collected in Table 3. The ratios [ox]/[red] for phthiocol and LP were obtained from A_{630}, where only LP absorbs, and A_{565}, where both LP and phthiocol absorb. At both wavelengths the light absorption of AQS is negligible. The average of $E_{m,7.0}$ for three batches of LP was −191±2 mV (−191, −193, −189).

Potentiometric determination of E_m. The solution of LP (27.3 μM) was deaerated as above and deaerated solution of AQS in buffer to give a 4-fold excess of AQS over LP was added. Helium was then continuously passed over the solution, while AQS was being reduced by H_2 which had been dissolved in the platinum black prior to its submersion into the solution. Reduced AQS subsequently reduced LP. This procedure avoided the exposure of the bright Pt electrode to H_2 and permitted potentiometric measurements. The potential between the bright Pt electrode and the calomel electrode was registered at regular intervals, as was the spectrum (Table 4).

Table 2. Proportions of the mediator (AQS) needed to reduce LP and HRP C2 by means of H_2/platinum black.

[Mediator]/[Enzyme]	LP	HRP C2
0.12	no reduction	reduction
0.28	no reduction	reduction
1.2	no reduction	reduction
1.91	no reduction	reduction
2.32	no reduction	reduction
2.80	slow reduction	reduction
4.76	reduction	reduction

Table 3. Optical determination of $E_{m,7.0}$. For details see text. $E_{m,7}$ phthiocol= −168 mV. 100 mM sodium phosphate pH 7.0, 25 °C.

% reduced LP 630 nm	$\frac{RT}{F} \cdot \ln\frac{[ox]}{[red]}$ LP at 630 nm mV	$\frac{RT}{2F} \cdot \ln\frac{[ox]}{[red]}$ phthiocol from 565 nm mV	$E_{m,7.0}$ mV
13.9	46.8	24.9	−189.9
23.2	30.7	8.5	−190.2
28.6	23.5	1.9	−189.6
37.7	12.9	− 9.8	−190.6
48.6	1.5	−23.6	−193.1
59.2	− 9.6	−32.4	−190.8
		Mv	−190.7±1.3 mV

Table 4. Potentiometric determination of $E_{m,7.0}$. For details see text. $E_{m,7}$ calomel electrode 249.9 mV. 100 mM sodium phosphate pH 7.0, 25 °C.

% reduced LP 630 nm	$\frac{RT}{F} \cdot \ln\frac{[ox]}{[red]}$ Mv	E_h mV	$E_{m,7.0}$ mV
0			
47.2	+ 2.9	−182.7	−185.6
60.2	−10.7	−198.1	−187.4
65.8	−16.8	−204.8	−188.0
73.0	−25.6	−214.7	−189.1
78.2	−30.3	−218.9	−188.6
100			
		Mv	−187.7±1.4 mV

A maximum of 80 % reduction of LP could be achieved with the amount of H_2 dissolved in the Pt black. At the end of the experiment an addition of a slight excess of $S_2O_4^{2-}$ was used to achieve 100 % reduction. The extent of reduction was measured at A_{630}, where the absorbance of AQS is negligible. The average of E_m from four experiments on different batches of LP was −188±1 mV (−188, −188, −187, −190).

The reduction rate of LP and mediator under the above described conditions is slow in relation to the transformation of LP Fe(II)-1 to the stable LP Fe(II)-2. The reduction potential in concern is therefore that of the equilibrium LP Fe(III)/LP Fe(II)-2. Plots of ln ($[LP]_{ox}/[LP]_{red}$) *versus* E_h from the two experiments detailed above gave straight lines shifted in parallel by 3 mV. The optical and potentiometric analyses gave n=1.05 and 1.11, respectively, the presumed value being n=1.

Effects of CETAB. Potentiometric titration in buffer with 0.5 % CETAB was carried out with sodium dithionite as reducing agent, presuming a higher rate of reduction than that of catalyzed disproportionation of the dithionite ion. Spectrophotometric titration of LP with AQS in the presence of 0.5 % CETAB reduced the ratio of AQS to LP from 2.9 to 2.4 in the optical test (*cf.* above). Proper potentiometric titration curves could be obtained with a 3.5-fold excess of AQS or AQS_2. The potentials obtained in single experiments were −182.9±1.9 and −179.2±0.8 mV, respectively. Nernst's plots gave straight lines, n=0.99 and 1.07. To permit comparison with the non-detergent experiments, LP was allowed to transform to LP Fe(II)-2 before potentiometric readings were made. The extent of reduction was calculated from A_{630}. The reason for the low increase, 10 mV, of the reduction potential cannot with certainty be identified. It may be attributed to detergent effects on the heme, or possibly on the equilibrium enzyme–mediator–electrode.

In summary, the Fe(III)/Fe(II)-2 reduction potential of LP has been determined as −189±2 mV in 100 mM sodium phosphate pH 7.0, 25 °C. $E_{m,7.0}$ for HRP A, HRP C, turnip peroxidase P_1 and chloroperoxidase is −212 mV, −263 mV, −218 mV[19] and −144 mV,[19] respectively. Thus, in spite of the similarities in spectrum between LP, 2,4-diacetyldeuteroheme HRP C, and turnip peroxidase P_7, and the alleged narrowness of the heme cleft in LP, $E_{m,7.0}$ of LP falls within a range common to several peroxidases also carrying protoheme as prosthetic group. There may be several reasons for the discrepancy between expected and observed E_m-values. Environmental and structural alterations to the heme may cause similar optical effects without influencing E_m to equal extents.

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Preparation and Hydrolysis of *N*-Glucuronides of Pyridine and Piperidine

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N-Glucuronides of pyridine and 3-methylpyridine were prepared and the chemical and enzymatic cleavage by base or β-glucuronidase studied respectively. A K_m value of 0.08 M was found using *E. coli* or *Helix pomatia* enzymes. Catalytic reduction of the *N*-glucuronide of pyridine caused cleavage of the glucuronide, except when the glucuronide was protected as methyl ester and peracetyl derivative. In this case the *N*-glucuronide of piperidine was identified by MS and NMR.

Glucuronides form a major class of compounds in the excretion of xenobiotics and endogenous metabolites. The presence of glucuronides is often proved by enzymatic methods utilizing β-glucuronidase (E.C.3.2.1.31) for the hydrolysis of the glucuronide conjugates.[1] As a result the more lipofilic aglycone can be determined more easily after extraction from a complex matrix. Structure elucidation and quantitation of intact conjugates have been performed, but it is a difficult task due to the administration of small doses and the numerous biotransformations a drug can undergo in a subject. Studies of the synthesis and stability of glucuronides may improve the possibilities for successful analysis of such compounds. The present work has been undertaken with the purpose of getting a more detailed picture of the *N*-glucuronides with regard to synthesis, hydrolysis and spectroscopic properties.

Administration of some xenobiotics results in the formation of *N*-glucuronides which are excreted in urine.[2–6] However, the *C*–*N* bond of *N*-glucuronides and other *N*-glycosides is liable to hydrolysis[7] and rearrangement[8–10] reactions depending on the functional group attached to nitrogen.

Ammonia and primary amines[7] react spontaneously in aqueous solution with glucuronic acid salts, while secondary amines, like piperidine, require methanolic solution for the successful conversion to *N*-glycosides.[11,12] Pyridine has been reacted with 1-bromo-1-deoxy-2,3,4,6-tetraacetyl glucose[13] and galactose[14] to give quaternary salts, glycosyl pyridinium bromides. A similar reaction between methyl 1-bromo-1-deoxy-2,3,4-triacetyl glucuronate and pyridine has been published.[15] Deesterification gave a hygroscopic compound, β-D-glucopyranuronosyl pyridinium bromide, which was used as model compound for glucuronidase studies. However, no details were given for the glucuronide, nor have any results been published on the action of glucuronidase on this compound.

The present paper describes a different work-up procedure for the preparation of 1-β-D-glucopyranuronosyl pyridinium hydroxide, as inner salt, which was suitable for further studies on the enzymatic and chemical hydrolysis, as well as the catalytic hydrogenation of the compound.

RESULTS AND DISCUSSION

The *N*-glucuronides (*5a,b*) of pyridine and 3-methylpyridine were prepared in good yields as pure β-anomers (Scheme 1). 2-Methylpyridine failed to react with *3*, probably due to steric hindrance caused by the methyl group. The structures were determined by ^{1}H NMR, IR, MS,

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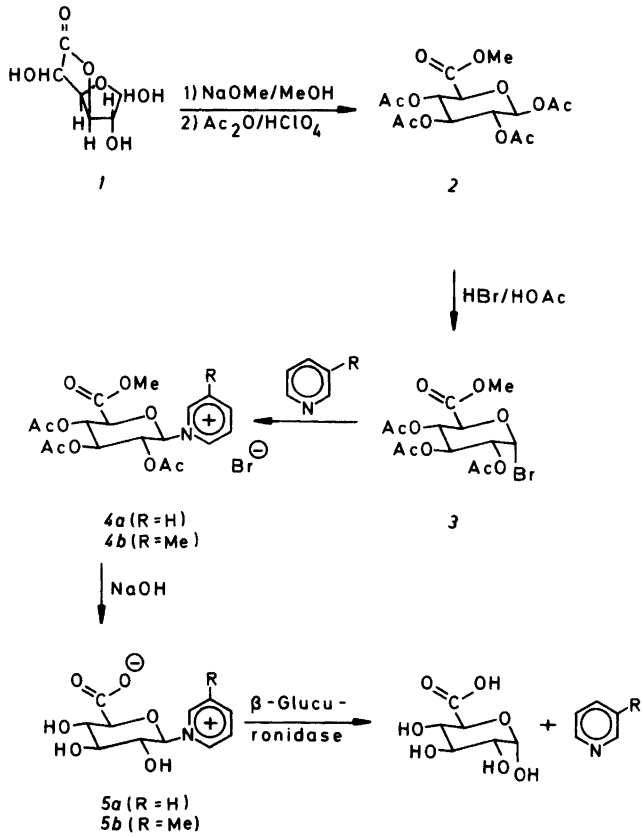


Scheme 1. Synthesis and hydrolysis of *N*-glucuronides of pyridines.
1. Glucurono-3,6-lactone.
2. Methyl 1-deoxy-1,2,3,4-tetracetyl-β-D-glucopyranuronate.
3. Methyl 1-bromo-1-deoxy-2,3,4-triacetyl-α-D-glucopyranuronate.
4a. 1-(Methyl 2,3,4-triacetyl-β-D-glucopyranuronosyl)-pyridinium bromide.
4b. 1-(Methyl 2,3,4-triacetyl-β-D-glucopyranuronosyl)-3-methylpyridinium bromide.
5a. 1-(β-D-Glucopyranuronosyl)-pyridinium hydroxide, inner salt.
5b. 1-(β-D-Glucopyranuronosyl)-3-methylpyridinium hydroxide, inner salt.

and elemental analysis. The presence of a broad doublet (J=8 Hz) at δ 5.70 ppm in D_2O of *5b* and a similar value for *5a* in DMSO-d_6 is evidence for the β-configuration at the anomeric carbon of the glucuronides. The enzymatic hydrolysis by β-glucuronidase is supporting the glycopyranosidic structure as well as the β-configuration, due to the high specificity encountered by this enzyme. IR showed the presence of a carboxylate group in the sample isolated in the ammonia eluate of a cation exchange column in agreement with the proposed inner salt structure. Acidification gave the free acid as shown by the IR resonance at 1740 cm^{-1}. Compounds *5a,b* were stable in solutions and as solids, except for basic solutions above pH 10, where slow hydrolysis occurred. A 0.1 M solution of *5a* was cleaved at a rate of 0.15 μM/min at 38 °C as determined by headspace analysis.

The Michaelis constant (K_m) and the rate of hydrolysis at saturation (V_{max}) were determined in a kinetic assay measuring the UV absorbance of the product formed by enzymatic hydrolysis using β-glucuronidase from *E. coli* and *Helix pomatia*. The product, pyridine, was measured at 254 nm and a graphic determination of K_m and

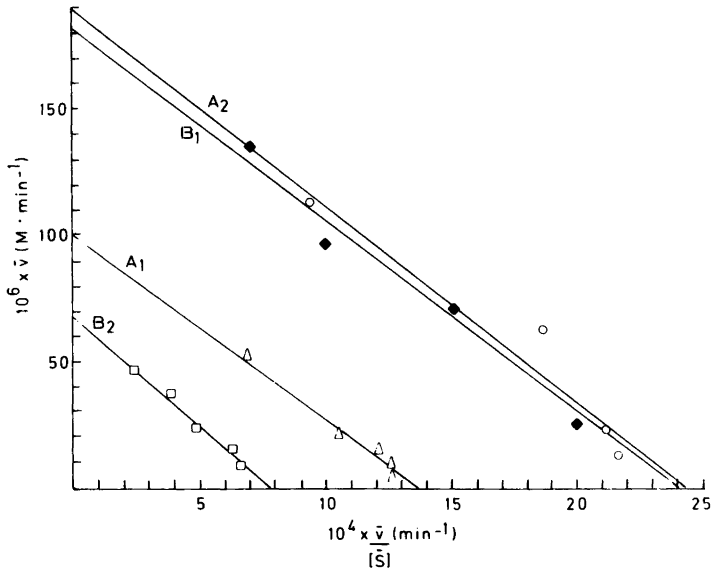


Fig. 1. v/[S] *versus* v plot of kinetic data. Spectrophotometric determination of pyridine obtained by hydrolysis of *5a* in the presence of β-glucuronidase from *E. coli*. The rate of product formation, $\bar{v}=[P]/t$(M min^{-1}) is taken as ordinate and the rate divided by mean substrate concentration, $[\bar{S}]=([S_o]+[S])/2$ (M) is taken as abscissa.
A_1 (K_m = 0.076 M, V_{max} = 1.0 · 10^{-4} M/min, r = 0.992).
A_2 (K_m = 0.078 M, V_{max} = 1.9 · 10^{-4} M/min, r = 0.968).
B_1 (K_m = 0.075 M, V_{max} = 1.8 · 10^{-4} M/min, r = 0.891).
B_2 (K_m = 0.087 M, V_{max} = 6.8 · 10^{-5} M/min, r = 0.984).

V_{max} (Fig. 1) was made using v/[S] *versus* v plot of the Michaelis-Menten model.[16] Reactions were allowed to proceed to about 5 % conversion to ensure sufficiently high absorbance values.

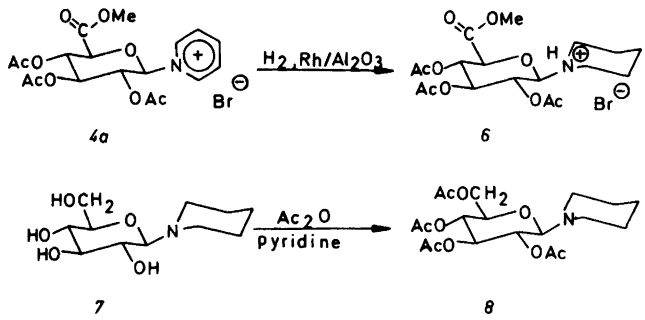
Usually less then 1–2 % conversion of the substrate is allowed in a kinetic assay where the initial rate (v = −d[S]/dt) is measured for a known substrate concentration [S_o]. However, it has been shown[17] that conversion of larger fractions of the substrate can be allowed with negligible error if v is replaced by a mean rate ($\bar{v}=P/t$) and [S_o] is replaced by $[\bar{S}]=([S_o]+[S])\times$ ½.

Four experiments utilizing *E. coli* enzyme having pH optimum at pH 7 and two samples from different preparations (A and B in Fig. 1) gave a mean K_m=0.08(1) M. A single determination of *Helix pomatia* glucuronidase with *5a* as substrate gave K_m=0.08 at the pH optimum of this enzyme (pH 5).

Further use of *5a,b* as substrates in the assay of β-glucuronidase in crude homogenates like faeces and cancer tissue, measuring pyridine by headspace gas chromatography, is described elsewhere.[18,19]

Preparation of the *N*-glucuronide of piperidine was attempted by hydrogenation of *5a* under mild conditions, but only piperidine formed by hydrolysis of the reduction product could be detected. However, hydrogenation of the protected *N*-glucuronide of pyridine, *4a*, successfully converted *4a* to the corresponding piperidine, *6*, determined by ^{1}H NMR and MS (Scheme 2). The electron impact MS spectrum showed a weak molecular ion and a more intense M−OAc at *m/z* 401 and 342, respectively. Cleavage at the anomeric center occurred, yielding *m/z* 317 due to loss of piperidine.

N-Glucosylpiperidine,[11] *7*, was prepared for comparison with regard to spectroscopic and hydrolytic behaviour (Scheme 2). This compound, like *6* showed no anomeric proton separate from other glycosidic protons in the ^{1}H NMR spectrum. The electron impact MS spec-



Scheme 2. Syntheses of peracetylated glycosylpiperidines.
6. 1-(Methyl 2,3,4-triacetyl-β-D-glucopyranuronosyl)-piperidinium bromide.
7. 1-(β-D-Glucopyranosyl)-piperidine.
8. 1-(2,3,4,6-Tetraacetyl-β-D-glucopyranosyl)-piperidine.

trum of the peracetylated derivative, *8*, showed a weak molecular ion at *m/z* 415 and more intense peaks at *m/z* 372 and 356 due to loss of acetyl and acetate, respectively. Compound *7* dissolved easily in water, yielding a solution of pH 7 which quickly rose to pH 11 indicative of a fast hydrolysis or rearrangement in agreement with the early findings of Hodge and Rist.[10]

EXPERIMENTAL

Materials. Solutions of β-glucuronidase (E.C.3.2.1.31) from *E. coli* (Boehringer) and *Helix pomatia* (Boehringer) were diluted with 1 M phosphate buffer prepared from NaH_2PO_4 in distilled water and 2 M NaOH to give a suitable pH.

Methods. Headspace analysis[18] was carried out using 4 ml vials fitted with screw cap and laminated rubber-teflon septum. All samples were thermostated at 38.0 °C in a water bath at least 30 min before sampling was allowed. A gas-tight 1 ml syringe (SGE) heated to 60 °C was used for sampling and injection on the Hewlett Packard 5730 gas chromatograph using FID detection. The column was a 1.5 m × 2 mm i.d. glass coil packed with 10 % Apiezon L and 4 % KOH on Chromosorb WHP 100–120 mesh. Nitrogen was used as carrier gas at a flow rate of 30 ml/min. Injector and detector were maintained at 200 °C and the column at 90 °C. Chromatograms were obtained with an Omniscribe recorder (Houston Instr).

UV spectrophotometry for determination K_m was performed on a Kontron Uvicon LCD 725 measuring absorbance at 254 nm in 1 cm cells. NMR spectra were obtained on a Varian EM 360 L spectrometer. MS spectra were run on a VG Micromass 7070 F (electron impact) or a modified Varian CH 5 (field desorption) spectrometer. IR spectra were run on a Perkin Elmer 457 grating spectrometer. Optical rotations were measured on a Perkin Elmer 141 polarimeter.

Determination of K_m using *5a* and β-glucuronidase from *E. coli*. Aqueous stock solutions of *5a* (0.1535 M and 0.394 M) and dilutions thereof were thermostated at 38 °C. These solutions (200 μl) were mixed with β-glucuronidase (200 μl) in 1 M phosphate buffer at pH 7.0. After a suitable time, 0.25 M glucaro-1,4-lactone (200 μl) was added followed by 1.0 ml chloroform. The vial was shaken and the content transferred to a bigger vial containing 4.0 ml chloroform. Absorbance was measured and pyridine concentration calculated from a standard curve. K_m was similarly determined using *Helix pomatia* juice at pH 5. In this case 1 ml 2 M NaOH was added before extraction with chloroform.

Preparation of 2 and 3 (Scheme 1). The commercially available glucuronic acid, γ-lactone (*1*) was used as starting material in some experiments. Alternatively 2 was purchased from Koch-Light. The detailed description of Bollenbach *et al.*[20] was followed in the syntheses of *2* and *3*, the formation of products being followed by TLC. Yields of *2* and *3* were about 70 % and 85 %, respectively, giving an overall yield of about 60 % of the theoretical.

Preparation of 4a. 2 g (5 mmol) of *3* were dissolved in 25 ml pyridine and left at room temperature for 2 d or heated to 80 °C for a few hours with somewhat lower yield. The mixture was filtrated and the crystals washed with diethyl ether. Yield 1.4 g (58 %), no well defined m.p. ^1H NMR (60 MHz, DMSO-d_6): δ 1.90 (3H,s,CH_3CO), 2.03 (3H,s,CH_3CO), 2.05

(3H,s,CH_3CO) 3.68 (3H,s,CH_3-OCO), 4.8–6 (4H,m,CHOAc), 6.5–6.7 (1H,m,anomeric proton), 8.4–9.6 (5H,m,pyridinium protons). MS (field desorption) *m/z* 396 (M–Br.).

Preparation of 4b. Preparation of *4b* followed the procedure for preparation of *4a* using 3-methylpyridine as reagent. Yield 1.9 g (73 %). ^{1}H NMR (60 MHz, D_20): δ 2.0 (3H,s,CH_3CO), 2.13 (3H,s,CH_3CO), 2.19 (3H,s,CH_3CO), 2.60 (3H,s,CH_3Ar), 3.88 (3H,s,CH_3–OCO), 5–6 (4H,m,CHOH), 6.3 (1H,d, J=8 Hz, anomeric proton), 8–9 (4H,m,Ar).

Preparation of 5a. An amount of 1 g (2.1 mmol) of *4a* was dissolved in 10 ml 1 M NaOH and left at room temperature overnight. The solution was applied to strongly acidic ion exchange resin (30 cm×1 cm i.d., Dowex 50×1, 50–100 mesh) and eluted with water to neutral reaction. Then elution was continued with 2 M ammonia and the first basic fractions (30 ml) containing U.V. absorbing material were collected. Evaporation of the solvent gave 450 mg (84 %) of *5a*. Anal. $C_{11}H_{13}NO_6$: C, H, N. $[\alpha]_D^{20}$+44.5 (c 1, water) ^{1}H NMR (60 MHz, DMSO-d_6): δ 3.5–4 (3H,m,CHOH), 4–5 (DOH), 5.8–6.1 (1H,m,anomeric proton), 8.2–9.4 (5H,m, pyridinium protons). MS (field desorption) of a sample treated with HCl *m/z* 256 (M+1). IR (KBr) cm^{-1} 1610(s) and 1630(s) (carboxylate). HCl treated sample: IR (KBr) cm^{-1} 1740 (carboxylic acid). Recrystallization of the product was done by dissolving the solid in water to give a saturated solution, approximately 0.3 M, and then adding ethanol to give a turbid mixture.

Preparation of 5b. *5b* was prepared as *5a* hydrolyzing *4b*. Yield 0.8 g (83 %), $[\alpha]_D^{20}$+45.96 (c 0.82, water). Anal. found: C 52.68, H 5.69, N 5.15; calc. for $C_{12}H_{15}NO_6$: C 53.54, H 5.62, N 5.20. ^{1}H NMR (60 MHz, D_20): δ 2.60 (3H,s,CH_3–Ar), 3.5–4.3 (4H,m,CHOH), 5.70 (1H, broad d, anomeric proton), 7.8–9 (4H,m,Ar).

Catalytic hydrogenation of 4a. 440 mg (0.92 mmol) of *4a* were disssolved in 40 ml distilled methanol. Hydrogenation was carried out in a glass vessel at 3 atm. using 5 % Rh on Alumina (Engelhardt), at room temperature overnight. The mixture was filtered through kieselguhr. The filtered solution was evaporated, giving 350 mg of compound *6*, which was used directly for spectroscopic analysis. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.5–2.3 (15H,m,), 2.9–3.5 (4H,m), 3.8 (3H,s), 4.5–5.6 (5H,m), MS (IP 70 eV, *m/z* (% rel. int.): 401(1,M), 342(1,M–OAc), 317(1,M–$C_5H_{10}N$), 228(7), 215(15), 186(38), 115(100).

Catalytic hydrogenation of 5a. 58 mg (0.23 mmol) of *5a* was dissolved in 15 ml of water and hydrogenated overnight at 3 atm. at room temperature using 5 % Rh on Alumina as catalyst. The mixture was filtered through kieselguhr and the solvent evaporated on a Rotavapor. TLC on silica using 2-propanol–water–acetic acid (8:3:1) as eluent. The plate was developed with ninhydrin in acetone. The visible spot had an R_F value (0.4) identical to piperidine.

Hydrolysis of 5a. A solution of *5a* (11.5 mM) and 2-methylpyridine (5 mM) in 1 M phosphate buffer pH 7 was mixed with β-glucuronidase from *E. coli* (100 μl of each solution) and the evolution of pyridine was measured by headspace analysis at intervals. When the ratio of pyridine and the internal standard remained constant the concentration was measured from a standard curve.

The nonenzymatic hydrolysis of *5a* in basic solution was determined by headspace analysis using a 0.134 M solution of *5a* in 0.01 M borax buffer pH 10.

Preparation of 7 *and* 8. Preparation of *7* and *8* followed the procedure of Hodge and Rist.[11] Compound 7: ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.4 (6H, broad s), 1.98 (3H,s,CH_3CO), 2.00 (3H,s,CH_3CO), 2.01 (3H,s,CH_3CO), 2.02 (3H,s,CH_3CO), 2.3–3.2 (4H,m,piperidine), 3.4–4.2 (4H,m,glycosidic), 4.8–5.2 (3H,m,glycosidic). MS (IP 70 eV, *m/z* (% rel. int.): 415 (5,M), 372 (1,M–Ac), 356 (19,M–OAc), 342 (14), 296 (1), 282 (3), 243 (9), 201 (1), 198 (3), 169 (19), 114 (100). m 342→282 obs. 232, calc. 232.5; 169→127 obs. 95.5, calc. 95.4, 156→114 obs. 83, calc. 83.3.

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A Novel Synthesis of β-Aminoalkylnitroamines. *rac*-β-Nitroaminoalanine and *N*-Nitroethylenediamine, Two Reported Metabolites from *Agaricus silvaticus*

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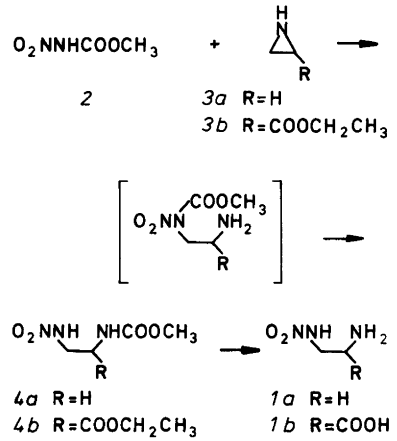
N-Nitroethylenediamine and *rac*-β-nitroaminoalanine have been prepared in good yields by the same general method, involving addition of methyl *N*-nitrocarbamate to aziridine or ethyl aziridine-2-carboxylate, respectively. Additive ^{13}C NMR shift parameters for the nitroamino group have been estimated.

In the course of an ongoing screening program for mutagens in larger fungi,[1] we noted a recent report[2,3] on the occurrence of *N*-nitroethylenediamine (*1a*) and β-nitroaminoalanine (*1b*) in two collects of *Agaricus silvaticus* Vit. ex Fr. from North Carolina and Puget Sound. The optical activity of the isolated sample of *1b* was not reported. The possible mutagenic or carcinogenic properties of monoalkylnitroamines themselves are little known, but they may undergo a chemical reduction, ultimately leading to diazoalkanes.[4] If a similar reduction were to occur under physiological conditions, alkylation of DNA might follow and result in gene damage. It should be noted that some tertiary nitroamines have recently been shown to be mutagenic to *Salmonella typhimurium*.[5] Since *A. silvaticus* is considered edible and palatable in many regions, we judged it desirable to subject its nitroamines to mutagenicity tests. However, we were unable to detect nitroamines in *A. silvaticus* from the Lund region, and we therefore had to obtain the pertinent compounds by synthesis.

N-Nitroethylenediamine (*1a*) has been prepared earlier,[6,7] *i.e. via* nitration of ethyl *N*-(2-aminoethyl) carbamate,[6] but the overall yield was very low and the general method seemed unsuitable for preparing β-nitroaminoalanine. In order to prepare the latter, we tried a Michael addition of methyl *N*-nitrocarbamate (*2*)[8,9] to ethyl-2-acetamidoacrylate, but the reaction failed, presumably due to the low nucleophilicity of the nitrocarbamic ester anion. However, an aziridinium ion was anticipated to be a sufficiently powerful electrophile to effect the desired aminoethylation, and we found that aziridine (*3a*) reacted cleanly with *2* to afford methyl *N*-(2-nitroaminoethyl) carbamate (*4a*) after initial salt formation and migration of the methoxycarbonyl group to the more basic α-amino group.



Scheme 1.

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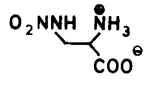
Alkaline hydrolysis of *4a* afforded *N*-nitroethylenediamine (*1a*) in a good yield. β-Nitroaminoalanine (*1b*) was obtained similarly in a 48 % overall yield from the reaction of ethyl 2-aziridinecarboxylate (*3b*) with *2* and subsequent alkaline hydrolysis of the intermediate carbamate *4b*. The product was identical (chromatography, electrophoresis and MS) with an authentic sample of β-nitroaminoalanine from *A. silvaticus*.* We found that in these reactions methyl *N*-nitrocarbamate (*2*) is to be preferred over the corresponding ethyl carbamate as starting material, since the former yields the intermediates *4* which are more readily hydrolysed.

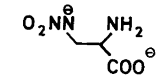
Nucleophilic ring-openings of 2-aziridinecarboxylic esters are ambiguous, since the nucleophile may enter either the α- or β-position with respect to the carboxylic group. It has been shown, however, that good nucleophiles like thiolate anions preferentially attack the α-position, and that weaker nucleophiles like water or chloride ion tend to enter the β-position.[10] The alkyl *N*-nitrocarbamate anions are apparently poor nucleophiles, as is indicated by their rather sluggish reaction with the aziridinium salt, and should consequently open the aziridine ring of *3b* to give the desired β-nitroamino intermediate *4b*. Further support for the correctness of the assigned structures *1b* and *4b* was obtained from a study of the acid catalysed decomposition of *1b* and from ^{13}C NMR data.

Primary alkylnitroamines readily eliminate dinitrogen oxide on warming with dilute mineral acids, forming products which would be expected from an alkyl cation intermediate.[11] When synthetic β-nitroaminoalanine was warmed with dilute hydrochloric acid, DL-serine was the main product, accompanied by traces of 2-amino-3-chloropropanoic acid. Neither isoserine nor 3-amino-2-chloropropanoic acid, the expected products from 3-amino-2-nitroaminopropanoic acid, were detected. The latter might conceivably decompose *via* intermediate formation of aziridine-2-carboxylic acid. but then a mixture of both aminochloropropanoic acids would be the expected products.[10]

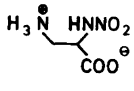
The ^{13}C NMR shifts of β-nitroaminoalanine are strongly influenced by the pH of the solution. The interpretation of the spectra in structual terms requires information on the additive shift parameters for the nitroamino group and its conjugate base, and such data seem not to have been reported in the literature so far. Most published ^{13}C NMR spectra concern open chain and cyclic compounds containing at least two nitroamino groups, all of which are undissociated[12] and there is just one report on an alkylnitroamine, methylnitroamine, (in 1, 2-dimethoxyethane) and its sodium salt (in D_2O).[13] Using the ^{13}C shift parameter set of Rabenstein and Sayer[14] for calculating carbon shifts of amines, carboxylic acids and amino acids in D_2O, shift increments of 11.3 and 17.3 ppm for methylnitroamine and its conjugate base, respectively, are obtained. This implies that deprotonation of the nitroamino group results in downfield shift of *ca.* 6 ppm for the attached carbon atom, making allowance for the different solvents used. Provisional α, β, and γ shift parameters for the nitroamino substituent in D_2O solution were determined using propylnitroamine as a model compund (Table 1). In conformity with the results of the previous investigators, a significant downfield shift (5.93 ppm) was observed for the α-carbon upon deprotonation of the nitroamino group, while the shift effects on the β and γ carbons were rather small, both for the nitroamine itself and its sodium salt. A solution of β-nitroaminoalanine in water had pH 3.8, with the solute probably best represented by formula *6a*. Titration with dilute sodium hydroxide indicated two dissociation steps with pK_a-values of 5.0 and 9.8. Since titration of propylnitroamine similarly gave pK_a 6.4, it may be assumed that the first dissociation step above primarily involves deprotonation of the nitroamino group of *6a*. The reported[15] pK_a-value for methylnitroamine in 50 % aqueous ethanol is 7.08.



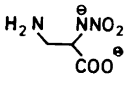
6a



6b



7a



7b

* The authentic sample was a kind gift from Prof. W.S. Chilton, University of Washington, Seattle, U.S.A.

Table 1. ^{13}C NMR (90.52 MHz) shifts for β-nitroaminoalanine (*1b*) and propylnitroamine (*5*) and their sodium salts in D_2O with sodium 3-(trimethylsilyl) propanesulfonate as internal standard. Calculated shifts for *1b* and 2-amino-3-nitroaminopropanoic acid, in different states of protonation (*6a,b* and *7a,b*, resp.).

	pD 3.9 Found		Calc.[a]		pD 12.8 Found		Calc.[a]	
	5	*1b*	*6a*	*7a*	*5*	*1b*	*6b*	*7b*
CO_2		174.4	175.3	181.5		183.7	184.2	185.0
C_α	50.2	57.1	54.2	67.7	56.2	57.1	56.1	78.2
C_β	22.1	48.8	50.2	42.6	23.1	58.8	60.7	44.5
C_δ	13.3				14.3			

[a] Using the parameter set of Ref. 14, the shift parameters for carbon atoms 1, 2 and 3 bonds removed from the nitroamino group as tentatively derived from observed shifts in *5* become: At pH 3.9, $\delta_{1bond}=14.15$, $\delta_{2bond}=-1.23$ and $\delta_{3bond}=-1.58$, at pH 12.5, $\delta_{1bond}=20.08$, $\delta_{2bond}=-0.21$ and $\delta_{3bond}=-0.59$.

On titration of β-nitroaminoalanine in D_2O solution from pD 3.8 to pD 5.7, the β-carbon triplet in the ^{13}C NMR spectrum shifted downfield 5.3 ppm, with much smaller increments of the α-carbon and carboxyl carbon shifts. This is in accord with the expected effects of a deprotonation of a nitroamino substituent in the β-position. Increasing the pD to 12.8 caused a further strong downfield shift of the β-carbon, as well as that of the carboxyl carbon, which is in conformity with the proposed structure and the known strong effects on the carboxyl and β-carbon shifts but small effect on the α-carbon shift upon deprotonation of an α-ammonium group of amino acids.[14] Using known shift parameters for amino acids[14] and tentative parameters derived from the propylnitroamine ^{13}C NMR spectrum, we have calculated ^{13}C NMR shifts for β-nitroaminoalanine (*6a,b*) as well as for the 2-amino-3-nitroaminopropanoic acid isomer (*7a,b*) at pD 3.9 and 12.5 (Table 1). These calculations show good agreement between found and calculated shifts for structure *6* and would seem to rule out the isomer *7*. However, interactions may occur between the rather congested and strongly polar substituents of these molecules, and thus care must be exercised in interpretations until more ^{13}C NMR shift data have been collected for nitroamines.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin Elmer model 257 instrument. ^1H NMR spectra were recorded at 60 MHz on a Jeol JNM-PMX 60 or at 360 MHz on a Nicolet WB 360 instrument and ^{13}C spectra at 15.09 MHz on a Jeol FX-60 or at 90.52 MHz on a Nicolet WB 360 instrument. Mass spectra were recorded on a Finnegan 4000 instrument, using chemical ionisation with methane as the reactant gas. Merck Kieselgel 60 (0.063–0.200 mm) was used for column chromatography and Merck Alufolie Kieselgel F_{254} or Cellulose F_{254} for TLC. Melting points were taken on a micro hot stage (Reichert) and are uncorrected.

Methyl N-*(2-nitroaminoethyl) carbamate* (4a). Aziridine (0.96 g, 22 mmol) was bubbled as a saturated vapor in nitrogen into a suspension of methyl *N*-nitrocarbamate[8,9] (2.76 g, 23 mmol) in chloroform (50 ml). After the addition was complete (4 h), the mixture was heated under reflux for 1 h. Evaporation of the solvent gave a solid residue which was purified on a silica gel column (120 ml) using 3 % ethanol in ether as eluant. Concentration of the eluate and trituration of the residue with a small volume of cold ether afforded chromatographically pure *4* (2.78 g, 73 %, m.p. 83–86 °C), which was used for hydrolysis to *1a*, m.p. 85–88 °C (from methanol). Anal. $C_4H_9N_3O_4$: C, H, N, O. ^1H NMR (60 MHz, deuterioacetone): δ 3.3–3.8 (4 H, m), 3.6 (3 H, s). ^{13}C NMR (15.09 MHz, deuterioacetone): δ 39.1 ($-CH_2-N-CO$), 46.6 ($N-N-CH_2$), 52.3 ($O-CH_3$), 158.3 (*CO*).

N-*Nitroethylenediamine* (1a). Methyl *N*-(2-nitroaminoethyl) carbamate (750 mg, 4.6 mmol) dissolved in 2.5 M sodium hydroxide (5 ml) was heated under reflux for 2 h and the reaction mixture was then neutralised with 2 M HCl and added to a Dowex 50WX4 (H form) column (38 ml). Elution with water afforded unreacted

carbamate (160 mg) and subsequent elution with 0.6 M aqueous ammonium hydroxide afforded chromatographically pure *N*-nitroethylenediamine, which was recrystallised from a small volume of water: Yield 350 mg (73 %, uncorrected for recovered starting material), m.p. 240–241 °C (lit.[6] 240 °C). IR and ^{1}H NMR were in agreement with published spectra:[2] TLC (Cellulose, BuOH-HOAc-H_2O 12:3:5) R_{Leu} 0.59 (lit.[2] 0.52).

Ethyl 2-(methoxycarbonylamino)-3-nitroaminopropionate (4b). A solution of ethyl aziridine-2-carboxylate[16] (*3b*) (500 mg, 4.35 mmol) in anhydrous dichloromethane (5 ml) was added slowly and with efficient stirring to a suspension of an excess of methyl *N*-nitrocarbamate[8,9] (*2*) (1.044 g, 8.70 mmol) in anhydrous dichloromethane (45 ml). The reaction mixture was heated under reflux until NMR indicated the absence of *3b* (16 h), and was then concentrated to give a solid mixture of crude *4b* and excess *2*, which was used as such for hydrolysis to *1b*.

A sample of crude *4b* dissolved in ethanol-ether (2:1) was purified by filtration through a short column of silica gel, concentration of the filtrate and two recrystallisations of the residue from water–ethanol (9:1) to afford pure *4b*, m.p. 84.0–84.5 °C. Anal. $C_7H_{13}N_3O_6$: C, H, N, O. ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.31 (3 H, t*),3.72 (3 H, s), 3.95 (1 H, dd broad, *J* 14.1 Hz and 6.2 Hz), 4.07 (1 H, dd, *J* 14.5 Hz and 5.1 Hz), 4.25 (2 H, m*), 4.56 (1 H, m), 5.87 (1 H, d, *J* 6.2 Hz), 9.80 (1 H, s). ^{13}C NMR (15.09 MHz, deuterioacetone): δ 14.3 (C–CH_3), 47.1 (C3), 52.4 (O–CH_3), 52.9 (C2), 62.2 (O–CH_2–), 157.5 (N–C=O), 170.4 (C1). IR (KBr): 3300 (s, NH), 1760 (s, O–C=O), 1700 (s, N–C=O), 1584 (m), 1450 (m), 1413 (m), 1380 (s), 1345 (s), 1260 (s, $N–NO_2$), 1208 (s), 1078 (s).

β-Nitroaminoalanine (1b). Crude ethyl 2-(methoxycarbonylamino)-3-nitroaminopropionate (*4b*) (1.71 g, 7.3 mmol) in 2 M sodium hydroxide (6 ml) was heated under reflux for 2 h. The reaction mixture was neutralised with dilute hydrochloric acid, and transferred to a Dowex 50WX4 (H form) column (38 ml), which was then eluted extensively with water until the effluent was no longer acidic. The β-nitroaminoalanine fraction, as located by TLC, was concentrated, and the residue recrystallised from dilute aqueous ethanol to afford pure *1b*: Yield 308 mg (48 % from *3b*), m.p. *ca.* 194 °C (dec.). Anal. $C_3H_7N_3O_4$: C, H, N, O. ^{1}H NMR (360 MHz, D_2O pD 3.8, $Me_3Si(CH_2)_3SO_3Na$): δ 3.90 (1 H, dd, *J* 15.5 Hz and 6.8 Hz), 4.05 (1 H, dd, *J* 6.8 Hz and 3.6 Hz), 4.15 (1 H, dd, *J* 15.5 Hz and 3.6 Hz). IR (KBr): 3345 (s), 2960 (s, broad), 1593 (s), 1413 (s), 1391 (s), 1275 (m), 1187 (m), 1145 (m), 1090 (m), 885 (m). MS CI: *m/e* (% rel. int. synth. *1b*, % rel. int. isol. *1b* from *A. silvaticus*): 70 (87, 100), 74 (22, 20), 75 (15, 20), 81 (14, 23), 87 (22, 8), 88 (100, 95), 90 (10, 48), 150 (33, 82). The identity of synthetic *1b* with an authentic sample from *A. silvaticus* was confirmed by TLC, MS and electrophoresis in pyridine–acetic acid buffer at pH 6.1.

Acid-catalysed reactions of β-nitroaminoalanine (1b). A solution of *1b* (25 mg) in 2 M hydrochloric acid (5 ml) was heated under reflux for 18 h and then passed through a Dowex 50WX4 (H form) column (10 ml). The resin was washed with water and then the amino acids were eluted with aqueous ammonia (7 %) and recovered by evaporation. TLC (cellulose, pyridine–water 85:15) indicated the presence of serine (R_f 0.53) and traces of 2-amino-3-chloropropanoic acid (R_f 0.71) but no isoserine (R_f 0.50) or 3-amino-2-chloropropanoic acid (R_f 0.66). Recrystallisation from water gave pure DL-serine (5.5 mg), identified by its IR spectrum.[17]

Propylnitroamine (5) Propylnitroamine was prepared using a modification of an early procedure.[18] Methyl propylcarbamate (14.5 g, 0.125 mol), obtained by the reaction of propylamine with methyl chloroformate in a 2:1 molar ratio in toluene, was dissolved in 95 % sulfuric acid (ice-bath) and then potassium nitrate (12.5 g, 0.125 mol) was added in portions. After 3 h at 0 °C the mixture was stirred into ice (400 g), extracted with ether and the ether solution washed with sodium sulfate solution and dried over sodium sulfate. On bubbling dry ammonia through the ether solution of methyl *N*-nitro-*N*-propylcarbamate, an oil separated which was washed with ether and then dissolved and carefully neutralised with 1 M sulfuric acid. Extraction with ether, drying (sodium sulfate) and subsequent distillation afforded colourless and spectroscopically (NMR) pure propylnitroamine; Yield 5.0 g (38 %), b.p. 47–48 °C/0.3 mmHg, n_D^{22} 1.4595 (lit.[18] b.p. 52–56 °C/0.1 mmHg, n_D^{20} 1.4610). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.0 (3 H, t, *J* 7.2 Hz), 1.6 (2 H, m), 3.6 (2 H, t, *J* 6.8 Hz), 9.3 (1 H, s). ^{13}C NMR (90.52 MHz, deuterioacetone): δ 11.8 (C3), 21.2 (C2), 47.9 (C1).

* The ethyl group gave rise to an ABX_3 spectrum, since the methylene protons are diastereotopic.

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Solvent Properties of Dichloromethane. 1. The Reactivity of Dichloromethane toward Some Ionic Nucleophiles. Dichloromethane as Solvent for Finkelstein Reactions

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The reactions of dichloromethane with various pseudohalide ions, N_3^-, NC^-, $NCTe^-$, $NCSe^-$, NCS^- and OCN^-, have been studied kinetically under homogeneous conditions in dichloromethane as solvent at 25.0 °C. In the 10^{-3} M concentration range the half-lives of the reactions are 70 min(N_3^-), 80 min(NC^-), 15 h($NCTe^-$), 60 h($NCSe^-$), 400 h(NCS^-) and 600 h(OCN^-). 1,2-Dichloroethane in 1,2-dichloroethane is slightly more reactive toward N_3^- and NCS^- with half-lives of 60 min and 150 h, respectively. CH_2Br_2 in CH_2Br_2 is 400 times as reactive as is CH_2Cl_2 in CH_2Cl_2 toward NCS^-; half-life 60 min.

The homogeneous Finkelstein reactions between methyl iodide and NCS^- and Cl^- in CH_2Cl_2 were studied kinetically at 25.0 °C. For equal initial concentrations of NCS^- (1.2×10^{-2} M) the reactivity order is [PNP]SCN>Ph_4AsSCN>Bu_4NSCN as anticipated from their association constants in CH_2Cl_2. When corrected for ion-pairing the second order rate constants are fairly independent of the counterion. The MeI-NCS^- and the MeI-Cl^- reactions proceed as rapidly in dichloromethane as in the usual dipolar aprotic solvents of considerably higher dielectric constants. The relative uncorrected second order rate constants for the MeI-NCS^- reaction in CH_2Cl_2, in 1,2-dichloroethane and in 1,1,2,2-tetrachloroethane are 1:0.8:0.2 at 25.0 °C.

Halogenated alkanes and especially dichloromethane have for a long time been used as solvents in synthesis and also as extraction agents due to their excellent solvent properties.[1] With the advent of synthetic procedures based upon phase-transfer catalysis[2–4] in which dichloromethane is the favourite organic co-solvent the use of this solvent has been greatly increased. In recent years, dichloromethane has also become a most useful solvent in inorganic chemistry since inorganic complexes which contain ligands with large organic groups, particularly aromatic groups, are readily soluble in this solvent.[5] Of no less importance is the general availability of this solvent, its low price and its fairly simple purification. When properly stored this solvent can be kept for months without decomposition.

However, all solvents have both their advantages and their disadvantages and dichloromethane is certainly not an exception to this rule. In this series we report on some of the characteristic properties of dichloromethane with special emphasis on its reactivity toward various species which causes some obvious limitations in its use as a solvent. Furthermore, its potential as solvent for various substitution reactions will be examined. For comparison other halogenated alkanes will also be considered.

In the present study we report on the reactivity of some anionic nucleophiles toward dichloromethane in dichloromethane as solvent. Purely nucleophilic species, N, are known to react by two consecutive nucleophilic displacement reactions[6,7] as shown by eqns. (1) and (2).

$$CH_2Cl_2 + N \xrightarrow{k_{2,1}} ClCH_2N + Cl^- \quad (1)$$

$$ClCH_2N + N \xrightarrow{k_{2,2}} NCH_2N + Cl^- \quad (2)$$

Presently, no detailed information is available with regard to the relative reactivity of $ClCH_2Cl$

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and $ClCH_2N$ toward nucleophiles, *i.e.* $k_{2,1}$ and $k_{2,2}$. $PhSeCH_2Cl$ is suggested to be more reactive toward $PhSe^-$ than is $ClCH_2Cl$[8] and for equal concentrations of $ClCH_2Cl$ and N in various solvents the final product, NCH_2N, is the major one; however, some exceptions are known.[6,7] In the majority of the reactions with nucleophiles performed under phase-transfer catalysis conditions the bis-product, NCH_2N, is the predominant one, but this may be due to prolonged reaction times.[9] In the reaction between diiodomethane and amines the second step is known to be the slower one.[10] In the present study in which [N], and thus $[ClCH_2N]$, was maximally 10^{-3} of that of the solvent, 15.50 M at 25.0 °C, one may safely assume the second step to be kinetically unimportant.

The reactivity of some pseudohalide ions, N_3^-, NC^-, $NCTe^-$, $NCSe^-$, NCS^- and NCO^- were studied under homogeneous conditions. These anions were chosen since the IR technique using liquid cells of various path lengths allowed rate constants to be determined with fairly high accuracy in different concentration ranges.[11–13] Data for the reactivity of most of these ions toward alkyl halides in various protic and aprotic solvents are available [11,14] allowing valuable comparisons to be made. As sources for the anions were used Bu_4NX, Ph_4AsX and $[(Ph_3P)_2N]X$; the latter abbreviated [PNP]X. Recently, association constants, K_A, for several of these salts in dichloromethane at 25.0 °C have been determined.[15,16] The corrected second order rate constants, $k_{2,1}$ (corr.), for some of the reactions could thus be calculated.

The limited reactivity of the thiocyanate ion toward dichloromethane allowed a kinetic study of the classical Finkelstein reaction between this ion and methyl iodide in this solvent, eqn. (3).

$$MeI + NCS^- \xrightarrow{ClCH_2Cl} MeSCN + I^- \qquad (3)$$

When corrected for association of the various ionic thiocyanates, the corrected second order rate constant in dichloromethane could be calculated. A comparison could thus be made with rate constants in other solvents. A similar study was also performed on the reaction between methyl iodide and chloride ion, eqn. (4).

$$MeI + Cl^- \xrightarrow{ClCH_2Cl} MeCl + I^- \qquad (4)$$

EXPERIMENTAL

Purification of dichloromethane. Dichloromethane, *technical grade,* was vigorously stirred for several days with small portions of concentrated sulfuric acid, 50 ml *per.* 5 l solvent, until the acid was colourless. The solvent was then washed with water until all acid had been removed, then vigorously stirred for some hours with 3 portions of 100 ml 1 M Na_2CO_3 and finally with distilled water of pH 6 until a neutral aqueous solution had been obtained. After drying with anhydrous $CaCl_2$, then with anhydrous K_2CO_3 and finally with CaH_2, the solvent was fractionated from CaH_2 in a dry argon atmosphere with a column of ~30 theoretical plates, b.p. 40.0–40.5 °C. The first and the last 10 % were discarded leaving a total yield of 3.5 l from 5 l. The purified product was stored over freshly activated Linde Molecular Sieves (4 Å) in 1/4 and 1/2 l bottles in darkness at −30 °C. No silicon grease was used during the purification process or during storage.

Several attempts were performed by GLC by various columns to analyze the purity of the solvent; however, no exact measure could be obtained. The ^1H NMR spectrum and the ^{13}C NMR spectrum (30000 accumulations) did not indicate any impurities; for comparison, dichloromethane contaminated with 1 % pentane or 1 % hexane gave distinct ^1H and ^{13}C signals. The specific conductivity of the solvent was less than 8.4×10^{-10} S cm^{-1} which was the lower limit of the conductivity equipment. No decomposition could be detected as viewed by kinetic results, by NMR spectra or by conductivity studies after several months when the purified solvent was stored as described above. No definite advantages with regard to the quality of the purified product could be observed when starting out from *p.A.* qualities instead of *technical grade.*

Halogenated solvents and reagents. Methyl iodide, Fluka *purum,* was washed with several portions of 5 % aqueous solution of K_2CO_3, then with water and was finally dried with anhydrous K_2CO_3. After filtration, the reagent was fractionated and stored in a black-painted bottle at −10 °C. 1,1,2,2-Tetrachloroethane, J. T. Baker, *Baker Grade,* was purified in a similar way and distilled twice prior to use. Dibromomethane, Koch-Light Labs., *puriss,* was flushed with argon for 2 h to remove traces of HBr and then distilled twice. 1,2-Dichloroethane, *Merck Spectroscopic Grade,* was fractionated from CaH_2 prior to use. Midfractions of the solvents were used for the kinetic studies.

Onium salts. The preparation and purification of the bis(triphenylphosphine)iminium salts,

[PNP]X, (X=$NCSe^-$, $NCTe^-$, NCO^-, N_3^-, NC^-, NCS^-, Cl^- and I^-) and tetraphenylarsonium thiocyanate, Ph_4AsSCN, was performed as previously described.[11,12,17] Tetramethylammonium thiocyanate, Me_4NSCN, was made from the corresponding chloride and dried KSCN in dry acetone and crystallized from this solvent and finally from acetonitrile, m.p. 293–296 °C (296–297 °C[18]). This salt was only used for the solubility study. Tetrabutylammonium thiocyanate, Bu_4NSCN, was made from the corresponding nitrate and dry KSCN in dry methanol. The salt was crystallized several times from ethyl acetate–hexane, m.p. 118–119 °C (119–120 °C[19]).

Solubility of onium thiocyanates in dichloromethane. Me_4NSCN: 100 ml of dichloromethane containing excess salt was stirred for 20 h at 25.0 °C; 20 h amount to only some 5 % of the half-life of the reaction. After filtration the solvent was removed in vacuum and the residue dissolved in 10 ml acetone. The concentration of ionic thiocyanate was determined by IR at 2058 cm^{-1} using 0.1 cm liquid cells and a calibration curve between peak height at this wave length *versus* the concentration of SCN^- in acetone. Bu_4NSCN: a saturated but very viscous solution was obtained at 25.0 °C, the concentration of which was determined by IR as for Me_4NSCN after 500 times dilution. Ph_4AsSCN and [PNP]SCN: saturated solutions could not be obtained; the solutions turned only more and more viscous upon addition of more salts allowing only minimum solubilities to be estimated.

Determination of rate constants. In Table 1 are listed some IR and UV data for the various compounds which formed the basis for the analytical methods for the determination of the rate constants. Also in Table 1 are listed the association constants, K_A, for the ionic thiocyanates and for some [PNP]-salts from a recent conductivity study.[16]

All reactions of dichloromethane with the pseudohalide ions, except the cyanide ion, were followed directly by IR in the 2000–2200 cm^{-1} region using liquid cells, *cf.* first column in Table 1. The reactions of N_3^-, NCS^- and $NCSe^-$ were examined in both the 10^{-3} and the 10^{-2} M concentration range, the CH_2Cl_2-$NCTe^-$ reaction only in the 10^{-3} M range owing to the limited solubility of [PNP]TeCN. Several attempts were made to follow the CH_2Cl_2-OCN^- reaction also in the 10^{-2} M range but poor and non-reproducible kinetics were observed in this concentration range.

The CH_2Cl_2-NC^- reaction could not be followed directly by IR owing to the very small extinction coefficient of the cyanide ion at 2060 cm^{-1}. The amount of unreacted ionic cyanide was therefore determined at appropriate time intervals as the selenocyanate ion by IR at 2067 cm^{-1} after selenization with a slight excess, 5 %, of triphenylphosphine selenide dissolved in a known volume of acetonitrile. Prior to the addition of the selenating agent, the solvent was rapidly removed in vacuum and the residue washed with diethyl ether. This change of solvent from dichloromethane to acetonitrile prior to the addition of Ph_3PSe was necessary since the equilibrium constant for the very rapid Ph_3PSe-NC^- reaction in dichloromethane forming Ph_3P and $NCSe^-$ was found to be only 18(2) for a concentration of [PNP]CN of 3×10^{-2} M. In acetonitrile, the corresponding equilibrium constant is very large, ⩾100, as viewed from IR studies of the reverse reaction, Ph_3P-$NCSe^-$, using a large excess of Ph_3P. Control experiments showed this method to determine concentrations of ionic cyanide in dichloromethane to be quantitative within 3 %.

The CH_2Cl_2-NC^- reaction mixture in the 10^{-3} M range attained after 2h, *i.e.* 2 half-lives, a slight yellow colour; after 24 h a weak pink colour could be observed. In the 10^{-2} M range this colourization of the reaction mixture was more

Table 1. IR, UV and association data in dichloromethane at 25.0 °C which formed the basis for the analytical methods applied for the determination of the second order rate constant.

	$\nu_{max}(cm^{-1})$ [a]	ε(250.5 nm)	$K_A\times10^{-3}$ $(1\,M^{-1})$ [c]
N_3^-	2005		
$NCTe^-$	2081		
$NCSe^-$	2067		
NCS^-	2058 [b]		
OCN^-	2140		
MeI		3.71×10^2	
I^-		1.08×10^4	
$[PNP]^+$		3.34×10^3	
Ph_4AsSCN			3.3(2)
Bu_4NSCN			27(5)
[PNP]Cl			1.7(3)
[PNP]SCN			1.8(2)
PNP]SeCN			1.2(2)
[PNP]OCN			1.5(2)
[PNP]CN			~0.5(1)

[a] Within experimental error, ± 2 cm^{-1}, the same frequencies as observed in acetonitrile, *cf.* Ref. 12. [b] In dibromomethane, in 1,2-dichloroethane and in 1,1,2,2-tetrachloroethane ν_{max} for NCS^- was also observed at 2058(2) cm^{-1}. [c] From Ref. 16.

rapid and the rate constant for this reaction was therefore only determined in the 10^{-3} M range. All experiments involving [PNP]CN and [PNP]TeCN were performed in solvent batches carefully flushed with argon.

The MeI-NCS^- reactions in dichloromethane, in 1,2-dichloroethane, and in 1,1,2,2-tetrachloroethane were also followed by IR. These reactions were studied under second order conditions with equal initial concentrations of MeI and of NCS^-, 1.2×10^{-2} M. Separate experiments showed the reactions of 1,2-dichloroethane and 1,1,2,2-tetrachloroethane with NCS^- to be sufficiently slow at 25.0 °C, *cf.* Table 2. The reaction between [PNP]Cl and methyl iodide in dichloromethane was also studied under second order conditions with initial concentrations of 1.2×10^{-2} M. The rate constant was calculated from the increase in absorption with time at 250.5 nm after the necessary dilution had been done. A distinct well in the absorption curve of the [PNP]-cation at this particular wavelength[17] made this rate determination possible, *cf.* Table 1. All rate constants were calculated from measurements performed at 25.0(1) °C. Up to 8 aliquots were withdrawn periodically for each kinetic run. With the exception of the CH_2Br_2-NCS-reaction all reactions were studied at least in duplicate and with separate weighing of the reactants.

Calculations. For the reaction between dichloromethane and an ionic nucleophile in dichloromethane as solvent as depicted by eqns. (1) and (2), pseudo-first order conditions exist since $[CH_2Cl_2]>10^3[N]$. When disregarding association and assuming the reaction according to eqn. (2) to be of no kinetic importance, the usual pseudo-first order equation is valid, eqn. (5).

$$\ln[a/(a-x)]=k_{2,1}Bt \qquad (5)$$

a is the initial concentration of the nucleophile, N, x is the concentration of products at time t and B is the molar concentration of CH_2Cl_2, 15.50 M at 25.0 °C. All kinetic runs were analyzed according to this rate equation and excellent rate plots were observed for more than 3 half-lives except for the CH_2Cl_2-OCN^- reaction in the 10^{-2} M concentration range as mentioned above. Attempts to include the reaction as depicted by eqn. (2) in the kinetics, *i.e.* $k_{2,2}x \sim k_{2,1}B$, caused significant curvature in the rate plots.

When taking association of the applied salts into account one may assume that there is no change in the association constant K_A during the reaction. This approximation may be justifiable since constant ionic strength is maintained during the kinetic runs and association constants of salts of large cations and small anions in dichloromethane are fairly independent upon the anion, *cf.* Table 1. When neglecting higher order aggregates[20] and also assuming the ion pairs to be non-reactive, the following rate equation is valid, eqn. (6).

$$-dx/dt=k_2B(2K_A)^{-1}[(1+4K_A(a-x))^{1/2}-1] \qquad (6)$$

k_2 is the second order rate constant corrected for association, $k_{2,1}$ (corr.), and $k_{2,2}$ is neglected as in eqn. (5). Upon integration one obtains

$$M=-k_2Bt+C \qquad (7)$$

in which

$$M=(1+4K_A(a-x))^{1/2}+\ln[(1+4K_A(a-x))^{1/2}-1]$$

and

$$C=(1+4K_A)^{1/2}+\ln[(1+4K_Aa)^{1/2}-1].$$

In the case of the Finkelstein reactions, MeI-NCS^- and MeI-Cl^-, eqns. (3) and (4), respectively, the usual second order rate equation applies for equal initial concentrations of the reactants when neglecting association, eqn. (8).

$$x/a(a-x)=k_2\,(\text{uncorr.})\,t \qquad (8)$$

When taking association into account but, as above, no higher order aggregates[20] and also assuming the ion-pairs to be non-reactive, the rate equation is

$$dx/dt=k_2(2K_A)^{-1}[(1+4K_A(a-x))^{1/2}(a-x)] \qquad (9)$$

in which k_2 is k_2 (corr.).
Upon integration one obtains

$$Q=k_2(2K_A)^{-1}\,t+D \qquad (10)$$

in which

$$Q=[(1+4K_A(a-x)^{1/2}-1]^{-1}+\tfrac{1}{2}\ln[((1+4K_A(a-x))^{1/2}+1][(1+4K_A(a-x))^{1/2}-1]^{-1}$$

$$D=[(1+4K_Aa)^{1/2}-1]^{-1}+\tfrac{1}{2}\ln[(1+4K_Aa)^{1/2}+1]\times[(1+4K_Aa)^{1/2}-1]^{-1}$$

A general program was written and used for the calculation of the rate constants according to eqns. (7)–(10).

RESULTS AND DISCUSSION

Solubility of onium thiocyanates. The solubility of tetramethylammonium thiocyanate,

Me_4NSCN, in dichloromethane at 25.0 °C was found to be only 8.3×10^{-4} M dm^3. The limited solubility of this salt is in general agreement with the results from solubility studies on various tetramethylammonium salts in 1,1- and 1,2-dichloroethane.[21,22] Tetramethylammonium salts can therefore in general not be used as sources of anions for homogeneous reactions in halogenated alkanes. Furthermore, owing to their limited solubilities in this class of solvents, tetramethylammonium salts have low extraction constants and are therefore of limited use in phase-transfer catalysis,[23] *cf.* Ref. 2.

The solubility of Bu_4NSCN is approximately 5.7 M dm^3 and this salt is thus close to four powers of ten more soluble in dichloromethane than is the corresponding Me_4N^+-salt. This observation is also in agreement with solubility studies in the dichloroethanes.[21,22] Tetraalkylammonium salts with symmetrical cations containing 16 or more carbon atoms are well known to be very soluble in solvents of fairly low dielectric constant and are also significantly better dissociated in this class of solvents than are tetraalkylammonium salts with smaller alkyl groups.[2,24] Presumably, this increase in solubility of tetraalkylammonium salts with increasing length of the carbon chain is due to the increase in the hydrophobicity of the cations per added CH_2 group[2] combined with the increasing ability of the larger cations to adapt configurations which enable a constant minimum cation–anion separation to be maintained.[25,26]

Ph_4AsSCN and [PNP]SCN are extremely soluble in dichloromethane at room temperature and only estimates of their solubilities in this solvent can be given, >6 M dm^{-3}. Actually, only few salts of these two cations can be crystallized from dichloromethane alone. The exceptional solubility of salts of these cations and of other cations containing several phenyl or aryl groups and also of non-ionic aromatic compounds in general is presumably due to specific interaction between the π-electrons of the phenyl groups and the hydrogen atoms of the solvent.[15,27–30] In spite of the fairly low dielectric constant of dichloromethane, 8.9 at 25.0 °C,[31,32] salts in which the cation or the anion contain several phenyl groups are readily dissolved in this solvent due to this unique solvation of the phenyl groups. As a result, these salts are also less associated in dichloromethane than are tetraalkylammonium salts, *cf.* Refs. 15 and 16 and Table 1. Hydrogen bond formation plays apparently a seemingly small role in the solvation of halide ions and other small anions in dichloromethane and other halogenated alkanes.[15,21,22] Solubilization and dissociation of salts in dichloromethane and related solvents are thus not assisted by solvation of the anions leaving these fairly "naked".[24] However, as is to be outlined below, this conclusion may not be valid for strongly basic anions.

It should be emphasized that this special preference for aryl groups as observed for dichloromethane is apparently not the case for 1,2-dichloroethane. Salts of phenyl-containing cations such as Ph_4P^+ and Ph_4As^+ are generally considerably less soluble in 1,2-dichloroethane than are the corresponding Bu_4N^+-salts and are only some two powers of ten more soluble than are the corresponding Me_4N^+-salts.[21,22] In this connection it is interesting to note that 1,2-dichloroethane and benzene give a fairly ideal solvent mixture with a slightly endothermic heat of mixing.[33] The interaction between dichloromethane and benzene, however, is exothermic and a non-ideal mixture is obtained.[33] 1,1-Dichloroethane exhibits even less solubilizing properties toward aromatic compounds than does 1,2-dichloroethane and Ph_4P^+ and Ph_4As^+-salts are generally significantly less soluble in this solvent than in the latter solvent.[21,22] From the thermodynamic parameters for the benzene-CH_2X_2 complexes[28] one may conclude that CH_2I_2 and CH_2Br_2 will behave fairly similarly toward phenyl-containing ions and aromatic compounds as does CH_2Cl_2.

The reactivity of dichloromethane toward the pseudohalide ions. In Table 2 are listed the second-order rate constants, $k_{2,1}$ (uncorr.), for the reactions between dichloromethane and the pseudohalide ions in dichloromethane at 25.0 °C. Table 2 also includes some rate constants of CH_2Br_2 and of 1,2-dichloroethane. [PNP]-salts were used as the source of the anions and the 10^{-3} M concentration range was common for all reactions. The listed second-order rate constants, $k_{2,1}$ (uncorr.), are uncorrected for ionic association, eqn. (5), and are based upon the molarity of the dihaloalkanes and not on the number of C–X bonds. For comparison with rate data of monohaloalkanes, RX, the rate constants in Table 2 and thus also in Tables 3–5 will have to be divided by two. In the last two columns in Table 2

Table 2. Rate constants uncorrected for ionic association, $k_{2,1}$ (uncorr.), half lives, τ, and relative rate constants for the reactions between [PNP]X and CH_2Cl_2 in CH_2Cl_2, between [PNP]SCN and CH_2Br_2 in CH_2Br_2 and between [PNP]X and $ClCH_2CH_2Cl$ in $ClCH_2CH_2Cl$ at 25.0 °C.

X^-	Substrate solvent	Conc. range (M)	$k_{2,1}$ (uncorr.) ($M^{-1}s^{-1}$)	τ(h)	Rel. rate[a]
N_3^-	CH_2Cl_2	10^{-3}	$1.09(4)\times10^{-5}$	1.13(5)	
		10^{-2}	$1.05(6)\times10^{-5}$	1.16(6)	300
NC^-	CH_2Cl_2	10^{-3}	$9.3(2)\times10^{-6}$	1.3(1)	270
$NCTe^-$	CH_2Cl_2	10^{-3}	$8.2(1)\times10^{-7}$	15(1)	24
$NCSe^-$	CH_2Cl_2	10^{-3}	$1.98(7)\times10^{-7}$	62(2)	6
		10^{-2}	$1.78(7)\times10^{-7}$	69(3)	
NCS^-	CH_2Cl_2	10^{-3}	$3.4(2)\times10^{-8}$	360(20)	1
		10^{-2}	$2.9(2)\times10^{-8}$	430(20)	
OCN^-	CH_2Cl_2	10^{-3}	$2.0(2)\times10^{-8}$	600(25)	0.6
NCS^-	CH_2Br_2	10^{-3}	$1.3(1)\times10^{-5}$	1.0(1)	360
NCS^-	$(ClCH_2)_2$	10^{-3}	$1.0(2)\times10^{-7}$	153(15)	3
N_3^-	$(ClCH_2)_2$	10^{-3}	$1.43(6)\times10^{-5}$	1.06(6)	360

[a] Rate constant for the NCS^--CH_2Cl_2 reaction in the 10^{-3} M concentration range defined as unity.

are listed the half-lives, τ, together with the relative rates of the reactions. (The rate constant for the CH_2Cl_2-NCS^- reaction in the 10^{-3} M concentration range is defined as unity.)

The data in Table 2 show that $NCTe^-$, $NCSe^-$, NCS^- and OCN^- react quite slowly with dichloromethane with half-lives ranging from 15 to 600 h at 25.0 °C. Dichloromethane and presumably also 1,2-dichloroethane may therefore prove to be important solvents for studies of these anions provided large aromatic cations or Bu_4N^+ are used. The half-lives for the CH_2Cl_2-N_3^- and CH_2Cl_2-NC^- reactions, however, are only some 70–80 min at room temperature which seriously limits the use of dichloromethane as solvent for studies of reactions involving these two ions. 1,2-dichloroethane is apparently not an alternative, *cf.* Table 3, last entry. Dibromomethane, CH_2Br_2, is 360 times as reactive as is dichloromethane toward the thiocyanate ion with a half-life of only some 60 min. The high reactivity of CH_2Br_2 toward carbophilic nucleophiles as experienced with NCS^- in the present study and also toward halophilic nucleophiles[34] suggests that CH_2Br_2 has only a limited potential as solvent for chemical reactions.

Table 3. A comparison between the second order rate constants for the MeI-X^- reactions in acetonitrile, k_2, and the corresponding uncorrected second order rate constants for the CH_2Cl_2-X^- reactions in dichloromethane in the 10^{-3} M range, $k_{2,1}$ (uncorr.), at 25.0 °C.

X^-	k_2[a] ($M^{-1}s^{-1}$)	$k_{2,1}$(uncorr.)[b] ($M^{-1}s^{-1}$)	$[k_{2,1}(\text{uncorr.})/k_2]\times10^6$
N_3^-	2.45×10^{-1}	1.1×10^{-5}	45
$NCTe^-$	~1.8[c]	8.2×10^{-7}	~0.4
$NCSe^-$	1.77×10^{-1}	2.0×10^{-7}	1.1
NCS^-	2.06×10^{-2}	3.4×10^{-8}	1.7
OCN^-	1.7×10^{-2}	2.0×10^{-8}	1.2
NC^-	26.5	9.3×10^{-6}	0.35

[a] From Ref. 13. [b] From Table 2. [c] Assuming the rate constant for the MeI-$NCTe^-$ reaction to be ten times the rate constant for the MeI-$NCSe^-$ reaction, *cf.* Ref. 12.

The association constants of [PNP]-salts in dichloromethane are fairly independent upon the anion.[15,16] Reactivities of anions in this solvent when [PNP]-salts are used may therefore be compared with reactivities in other solvents in which the applied salts are completely dissociated. Table 3 presents such a comparison between the rate constants as determined in the present study in the 10^{-3} M concentration range and the rate constants for the reactions between the anions and methyl iodide in acetonitrile at 25.0 °C. The ratios between the rate constants, last column in Table 3, show that the anions except the azide ion, N_3^-, and possibly the cyanide ion, NC^-, respond quite similarly to the change in both substrate and solvent although CH_2Cl_2 in CH_2Cl_2 is some six powers of ten less reactive than is MeI in MeCN.

The high rate of the CH_2Cl_2-N_3^- reaction prevents accurate conductivity studies on ionic azides in dichloromethane to be performed. Since all [PNP]-salts, however, appear to have association constants in dichloromethane at 25.0 °C in the $1-2\times10^3$ M^{-1} range[16] one may conclude that the high reactivity of the azide ion in this solvent is not caused by an exceptionally low association constant of $[PNP]N_3$. The question therefore arises whether specific solvation of the anions in dichloromethane is a determining factor with regard to the reactivity of anions in this solvent. While $NCTe^-$, $NCSe^-$ and NCS^-, the three weakly basic and soft nucleophiles, may be solvated through the chlorine atoms,[35] the two basic anions, NC^- and OCN^-, are presumably solvated through interaction with the hydrogen atoms. The high rate of the CH_2Cl_2-N_3^- reaction may well be due to a poor interaction between the azide anion and the solvent molecules. The solvation of the azide ion in dichloromethane may differ from that of the cyanate ion since oxygen atoms seem to interact more strongly than nitrogen atoms with the hydrogen atoms of this solvent; *cf.* the thermodynamic studies on the R_2O-CH_2Cl_2 and the R_3N-CH_2Cl_2 adducts.[36,37] Oxygen nucleophiles like phenoxide ions [43] are known to be distinctly less reactive in dichloromethane than in the usual dipolar aprotic solvents while nitrogen nucleophiles like the azide ion and pyridine do not suffer a similar decrease in reactivity.[39] In short, dichloromethane and other halogenated alkanes seem to behave as protic solvents toward highly basic species, *i.e.* the cyanide ion as mentioned above, and toward nucleophiles containing an oxygen atom, *i.e.* OCN^- and ArO^-.[38] It is notable that ionic fluorides may be crystallized from dichloromethane without any reaction taking place.[17,40]

No data are presently available with regard to the acidity of dichloromethane. This compound is undoubtedly a very weak acid and is probably less acidic than is acetonitrile.[41,42] The mere fact that the tellurocyanate ion does not decompose in dichloromethane is evidence for the weakly acidic properties of this solvent.[43] The very basic cyanide ion does not seem to deprotonate dichloromethane to a significant extent since a freshly prepared solution of [PNP]CN in this solvent was found to give a quantitative yield of ionic selenocyanate after treatment with a slight excess of triphenylphosphine selenide in acetonitrile, *cf.* eqn (11).

$$[PNP]CN+SePPh_3 \longrightarrow [PNP]SeCN+PPh_3 \quad (11)$$

Actually, the cyanide ion in dichloromethane is apparently not sufficiently basic to deprotonate even $ClCH_2CN$, the first formed product from the CH_2Cl_2-NC^- reaction, to an extent which is kinetically detectable. If this were the case, a second molecule of ionic cyanide would be consumed leading to a rate equation as shown in eqn. (12).

$$\ln[a/(a-2x)]=2k_{2,1}Bt \quad (12)$$

The rate data for the CH_2Cl_2-NC^- reaction were in no case found to obey eqn. (12); all rate data could actually be treated successfully according to the usual rate equation, eqn. (5), for more than three half-lives. The weakly yellow colourization of the reaction mixture after some two half-lives, however, suggests that some deprotonation reactions take place. Proton transfer may also be the cause for the poor kinetics observed for the CH_2Cl_2-OCN^- reaction in the more concentrated runs, *cf.* Experimental.

The equilibrium constants for the reaction as depicted by eqn. (11) are distinctly different in dichloromethane and in acetonitrile, 18(2) and $\gg$100, respectively. The lower equilibrium constant in dichloromethane may partly be due to association of [PNP]CN in this solvent leaving fewer free cyanide ions available in dichloromethane than in acetonitrile. The lower equilib-

rium constant in dichloromethane, however, may be considered as further evidence for the suggestion that the cyanide ion is stabilized in this solvent. The fairly low estimate of the equivalent conductivity of the cyanide ion in dichloromethane [16] and the low association constant of [PNP]CN as compared with other [PNP]-salts, *cf.* Table 1, accord with this suggestion.

With regard to reactions of dichloromethane with nucleophiles, it should be emphasized that other factors than solvation of the nucleophiles may be responsible for the observed reactivity differences. The well documented reactivity decrease from monohalomethanes to dihalomethanes is generally supposed to be due to the polar effects of the halogen atoms and their steric demands to which the various nucleophiles respond differently.[44] The electronegative nature of the halogen atoms in dihalomethanes seems to favour attack by hard nucleophiles, *cf.* the considerable reactivity of amines, R_3N,[45] as compared with the poor reactivity of the generally very nucleophilic phosphines, R_3P.[45,46] The exceptional reactivity of the azide ion toward S_N1-type substrates is well-known, *cf.* the azide probe.[47] Since the steric demands of the one non-reacting halogen atom will prevent the formation of the ideal linear transition state, it is natural to assume that nucleophiles with small nucleophilic atoms are to be favoured. Recently [48] it has been suggested that the major interaction responsible for the deactivating effect of an α-chlorine substituent is the four-electron repulsion between a *p*-lone pair on the chlorine atom with the high-lying π-type orbital associated with the reaction coordinate at the S_N2 transition state. All factors taken into consideration the azide ion seems to be ideal for substitution reactions with dihalomethanes. It is apparent that nucleophilicity scales derived from reactions with methyl halides [49] will be highly unreliable when attempting to predict the reactivity of nucleophiles toward dihalomethanes.

Ion-pairs, reactive or non-reactive? A mechanistically important question is whether the predominant nucleophiles in the CH_2Cl_2-X^- reactions are the ion-pairs of the free dissociated ions, *cf.* Refs. 2 and 50 for recent critical discussions of substitution reactions in solvents of low dielectric constants. Owing to the fairly large association constants of salts in dichloromethane, *cf.* Table 1, a considerable fraction of the anions do not exist as free dissociated ions but as ion-pairs. One of the basic assumptions made when evaluating the various rate equations for the CH_2Cl_2-X^- reactions was that the ion-pairs are non-reactive, *cf.* Experimental.

The uncorrected second order rate constants for the CH_2Cl_2-NCS^- and the CH_2Cl_2-$NCSe^-$ reactions as shown in Table 2 are slightly but probably significantly larger in the 10^{-3} M concentration range than in the 10^{-2} M range. This observation is as expected if the free dissociated anions were the predominant nucleophiles in the reactions. If the association constants of [PNP]SCN and [PNP]SeCN in dichloromethane were the same in the two concentration ranges and the second order rate constants were independent upon the ionic strength, the rate constant as calculated by eqn. (7) should be independent upon the concentration range provided the ion-pairs were non-reactive. As shown in Table 4, the corrected rate constants, k_2(corr.), for the CH_2Cl_2-NCS^- and the CH_2Cl_2-$NCSe^-$ reactions are significantly different. Apparently, one or several assumptions made when evaluat-

Table 4. Observed and uncorrected rate constants, $k_{2,1}$(uncorr.), and rate constants corrected for association, $k_{2,1}$(corr.), for the reaction between [PNP]SCN and [PNP]SeCN and CH_2Cl_2 in CH_2Cl_2 at 25.0 °C.

Conc. range	$k_{2,1}$(uncorr.)$\times 10^8$ [a] ($M^{-1}s^{-1}$)	$k_{2,1}$(corr.)$\times 10^8$ [b] ($M^{-1}s^{-1}$)
10^{-2}(NCS^-)	2.86(17)	31(2)
10^{-3}(NCS^-)	3.43(17)	14(1)
10^{-2}($NCSe^-$)	17.8(7)	168(9)
10^{-3}($NCSe^-$)	19.8(7)	67(4)

[a] Calculated according to eqn. (5). [b] Calculated according to eqn. (7).

ing eqn. (7) are not valid.

According to the Acree equation [51] an observed rate constant may be considered as the sum of the rates due to the free ions, k_i, and to the ion-pairs, k_m,[52–55] eqn. (13),

$$k_2(\text{obs.}) = \alpha k_i + (1-\alpha)k_m \quad (13)$$

in which α, the degree of dissociation, is given by eqn. (14),

$$K_A = (1-\alpha)/c\alpha^2 \quad (14)$$

and c is the concentration of the electrolyte. Since α is dependent upon the concentration, one may for the evaluation of k_i and k_m apply an average value of α as calculated from the average concentration of the applied salts during the kinetic runs for the two concentration ranges, 6×10^{-3} and 6×10^{-2} M, respectively. From the rate data in Table 4 the following rate constants may then be calculated; NCS^-: $k_i = 6.0\times10^{-8}$ $M^{-1}s^{-1}$ and $k_m = 2.5\times10^{-8}$ $M^{-1}s^{-1}$; $NCSe^-$: $k_i = 2.7\times10^{-7}$ $M^{-1}s^{-1}$ and $k_m = 1.7\times10^{-7}$ $M^{-1}s^{-1}$. Thus, the reactivity of the ion-pairs appears to be some 40 and 60 % of the reactivity of the free NCS^- and $NCSe^-$ ions, respectively. Beronius and co-workers [52] have concluded that the nucleophilicity of the Bu_4N^+-Br^- ion-pairs is some 20 % of the nucleophilicity of the free Br^- ions toward 4-nitrobenzyl bromide in acetone. Since the k_i/k_m ratio is anticipated to decrease with decreasing dielectric constant of the solvent it may not be surprising to find that k_m is not negligible in a solvent like dichloromethane.

However, the numerous assumptions made when treating the kinetic data according to the Acree equation should be born in mind. Furthermore, the larger corrected rate constants in the 10^{-2} M range than in the 10^{-3} M range may be due to a positive salt effect and not to an increase in the relative concentration of the ion-pairs. Since no reliable data on salt effects on Finkelstein reactions in solvents of low dielectric constant are presently available, it is to be concluded that the Acree equation [5] is to be applied with caution for reaction systems of the type described in the present study. The ambiguity with regard to the possible reactivity of ion-pairs suggests that when rate data from kinetic studies involving weakly dissociated reactants are to be presented concentrations, counterions *etc.* are to be carefully listed in order to avoid futural invalid comparisons.

The MeI-NCS^- and MeI-Cl^- reactions. The results for these Finkelstein reactions are summarized in Table 5. In the first column are listed the uncorrected rate constants, k_2 (uncorr.), as calculated according to eqn. (8). In the last column are listed the corrected rate constant, k_2 (korr.), as calculated according to eqn. (10) when the necessary association constants were available.

As anticipated from the association constants, *cf.* Refs. 15 and 16 and Table 1, the uncorrected rate constant for the MeI-NCS^- reaction is dependent upon the cation and increases from Bu_4NSCN to [PNP]SCN; Ph_4AsSCN being intermediate. When corrected for association fairly similar values for k_2 (corr.) are obtained, *cf.* the last column in Table 5. This observation suggests that the ion-pairs are fairly non-reactive toward

Table 5. Second order rate constants for the reactions between some onium thiocyanates and methyl iodide in CH_2Cl_2, between [PNP]SCN and methyl iodide in 1,2-dichloroethane and in 1,1,2,2-tetrachloroethane and between [PNP]Cl and methyl iodide in CH_2Cl_2 at 25.0 °C. (Initial concentrations of both reactants in the $1.14-1.25\times10^{-2}$ M range).

Salt	Solvent	k_2(uncorr.)$\times10^2$ [a] ($M^{-1}s^{-1}$)	k_2(corr.)$\times10^2$ [b] ($M^{-1}s^{-1}$)
Bu_4NSCN	CH_2Cl_2	2.3(3)	25(5)
Ph_4AsSCN	CH_2Cl_2	4.5(3)	23(2)
[PNP]SCN	CH_2Cl_2	6.1(5)	23(2)
[PNP]SCN	$C_2H_4Cl_2$ [c]	4.8(3)	[d]
[PNP]SCN	$C_2H_2Cl_4$ [e]	1.27(8)	[d]
[PNP]Cl	CH_2Cl_2	14(2)	56(4)

[a] Calculated according to eqn. (8). [b] Calculated according to eqn. (10). [c] 1,2-Dichloroethane. [d] K_A not available. [e] 1,1,2,2-Tetrachloroethane.

Table 6. A comparison between the corrected second order rate constants for X^- +MeI→MeX+I^- in various solvents at 25.0 °C. (X=NCS^- and Cl^-).

Solvent[a]	X=NCS^- $k_2(M^{-1}s^{-1})$	X=Cl^- $k_2(M^{-1}s^{-1})$
H_2O	3.16×10^{-4}	3.54×10^{-6}
MeOH	5.01×10^{-4}	3.16×10^{-6}
Formamide	1.58×10^{-3}	5.01×10^{-5}
DMF	7.9×10^{-2}	2.51
DMA	1.6×10^{-1}	7.9
MeCN	1.2×10^{-2}	1.2×10^{-1}
N-MePy[b]	1.58	25.1
Acetone	1.6×10^{-2}	5.01
CH_3NO_2	1×10^{-2}	5×10^{-2}
CH_2Cl_2[c]	2.3×10^{-1}	5.6×10^{-1}

[a] From the survey in Ref. 14. [b] N-MePy is *N*-methylpyrrolidone. [c] This study.

methyl iodide as compared with the free thiocyanate ions since Bu_4NSCN is significantly more associated than are Ph_4AsSCN and [PNP]SCN, *cf.* Table 1.

The uncorrected rate constants for the MeI-NCS^- reaction in 1,2-dichloroethane and in 1,1,2,2-tetrachloroethane are slightly lower than in dichloromethane. The present lack of association constants for [PNP]SCN in these two solvents prevents the calculation of the corrected second order rate constants. However, the slight differences in the uncorrected rate constants may suggest that these solvents are equally valuable as solvents for organic substitution reactions.

In Table 6 a comparison is made between the corrected second order rate constants for the MeI-NCS^- and the MeI-Cl^- reactions in several protic and aprotic solvents including dichloromethane at 25.0 °C. In the case of Finkelstein reactions dichloromethane appears to be a significantly better solvent than are all the protic solvents and is actually comparable with the usual dipolar aprotic solvents. A similar observation has recently been made for a Menschutkin reaction, the EtI-pyridine reaction.[39] Apparently, rates of this type of substitution reactions are poorly correlated with the dielectric constant of the solvents.[56,57] The lack of any reliable correlation between the dielectric constant of the solvent and reaction rates was actually suggested by Hinshelwood nearly 50 years ago.[58]

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N-Quaternary Compounds. Part LV.* Synthetic Studies of the 2,3-Dihydrothiazolo[3,2-*c*]pyrimidinium-8-olate System

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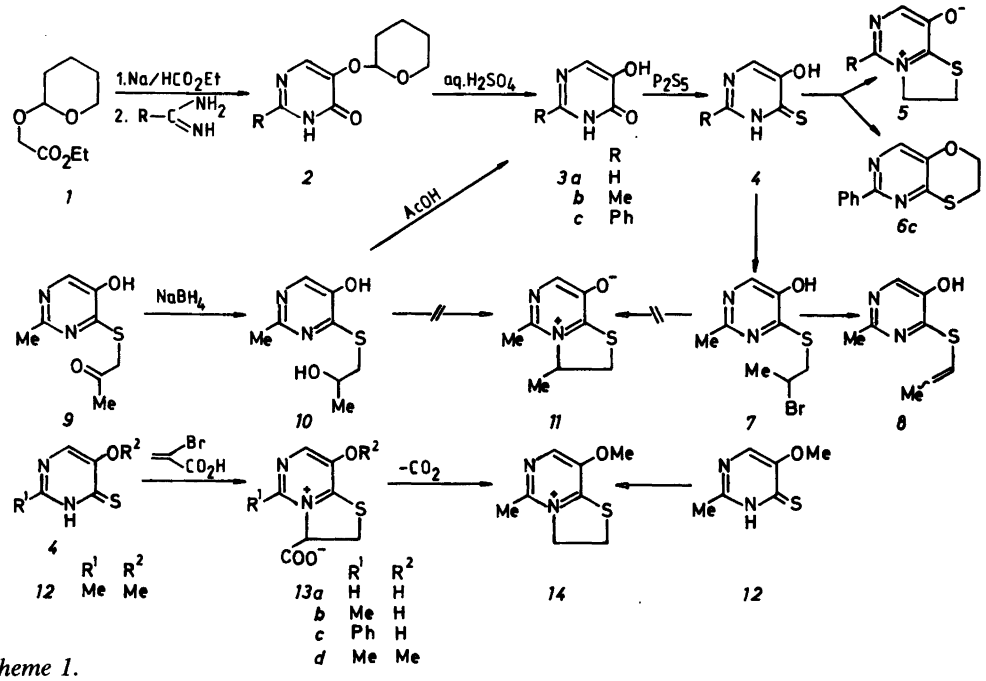
5-Hydroxy-4-pyrimidinethiones form the novel 2,3-dihydrothiazolo[3,2-*c*]pyrimidinium-8-olate system on reaction with vicinal dibromides or with 2-bromopropenoic acid. Steric or electronic effects may change the reaction path towards the formation of a 2,3-dihydro[1,4]oxathiino[5,6-*d*]-pyrimidine or may lead to *S*-vinylation.

For further comparisons of chemical and physical properties of the novel dihydrothiazolo[3,2-*a*]-pyridinium-8-olate system[2] with the aza-analogous dihydrothiazolo[3,2-*c*]pyrimidinium-8-olate system,[3] we herein report synthetic work leading to the latter system (Scheme 1).

The protected glycolic acid ester *1* is formylated on the methylene carbon and condensed with an amidine to yield the pyrimidine *2* with the desired 2-substituent. The latter can be isolated, or may advantageously have the protecting group removed *in situ* by aqueous acid. Phosphorus pentasulfide is used for the thiation. The reactivity depends on the 2-substituent; thus the 2-

* Part LIV, see Ref. 1.



Scheme 1.

0302-4369/83 $2.50

unsubstituted lactam *3a* requires heating in refluxing pyridine for 1 d whereas the reaction for the 2-phenyl derivative is complete after 7 d. *4a* reacts with 1,2-dibromoethane in the same manner as reported for *4b*.[3] The 2-phenyl derivative *4c* gives the same type of product *5c* in methanolic sodium methoxide; with sodium carbonate in DMF several products are formed, a major one being the bicyclic oxathiin *6c*. In the pyridine series the latter type of cyclization occurs only under strongly acid conditions.[2] The formation of *6c* is attributed to the steric effects of the vicinal phenyl substituent as well as to the low nucleophilicity of the pyrimidine nitrogen.

The 3,5-dimethyl derivative *11* could not be obtained by simple cycloalkylation reactions which we attribute to steric interaction between these substituents. The corresponding pyridine, however, can be formed in such reactions although there is a notable steric retardation.[2]

In the alkylation of *4b* by means of 1,2-dibromopropane, the *S*-vinyl derivative *8* was obtained as a 1:1 *cis–trans* mixture (^{1}H NMR). Formation of *8* by an elimination reaction from the desired bicyclic compound *11* present as an intermediate can be excluded since ring opening reactions of *11* would lead to a mixture of *N*- and *S*-vinyl derivatives by analogy to the behaviour of pyridine analogues.[2] Instead the formation of *8* corresponds to selective substitution at C–1 of the alkylating agent with subsequent HBr elimination; the pyridine corresponding to *7*, however, undergoes cycloalkylation to form the pyridine analogue of *11*.[2] The different reaction paths for the two azine systems we attribute to differences in the nucleophilicities of the heterocyclic nitrogens.

Assignment of structure *8* to the vinyl product is based on its UV absorption bands at 314 and 234 nm; isomeric *N*-vinyl 4-pyrimidinethiones absorb at 350–360 and *ca.* 280 nm.[4]

In an alternative approach to the preparation of *11*, the thiolactam *4b* was *S*-alkylated (*9*) by bromoacetone and the oxo function reduced by sodium borohydride to the alcohol *10*. Attempts to effect acid catalyzed cyclization as for the pyridine analogues, however, met with little success due to the ease of nucleophilic displacement of the 4-thioether group which results in the formation of the lactam *3b*.

The thiolactam *4b* will add in a Michael fashion over the sulfur to 2-bromopropenoic acid with subsequent cyclization to form the bicyclic pyrimidinium salt *13b*.[3] The thiolactam *4a* reacts in the same manner. The rate of this reaction is sensitive to the nucleophilicity of the Michael addend; the reaction time for the lactam *4a*, which is without a 2-substituent, is considerably increased over that for the 2-methyl derivative *4b* whereas the 2-phenyl thiolactam *4c* failed to react. The 5-methoxythiolactam *12* reacts with 2-bromopropenoic acid to form *13d*; the latter is very readily decarboxylated due to the activation from the quaternary nitrogen. The methoxy group does not counteract the electron deficiency of the pyrimidine ring to the same extent as does the hydroxy group, and hence the tendency for decarboxylation is greater in *13d* than in *13a* and *13b*. The product isolated from the reaction of *12* was therefore the decarboxylated product *14*; the latter is also available by the direct alkylation of *12* by 1,2-dibromoethane.

The mass spectra of the betaines *5* all have a molecular ion with mass number corresponding to the mass of the betaine. By analogy to our previous structure analyses of gaseous species from betaines in the gas phase in the mass spectrometer using appearance potentials,[5] the compounds *5* will go into the gas phase without any structure rearrangement. This is supported by the different fragmentation pattern of the phenyl isomers *5c* and *6c* and the different fragmentation of *5a* and *13a*. The latter is decarboxylated in the mass spectrometer to give a molecular ion of the same mass as for *5a*. The fragmentation pattern, however, is different because of ring-opening and formation of the corresponding *N*-vinyl isomer.

EXPERIMENTAL

The mass spectra are presented as MS[70 eV; *m/z* (% rel. int.)].

2-Phenyl-5-(tetrahydro-2-pyranyloxy)-4-pyrimidinone 2c. Ethyl (tetrahydro-2-pyranyloxy) acetate[6] (18.8 g, 0.1 mol) was added dropwise to a well-stirred mixture from sodium (2.3 g, 0.1 mol) and ethyl formate (7.4 g, 0.1 mol) in dry ether (100 ml) at 0 °C. The mixture was stirred at room temperature for 4 h before benzamidine (0.1 mol) in ethanol (100 ml) was added. The mixture was heated under reflux for 16 h, the solvent evaporated, water (300 ml) added, the pH adjusted to *ca.* 5 with acetic acid, the mixture

extracted with chloroform (3 × 90 ml), the chloroform solution washed with water and the dried ($MgSO_4$) solution evaporated; yield 14.3 g (53 %), m.p. 174 °C (EtOH). Anal. $C_{15}H_{16}N_2O_3$: C, H. ^{1}H NMR ($CDCl_3$): δ 1.8, 3.8, 5.65 (pyranyl), 7.8 (Ph, H–6) MS: (M, O), 188 (100), 116 (22), 104 (37), 85 (60), 84 (14), 77 (20), 57 (22).

5-Hydroxy-2-methyl-4-pyrimidinone 3b.[7] Ethyl (tetrahydro-2-pyranyloxy)acetate[6] (145.0 g, 0.77 mol) was added dropwise to a mixture from sodium (17.7 g, 0.77 mol) and ethyl formate (57.0 g, 0.77 mol) in dry ether (400 ml) at 0 °C. The mixture was stirred at room temperature for 4 h before acetamidine hydrochloride (72.8 g, 0.77 mol) in ethanol (500 ml) was added. The mixture was heated under reflux for 21 h, the solvent evaporated at reduced pressure, the residue dissolved in water (600 ml), sulfuric acid (20 ml) added, the mixture stirred for 2 h before addition of sodium carbonate to pH 5 and the precipitated product triturated with water (500 ml) to redissolve any inorganic salt; yield 41.4 g (43 %), m.p. 285 °C (H_2O).

5-Hydroxy-2-phenyl-4-pyrimidinone 3c.[8] 2-Phenyl-5-(tetrahydro-2-pyranyloxy)-4-pyrimidinone (20.0 g, 0.073 mol) was added to 1 M H_2SO_4 and the mixture stirred for 2 h before sodium carbonate was added to pH 5. The precipitate was recrystallized from water–ethanol (2:1); yield 11.4 g (83 %), m.p. 220–222 °C.

5-Hydroxy-4-pyrimidinethione 4a. A well-stirred mixture of 5-hydroxy-4-pyrimidinone[9] (10.0 g, 0.09 mol) and phosphorus pentasulfide (22.2 g, 0.1 mol) in dry pyridine (250 ml) was heated under reflux for 24 h. Water (150 ml) was added to the cold reaction mixture and the heating under reflux was resumed for 2 h to destroy excess phosphorus pentasulfide. Concentration of the solution to *ca.* 100 ml at reduced pressure precipitated the product which was recrystallized from water; yield 6.6 g (57 %), m.p. 212 °C (decomp.). Anal. $C_4H_4N_2OS$: C, H. ^{1}H NMR (TFA): δ 7.60 (H–6), 8.85 (H–2). MS: 128 (100, M), 100 (3), 95 (5), 84 (7), 73 (6), 68 (14).

5-Hydroxy-2-phenyl-4-pyrimidinethione 4c. 5-Hydroxy-2-phenyl-4-pyrimidinone (10.0 g, 0.053 mol) and phosphorus pentasulfide (15.6 g, 0.07 mol) were heated together with stirring in boiling pyridine (150 ml) for 7 d. Water (50 ml) was added to the cold mixture, the mixture refluxed for another 2 h, the solvents distilled off, the residue triturated with water and the solid recrystallized from H_2O–EtOH (3:1), yield 9.5 g (88 %), m.p. 195–196 °C. Anal. $C_{10}H_8N_2OS$: C, H. ^{1}H NMR (TFA): δ 7.8 (Ph and H–6). MS: 204 (100, M), 188 (55), 171 (15), 144 (40), 116 (57), 115 (11), 101 (13), 89 (26), 77 (34).

2,3-Dihydrothiazolo[3,2-c]pyrimidinium-8-olate 5a. 5-Hydroxy-4-pyrimidinethione (6.5 g, 0.05 mol), sodium carbonate (5.3 g, 0.05 mol) and 1,2-dibromoethane (9.4 g, 0.05 mol) in dry DMF (100 ml) were heated at 55 °C for 3 h. The mixture was evaporated, the residue dissolved in the minimum amount of boiling water, the pH brought to *ca.* 4, the precipitate was redissolved in water and passed through a DEAE-Sephadex A-25 column in the formate form and the title compound eluted with 0.01 M formic acid; yield 3.2 g (42 %), m.p. 213 °C. Anal. $C_6H_6N_2OS$: C, H. ^{1}H NMR (TFA): δ 4.02 (2H-2, t), 5.38 (2H-3, t), 8.57 (H-7), 9.13 (H-5), MS: 154 (100, M), 126 (21), 82 (22), 80 (22), 71 (41), 68 (17), 60 (36).

5-Phenyl-2,3-dihydrothiazolo[3,2-c]pyrimidinium-8-olate 5c. 1,2-Dibromoethane (6.0 g, 0.032 mol) in methanol (35 ml) was added to a solution from 5-hydroxy-2-phenyl-4-pyrimidinethione (6.6 g, 0.032 mol) in methanolic sodium methoxide (100 ml; 0.064 mol) and the mixture refluxed for 24 h. The solvent was then distilled off at reduced pressure, water added to the residue, the solution washed with chloroform (5×), the aqueous solution evaporated at reduced pressure, the dry residue extracted with 2-propanol, the alcohol evaporated and the residue recrystallized from water; yield 0.8 g (11 %), m.p. 237 °C. Anal. $C_{12}H_{10}N_2OS$: C, H. ^{1}H NMR (TFA): δ 3.92 (2H-2, t), 5.20 (2H-3, t), 7.63 (5-Ph, s), 8.70 (H-7). MS: 230 (100, M), 204 (79), 172 (30), 147 (63), 144 (30), 116 (35), 115 (24), 105 (24), 104 (49), 103 (81), 77 (40).

6-Phenyl-2,3-dihydro[1,4]oxathiino[5,6-d]pyrimidine 6c. 1,2-Dibromoethane (2.8 g, 0.015 mol) in dry DMF (15 ml) was added dropwise to a mixture of 5-hydroxy-2-phenyl-4-pyrimidinethione (3.0 g, 0.015 mol) and sodium carbonate (1.6 g, 0.015 mol) in dry DMF (100 ml) at room temperature. The mixture was stirred at 55 °C for 4 d, the DMF distilled off at reduced pressure, water added to the residue and the mixture extracted with chloroform (5×), the chloroform extracts washed with water, the dried ($MgSO_4$) solution concentrated and chromatographed on a silica gel column eluting with chloroform. The first fraction contained the title compound which was recrystallized from ethanol; yield 0,2 g (6 %), m.p. 109 °C. Anal. $C_{12}H_{10}N_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 3.27 (2H-3, t), 4.39 (2H-2, t), 7.8 (Ph, H-8). MS: 230 (100, M), 170 (12), 149 (13), 147 (62), 115 (19), 103 (40), 77 (9).

cis/trans-*5-Hydroxy-2-methyl-4-propenylthiopyrimidine* 8. 1,2-Dibromopropane (1.4 g, 0.007 mol) in dry DMF (20 ml) was added dropwise to a mixture of 5-hydroxy-2-methyl-4-pyrimidinethione[3] (1.0 g, 0.007 mol) and sodium carbonate (0.74 g, 0.007 mol) in dry DMF (50 ml). The

mixture was stirred at 60 °C for 4 h, the solvent was distilled off at reduced pressure, water added to the residue and the mixture extracted with ether (5×), the ether washed and dried ($MgSO_4$) and the ether distilled off leaving the title compound as a 1:1 *cis–trans* mixture; yield 0.67 g (53 %). The product can be further purified by sublimation at 100 °C/0.05 mmHg. Anal. $C_8H_{10}N_2OS$: C, H. ^{1}H NMR ($CDCl_3$; 200 MHz): δ 1.85–1.95 (*cis/trans* β-Me, $J_{al.}$ 1.6 Hz, $J_{vic.}$ 7 and 10 Mz), 5.95–6.15 (H–β), 6.82 (H_α, J_{trans} 16 Hz), 7.06 (H_α, J_{cis} 10 Hz), 7.63 and 7.67 (C–6; *cis/trans* isomers). UV (EtOH, log ε): 314 (4.08), 234 nm (3.65). MS: 182 (8, M), 167 (100), 126 (24), 109 (15), 82 (20), 54 (17).

1-(5-Hydroxy-2-methyl-4-pyrimidinylthio)-2-propanone 9. Bromoacetone (3.4 g, 0.025 mol) in methanol (15 ml) was added dropwise to a solution from 5-hydroxy-2-methyl-4-pyrimidinethione [3] (2.9 g, 0.02 mol) in 0.4 M methanolic sodium methoxide (50 ml). The reaction mixture was stirred at room temperature for 4 h, the solvent distilled off, water added to the residue and the mixture extracted with chloroform (5 × 50 ml), the washed and dried ($MgSO_4$) chloroform solution evaporated and the residue recrystallized from ethanol–benzene 1:1; yield 3.0 g (76 %), m.p. 153 °C (decomp.). Anal. $C_8H_{10}N_2O_2S$: C, H. ^{1}H NMR (TFA): δ 2.58 (MeCO), 2.80 (2-Me), 4.37 (CH_2), 7.97 (H-6). MS: 198 (12, M), 157 (11), 156 (89), 155 (100), 123 (36), 109 (29), 82 (26), 78 (30).

1-(5-Hydroxy-2-methyl-4-pyrimidinylthio)-2-propanol 10. Sodium borohydride (0.8 g, 0.021 mol) was added to a solution of 1-(5-hydroxy-2-methyl-4-pyrimidinylthio)-2-propanone (3.2 g, 0.016 mol) in 2-propanol (150 ml) and the mixture stirred at room temperature for 2 h. The solvent was then removed at reduced pressure, water added to the residue, the pH brought to *ca.* 6 with HCl, the mixture extracted with ethyl acetate (10 × 75 ml), the washed and dried ($MgSO_4$) extracts evaporated and a chloroform solution of the residue filtered through a silica gel column; yield 2.3 g (72 %) of oily material. Anal. $C_8H_{12}N_2O_2S$: C, H: ^{1}NMR (TFA): δ 1.52 (β-Me), 2.82 (2-Me), 3.63 (CH_2), 4.4 (CH), 7.82 (H-6). MS: 200 (0.8, M), 167 (16), 156 (52), 143 (17), 142 (100), 126 (13), 123 (49), 109 (34).

5-Hydroxy-2-methyl-4-pyrimidinone 3b *from* 10. A solution of 1-(5-hydroxy-2-methyl-4-pyrimidinylthio)-2-propanol (0.005 mol) in acetic acid (20 ml) was heated under reflux for 24 h before the acetic acid was removed by distillation. The residue was heated in 6 M hydrochloric acid for 1 h to hydrolyze any acetate. Evaporation and trituration with sodium carbonate solution left the lactam *3b* in 70 % yield.

8-Hydroxy-2,3-dihydrothiazolo[3,2-c]pyrimidinium-3-carboxylate 13a. A solution of 5-hydroxy-4-pyrimidinethione (1.0 g, 0.008 mol) and 2-bromopropenoic acid (1.5 g, 0.01 mol) in aqueous (1:1) methanol (50 ml) was stirred at room temperature for 14 d. During this period additional 2-bromopropenoic acid (4 × 0.9 g) was added. Evaporation of the reaction mixture and trituration with ethyl acetate left the hydrobromide of the title compound. The zwitterion was obtained from this salt using a DEAE-Sephadex A-25 column in the formate form and 2 % formic acid; yield 0.55 g (35 %), m.p. 150 °C (decomp.); (EtOH). Anal. $C_7H_6N_2O_3S$: C, H. ^{1}H NMR (TFA): δ 4.44 (2H-2), 6.53 (H-3), 8.67 (H-7), 9.34 (H-5). MS: (M, O), 154 (47), 153 (61), 128 (20), 68 (14), 44 (100).

8-Methoxy-5-methyl-2,3-dihydrothiazolo[3,2-c]pyrimidinium bromide 14. Method A: 5-Methoxy-2-methyl-4-pyrimidinethione [10] (6.0 g, 0.038 mol), sodium carbonate (2.0 g, 0.038 mol) and 1,2-dibromoethane (7.1 g, 0.038 mol) in dry DMF (100 ml) were heated together at 70 °C for 4 h. The mixture was then concentrated to *ca.* 50 ml, the precipitate collected and triturated with a little water to dissolve inorganic material; yield 4.9 g (49 %), m.p. 220–222 °C (MeOH). Anal. $C_8H_{11}BrN_2OS$: C, H. ^{1}H NMR (TFA): δ 3.03 (5–Me), 4.10 (2H–2), 4.23 (OMe), 5.38 (2H–3), 8.48 (H–7), MS: 183 (39, M), 182 (87), 181 (100), 168 (33), 167 (25), 156 (13), 123 (23), 96 (35), 94 (37).

Method B: 5-Methoxy-2-methyl-4-pyrimidinethione (3.0 g, 0.019 mol) and 2-bromopropenoic acid (4.4 g, 0.029 mol) were heated together at 60 °C in methanol (140 ml) for 5 d. Trituration of the precipitates with water left the title compound; yield 1,3 g (26 %).

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Short Communications

The Reaction between Acetic Formic Anhydride and Salicylamide

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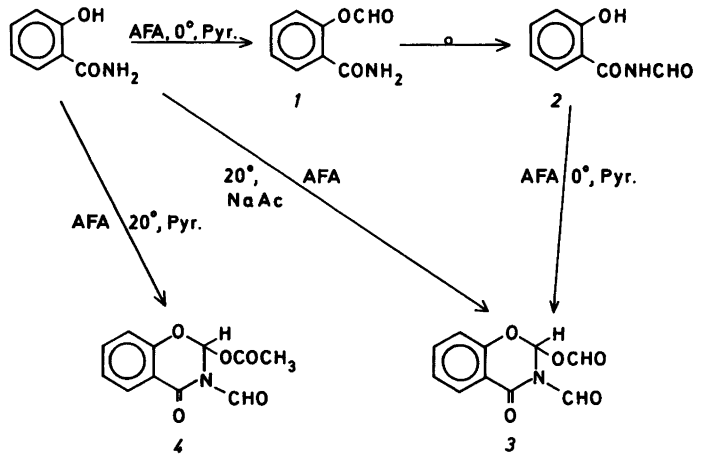
In connection with our interest in the reaction between salicylamide and triethyl orthoformate[1] we became aware of some interesting reactions between salicylamide and acetic formic anhydride, in which a 2,3-dihydro-1,3-benzoxazine-4-one structure was formed.

Formylation of salicylamide cannot be carried out by formic acid or ethyl formate, but acetic formic anhydride formylates salicylamide rapidly when a basic catalyst is used. If the formylation is carried out at 0 °C in chloroform solution with a few drops of pyridine as catalyst *O*-formylsalicylamide *1* is obtained in good yield (Scheme 1). Like the analogous *O*-acetylsalicylamide[2] *O*-formylsalicylamide rearranges readily to the *N*-formylsalicylamide. In DMSO-d_6 solution the rearrangement proceeds to 50 % within 40 min at room temperature. Reflux in toluene with a few drops of pyridine causes the *O*-formylsalicylamide to rearrange within a few min, which indicates that the formyl group migrates faster than the acetyl group.[2]

When the formylation reaction was carried out at room temperature with sodium acetate or very small amounts of pyridine as catalyst a compound with the composition corresponding to the introduction of three formyl groups into salicylamide was formed. The ^{1}H NMR spectrum of the compound showed three different C*H* signals at 6.95, 8.20 and 9.44 ppm and the ^{13}C NMR spectrum showed one significant diamagnetic shifted signal at 90.6 ppm, corresponding to an orthoester type carbon atom.

The structure of the compound must therefore be 2-formyloxy-3-formyl-2,3-dihydro-1,3-benzoxazin-4-one *3* (Scheme 1). Reaction between acetic formic anhydride and salicylamide carried out at room temperature in chloroform with greater amounts of pyridine as catalyst gave an analogous compound *4* with one formyl group and one acetyl group attached to the 2,3-dihydro-1,3-benzoxazin-4-one system. The IR spectra of *3* and *4* were similar to each other. The only differences were: the intensities of the absorp-



Scheme 1. (AFA=Acetic formic anhydride, NaAc=sodium acetate, Pyr.=pyridine).

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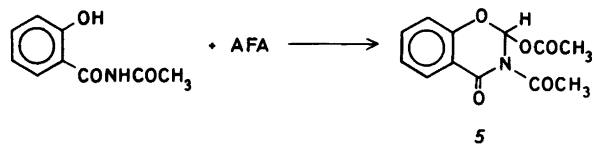

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Scheme 2.

tions, shifting of the 1750 cm^{-1} signal in *3* to 1770 cm^{-1} in *4* and some smaller pattern differences at lower wavelengths. From the ^1H NMR spectrum it could be seen that *4* was a *N*-formyl and not a *N*-acetyl compound because the formyl C*H* signal was found at 9.40 ppm. The C*H* signal for *O*-formyl is normally found at 8.15 ppm.

N-Acetylsalicylamide reacts with acetic formic anhydride to give compound *5* with two acetyl groups. At the same time *N,O*-diacetylsalicylamide is formed in almost the same yield (Scheme 2). From the ^1H NMR chemical shifts of the methyl groups it can be seen, that one of the acetyl groups in *5* is an *N*-acetyl group at 2.64 ppm and the other at 1.97 ppm an *O*-acetyl as in *4*, where the acetyl group was found at 2.01 ppm.

N-Formylsalicylamide treated in the same manner as *N*-acetylsalicylamide with acetic formic anhydride in chloroform solution with pyridine catalysis gave compound *3* and no acetyl group is introduced in the predominating reaction product.

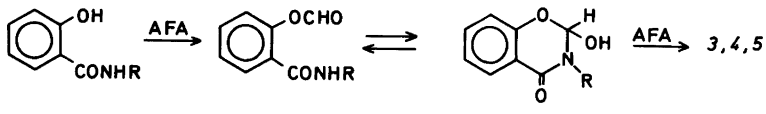
The *O*-formyl group seems essential for the reaction leading to dihydrobenzoxazine. Neither *O*-acetyl- nor *N,O*-diacetylsalicylamide or benzamide could react with acetic formic anhydride. This indicates that the free phenolic group is essential for initiation of the formylation with acetic formic anhydride. Since *N*-acetylsalicylamide reacts to dihydrobenzoxazine even at 0 °C, and the reaction does not stop at the *O*-formyl step as it does for unsubstituted salicylamide, the *N*-acetylation seems to accelerate the ring closure.

The mechanism for these reactions could therefore be explained by an initial *O*-formylation followed by an equilibrium between the *O*-formyl and the dihydrobenzoxazine structure. Dependent on temperature, catalyst and substitution of the salicylamide the next steps are formylation and/or acetylation (Scheme 3).

Experimental. The experimental equipment was reported earlier.[3] Melting points are uncorrected.

O-*Formylsalicylamide* 1. Salicylamide (0.05 mol) was dissolved in chloroform (50 ml), ten drops of pyridine were added, the solution cooled to 0 °C and acetic formic anhydride (0.15 mol) added during 30 min. After 1 h at 0 °C the unreacted salicylamide was filtered off and the filtrate cooled to −18 °C overnight. The *O*-formylsalicylamide was filtered off giving 53 % yield based on dissolved salicylamide. M.p. 97–99 °C. Anal. $C_8H_7NO_3$: C, H, N. ^1H NMR (DMSO-d_6): δ 6.90–8.03 (6 H, m), 8.45 (1 H, s). ^{13}C NMR (DMSO-d_6): δ 167.0, 160.6, 147.4, 131.5, 129.2, 126.2, 122.9. IR (KBr, cm^{-1}): 3320 (m), 3150 (m), 1730 (s), 1720 (s), 1660 (s), 1620 (s), 1400 (m), 1190 (s), 1110 (s). IR ($CHCl_3$, cm^{-1}): 3530 (w), 3400 (w), 1750 (m), 1675 (s).

2-*Formyloxy*-3-*formyl*-2,3-*dihydro*-1,3-*benzoxazin*-4-*one* 3 was prepared from salicylamide (0.05 mol), acetic formic anhydride (15 ml) and sodium acetate (5 g) in chloroform (65 ml). The mixture was stirred at room temperature for 3 h. The sodium acetate was filtered off and the filtrate washed with 5 ml ice-water. The chloroform-phase was dried over $MgSO_4$, evaporated to dryness and recrystallized from light petroleum giving *3* in 41 % yield. M.p. 86–88 °C. Anal. $C_{10}H_7NO_5$: C, H, N. ^1H NMR ($CDCl_3$): δ 6.95–8.20 (6 H, m), 9.44 (1 H, s). ^{13}C NMR ($CDCl_3$): δ 159.5, 159.1, 157.7, 153.9, 137.1, 128.6, 124.5, 117.8, 115.3, 90.6. IR ($CHCl_3$, cm^{-1}): 3020 (w), 1750 (s), 1725 (s), 1710 (s), 1610 (s), 1465 (s), 1390 (s), 1370 (m), 1320 (m), 1300 (s), 1260 (s), 1245 (s). *3* was also prepared from *N*-formylsalicylamide (0.02 mol) in chloroform (20 ml) with 10 drops of pyridine and acetic formic anhydride added in 15 min. The temperature was kept below 5 °C. After termination of the reaction all *N*-formylsalicylamide had dis-



Scheme 3.

solved and the reaction mixture was cooled to −18 °C giving 75 % yield of *3*.

2-Acetyloxy-3-formyl-2,3-dihydro-1,3-benzoxazin-4-one 4 was prepared from salicylamide (0.02 mol) and acetic formic anhydride (0.10 mol) in chloroform (20 ml) with 10 drops of pyridine. The mixture was stirred at room temperature. After 30 min all the salicylamide had reacted. The reaction mixture was evaporated to dryness and recrystallized from light petroleum-ethyl acetate. Yield 62 %, m.p. 115−117 °C. Anal. $C_{11}H_9NO_5$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 2.00 (3 H, s), 6.90−8.20 (5 H, m), 9.45 (1 H, s). ^{13}C NMR ($CDCl_3$): δ 168.0, 159.6, 154.1, 137.0, 128.6, 124.1, 117.8, 115.3, 91.0, 20.5. IR ($CHCl_3$, cm^{-1}): 3020 (w), 1770 (m), 1725 (s), 1710 (s), 1610 (m), 1465 (s), 1390 (m), 1370 (m), 1325 (m), 1305 (m), 1260 (m), 1250 (m).

2-Acetyloxy-3-acetyl-2,3-dihydro-1,3-benzoxazin-4-one 5 was prepared from *N*-acetylsalicylamide (0.02 mol) in chloroform (30 ml) with 10 drops of pyridine. Acetic formic anhydride (0.06 mol) was added at 0 °C in 30 min. After 1 h the reaction mixture was cooled to −18 °C and the precipitate recrystallized from light petroleum-ethyl acetate giving a yield of 37 % of *5*. M.p. 110−115 °C. Anal. $C_{12}H_{11}NO_5$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.97 (3 H, s), 2.64 (3 H, s), 6.95−8.20 (5 H, m). ^{13}C NMR ($CDCl_3$): δ 171.1, 167.9, 159.9, 153.9, 136.3, 128.7, 123.7, 117.4, 116.3, 92.9, 26.9, 20.5. IR ($CHCl_3$, cm^{-1}): 3010 (w), 1760 (s), 1720 (s), 1610 (s), 1460 (s), 1380 (m), 1365 (s), 1320 (m), 1305 (m).

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Studies of α-Phenyl-β-amidoethanols. 2.* Internal Rotational Barriers of Some Phenyl Substituted Derivatives

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Recently we reported on the α-phenyl-β-(*N*-methylacetamido)ethanol system[1]. The amide equilibrium position was governed by (a) the choice of solvent, (b) the concentration and (c) the *para* substituent on the phenyl ring. The *E* and *Z* isomer populations are approximatively equal in a polar aprotic solvent like dimethylsulfoxide-d_6 (DMSO-d_6), while the *Z* isomer is favoured in chloroform-*d* ($CDCl_3$). The overall *E*/*Z* equilibrium is changed towards the intramolecular hydrogen bonded *Z* isomer by decreasing the substrate concentration in a nonpolar solvent. This isomer is also preferred when electron-withdrawing *para* substituents are attached, an observation valid in both DMSO-d_6 and $CDCl_3$.

Hence, in the present system it could be of interest to investigate to what extent the intramolecular hydrogen bond of the *Z* isomer influences the rotation barrier about the amide bond.

Intramolecular hydrogen bonds have been found to lower $\Delta G^{\neq}$ in *o*-hydroxy and *o*-amino-*N*,*N*-dimethylbenzamides.[2] In these systems it is assumed that the intramolecular hydrogen bond maintains the coplanarity of the $C(O)NMe_2$ moiety and the aromatic ring. Consequently there is an enhanced conjugative stabilization of the transition state. This has also been claimed for *o*-hydroxy-*N*,*N*-dimethylthiobenzamides.[3]

Since the −OH and $-CH_3$ groups occupy roughly the same van der Waals volume,[4] a substitution of −OH for $-CH_3$ could serve as a tool for probing the hydrogen bonding contribution to the rotational process without changing the steric influence.

Also, it would be of interest to observe whether a change of *para* phenyl substituent has any pronounced effect on the dynamic behaviour. For example a *para* substituent might modify the −OH proton donating ability and also change a field induced stabilization of the ground state and/or the transition state.

Experimental. α-Phenyl-β-(*N*-methylacetamido)ethanols were prepared as reported earlier.[1] These compounds show IR absorptions (Perkin Elmer 681, 0.010 M in $C_2D_2Cl_4$) due to free, intramolecular hydrogen bonded and intermolecular hydrogen bonded hydroxyl groups at ~3610, ~3586 and ~3340 cm^{-1}, respectively.[5]

N-methyl-*N*-(2-phenyl)propylacetamide was obtained by reaction of 2-phenylpropyl bromide (10 mmol) with a tenfold excess of methylamine (40 % H_2O solution) at 115 °C in a sealed ampoule using ethanol as solvent. The reaction proceeded overnight and the obtained amine was then extracted with chloroform. The chloroform solution was extracted with 6 M HCl, the solution made basic with NaOH and finally extracted with chloroform. After drying with $MgSO_4$ and evaporation, 47 mmole of *N*-methyl-*N*-(2-phenyl) propylamine were obtained. The amine was treated with acetic anhydride to give the desired product, which was purified by column chromatography (silica gel-60, $CHCl_3$).

^{1}H NMR (Bruker WM-250, 250 MHz, $CDCl_3$): 1.26, *J*=7 Hz (*E*-CH_3), 1.33, *J*=7 Hz (*Z*-CH_3), 1.84 (*E*-$C(O)CH_3$), 2.03 (*Z*-$C(O)CH_3$), 2.72 (*Z*-NCH_3), 2.87 (*E*-NCH_3), 2.99–3.47 (*E*, *Z*-CH_2, m), 3.78–3.89 (*E*, *Z*-CH, m), 7.17–7.37 (*E*, *Z*-aromatic region). IR (Perkin-Elmer 681, $CDCl_3$, cm^{-1}): 3035, 3070, 3091 (sp^2 C–H); 2880, 2938, 2974 (sp^3 C–H); 1666 (broad, carbonyls). MS (Finnigan 4000, 70 eV): *m/e* 191 (% rel. int. 33), 176 (2, M−CH_3), 148 (1, M−$C(O)CH_3$), 105 (14, M−C_4H_8NO).

Variable temperature measurements, by monitoring the $C(O)CH_3$ resonances, were performed on a Bruker WM-250 operating at 250 MHz and equipped with a temperature unit B–VT1000. The concentrations of the test solutions were held

* Part 1. See Ref. 1.

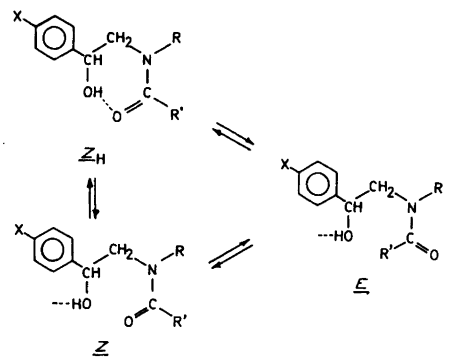

Fig. 1. The α-phenyl-β-(*N*-alkylamido)ethanol system where the hydroxyl groups of the *E* and *Z* isomers are either free or intermolecularly hydrogen bonded and Z_H is intramolecularly hydrogen bonded.

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Table 1. Free energies of activation[a] for the amide rotation in α-(*p*-X)phenyl-β-(*N*-methylacetamido)ethanol and *N*-methyl-*N*-(2-phenyl)propylacetamide (B) at 0.010 M.

Compound	$C_2D_2Cl_4$ $\Delta G^{\ddagger}_{Z\rightarrow E}$	$\Delta G°$	DMSO-d_6 $\Delta G^{\ddagger}_{Z\rightarrow E}$	$\Delta G°$
p-H	78.78 (0.46)	4.09 (0.026)	78.44 (0.43)	−0.78 (0.34)
p-Br	77.63 (0.34)	4.52 (0.046)	−[b]	−[b]
p-NO_2	77.20 (0.26)	5.42 (0.039)	78.30 (0.26)	0.02 (0.025)
B	78.83 (0.15)	0.07 (0.035)	78.05 (0.30)	0.02 (0.034)

[a] In kJ/mol with standard deviation in parenthesis. [b] Not obtained.

constant at 0.010 M. Temperatures were measured immediately before and after each experiment (by a thermocouple inside an NMR tube containing heat exchange paste) and are considered accurate to within 0.5 °C. The solvents used in this study, tetrachloroethane-d_2 and dimethylsulfoxide-d_6, were dried over 4Å molecular sieves.

The evaluation of T_2 was performed as described in the literature, using TMS as internal reference.[6]

The populations and rate constants were evaluated by superposition of the calculated and experimental spectra. The free energies of activation were calculated using the Eyring equation,[7] assuming a transmission coefficient of unity.

The NMR lineshape calculations were computed using the McConnell equation for an uncoupled two-site exchange,[8] and they were performed at the Computer Graphics Laboratory at the Chemical Center of Lund.

Results and discussion. The free energy of activation for the amides investigated are shown in Table 1. $\Delta G^{\ddagger}_{Z\rightarrow E}$ has the same magnitude for all compounds in DMSO-d_6. In this context it is important to consider the slightly different isomer distribution when comparing the *para* NO_2 substituted β-amidoethanol with the parent unsubstituted compound. The population of the *Z* isomer (for *p*-H) is 0.43 compared to the *Z* population (for *p*-NO_2) of 0.50 (T=341 K).

Based on the *E* and *Z* isomer distribution for *para* substituted α- phenyl-β-amidoethanols,[1] and results in similar systems,[9] the population difference in DMSO-d_6 was interpreted as if an intramolecular hydrogen bond in the *Z* isomer still exists. However, the free energies of activation for the listed compounds (Table 1) suggest that the intramolecular hydrogen bond of the *Z* isomer is not present in that medium. Thus the observed barrier heights are comparable to ordinary *N*,*N*-disubstituted amide barriers.[10]

In the absence of an intramolecular hydrogen bond, the dipole–dipole type, ground state stabilization of the *p*-NO_2 *Z* isomer relative to the *p*-H *Z* isomer is quite likely. Moreover, our obtained values for $\Delta G^{\ddagger}_{Z\rightarrow E}$ (78.44 and 78.30 kJ/mol for the *p*-H and *p*-NO_2 β-amidoethanol respectively) suggest that the *Z* isomer transition state receives a similar stabilization. Alternatively, the *p*-NO_2 *E* isomer is less stable relative to the *p*-H *E* isomer.

An inspection of the data in the nonpolar solvent $C_2D_2Cl_4$ reveals that $\Delta G^{\ddagger}_{Z\rightarrow E}$ is lowest for the nitrosubstituted compound. This derivative shows the largest fraction of the thermodynamically more stable *Z* isomer. The intramolecular hydrogen bond in this medium can, however, stabilize the ground state as well as the transition state.

Interestingly, *N*-methyl-*N*-(2-phenyl)propylacetamide has the same barrier to rotation as the parent unsubstituted β-amidoethanol. A plausible explanation for this observed trend is an equal stabilization of the ground state and transition state in the case of *p*-H, while on the other hand, stabilization of transition state relative to ground state increases in the order *p*-H<*p*-Br<*p*-NO_2.

An alternative explanation would be a total lack of influence of the intramolecular hydrogen bond on the barrier height. In this case the dipole in the phenyl moiety (for *p*-Br and *p*-NO_2) would interact with the amide function, thus lowering $\Delta G^{\ddagger}_{Z\rightarrow E}$.

N-methyl-*N*-(2-phenyl)propylacetamide shows a slightly larger (0.78 kJ/mole) free energy of activation in $C_2D_2Cl_4$ compared to DMSO-d_6. At least two types of solvation phenomena can be operative in amide systems in these solvents. $C_2D_2Cl_4$ can act as a proton (deuteron) donor, thus interacting with amide oxygen function (in a similar fashion as has been noted for chloroform[11]). The DMSO-d_6 interaction is of the same nature as a previously reported amide–amide self-association.[12] Thus for *N*-methyl-*N*-(2-phenyl)propylacetamide, $C_2D_2Cl_4$ might cause a hydrogen (deuterium) bonded stabilization of the

ground state which is slightly larger in magnitude than the dipole–dipole stabilization in DMSO-d_6.

Finally, it must be emphasized that the observed differences in thermodynamic and kinetic parameters represent small energy differences. Hence, care should be exercised not to overinterpret the experimental data.

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