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Silyl Nitronates in Organic Synthesis. Routes to Heterocycles and Cyclopentanoids. Synthesis of Allethrolone and Calythrone. Acylation and Cyanohydroxylation of Double Bonds. An Exploratory Study

SØREN H. ANDERSEN, NALIN B. DAS, RUTH D. JØRGENSEN, GUNHILD KJELDSEN, JES S. KNUDSEN†, SUBHASH C. SHARMA and KURT B. G. TORSSELL*,**

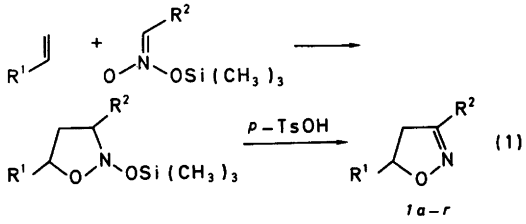
Chemical Institute, Department of Organic Chemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

Silyl nitronates are versatile reagents for the preparation of heterocycles by dipolar addition to double bonds. The intermediate isoxazolidines can be transformed to 2-isoxazolines, isoxazoles, furans, dihydrofuranones, pyrazoles, pyridazines and pyridazones. Reduction of 2-isoxazolines with Ti^{3+} leads to hydroxylated 1,4-diketones, which subsequently can be cyclized to cyclopentenones. Routes to calythrone, rethrolones, prostanoids and a number of naturally occurring dihydrofuranones are devised, as well as synthetic procedures for acylation, preparation of endiones, hydroxyacylation, cyanation and hydroxycyanation of double bonds.

In earlier papers[1,2a] preparations and properties of trimethylsilyl nitronates were described. Of special interest is their use as starting material for preparation of isoxazolidines which can be transformed to a number of heterocycles including 2-isoxazolines, isoxazoles, pyrazoles and pyrrolidones. This work is extended to the preparation of some furans, pyridazines and pyridazones. In this connection we investigated an alternative route to muscimol analogues.

Our isoxazoline synthesis is closely related to Mukaiyama-Hoshino's procedure which generates nitrile oxides by dehydration of primary nitro compounds with phenylisocyanate and catalytic amounts of triethylamine.[2b]

It was envisaged that the 2-isoxazolines obtained from vinyl ketones and silyl nitronates on selective reduction of the N – O bond, hydrolysis, and base catalyzed cyclization should give cyclopentenones, which are important structural units of many natural products of different biogenetic origins. These reactions and a few other modifications of 2-isoxazolines are the subject of the present communication.



1,3-Dipolar addition preparation of starting materials. The three-step sequence: silylation, dipolar addition and silanol elimination that leads to the 2-isoxazolines (1) can with advantage be carried out as a one pot reaction (Table 1). This is, in fact, the only procedure that worked for preparation of *1e* and *1f*. An attempt to prepare the silyl nitronates of nitro-olefins separately gave a product which did not show the presence of olefinic protons, indicating that intramolecular cyclization may have occurred, presumably to a bicyclic product (2). The silylation was therefore performed in the presence of the reactive methyl vinyl ketone, which trapped the intermediate nitronic ester, and *1e,f* were obtained in satisfactory yields. Most of the isoxazolines were prepared in benzene as solvent.

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**To whom inquiries are to be sent.

† Deceased.

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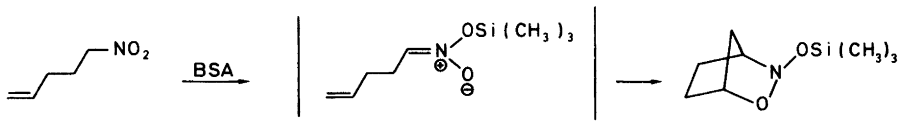
Table 1. Synthesis of 2-isoxazolines.

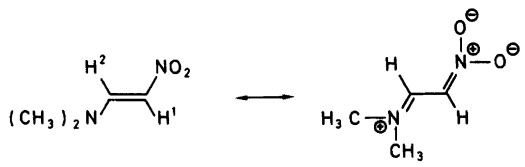
	R^1	R^2	Yield
1a	$COCH_3$	C_2H_5	55
1b	COC_2H_5	C_2H_5	58
1c	$CH=CH_2$	C_2H_5	30
1d	$CH=CHCOOCH_3$	C_2H_5	31
1e	$COCH_3$	$(CH_2)_2CH=CH_2$	55
1f	$COCH_3$	$(CH_2)_3CH=CH_2$	60
1g	$COCH_2OAc$	C_2H_5	47
1h	$COCH_3$	$(CH_2)_2COOCH_3$	52
1i	$COOCH_3$	$CH=CHCOCH_3$	~5
1j	$COOCH_3$	OTs	~10
1k	$COCH_3$	CH_3	76
1l	$COOCH_3$	CH_3	52
1m	$COOCH_3$	C_2H_5	86
1n	$COOCH_3$	C_5H_{11}	81
1o	C_6H_5	CH_3	56
1p	C_6H_5	C_2H_5	79
1q	$OSi(CH_3)_3$, OH	C_5H_{11}	~60
1r	$COCH_3$	$(CH_2)_6COOCH_3$	~100 (crude)



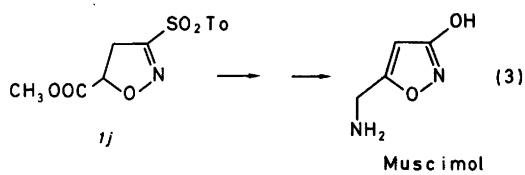
(2)

We later found that the use of acetonitrile or mixtures of acetonitrile and benzene as solvent improved the yield.[2c]

The nitro-olefins required for *1e,f* were prepared from the corresponding bromides. Methyl-3-nitropropanoate was prepared by Michael addition of nitromethane to methyl acrylate and 1-nitropent-2-en-4-one[3] by addition of acetone to 2-dimethylamino-1-nitroethene. This nitroethene derivative shows a small H^1H^2 spin coupling, 10.6 Hz, indicative of a *cis* configuration, but the strongly dipolar nature of the compound could account for this exceptionally small value of the more plausible *trans*-structure. The problem was eventually solved by an X-ray determination proving the *trans* structure.[4]

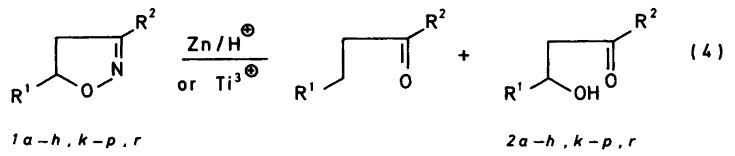


p-Tolyl nitromethyl sulfone was prepared from nitromethane, iodine, and sodium *p*-tolylsulfinate.[5] The nitromethyl sulfone was of interest in context of preparation of muscimol analogues. *1j* could in principle be transformed to muscimols by nucleophilic substitution of the sulfone group[6] by hydroxyl and reduction of the carbomethoxy group to an aminomethyl group (3).[7] The yield of *1j* was disappointingly low, however, so this synthetic route was abandoned. The acetoxymethyl vinyl ketone[8] was prepared according to a slightly modified procedure to avoid polymerization and to free the compound from moisture and acetic acid. Methyl 8-nitrooctanoate was prepared by nitration of cyclooctanone.[9]



1j Muscimol (3)

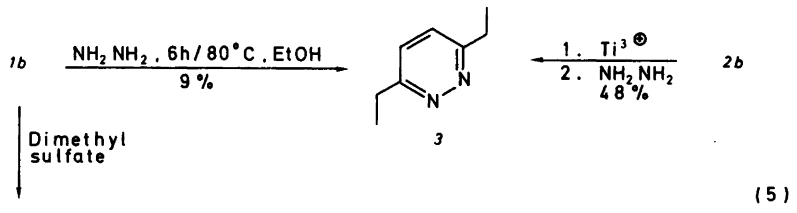
Reduction. The selective reduction presented some problems. Hydrogenation of isoxazolines with Raney-Ni or Pd/C catalysts has been reported to cleave the N–O bond[1,10,11] but is less suitable for the alkene derivatives, *1e* and *f*. 2-Isoxazolines proved to be rather resistant to zinc in acetic acid

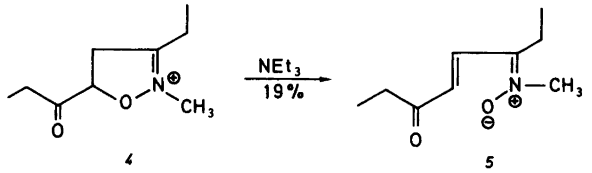


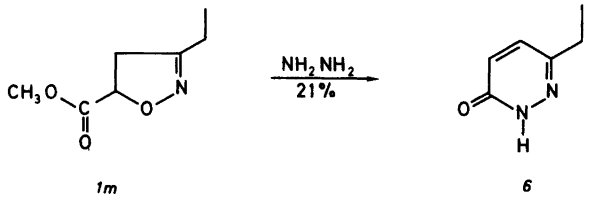
but reduction over a few days or at higher temperatures did cause ring opening at the expense of considerable C–O cleavage (4). The deoxy compound turned out to be the major product. Reduction with titanous ions [12] was slow but selective and the hydroxy derivatives became the major product and were obtained in good yields. The reduction proceeds faster and more satisfactorily at pH 3–4 than under more acidic conditions. The reduction of the acetoxy derivative *1g* turned out to be very sensitive to the reaction conditions. The pH had to be carefully controlled and only the theoretical amount of reducing agent should be used to avoid excessive reductive elimination of the acetoxy group or hydrolysis of the ester. The expected product *2g* was always obtained admixed with *2a* and the corresponding 1-hydroxy derivative.

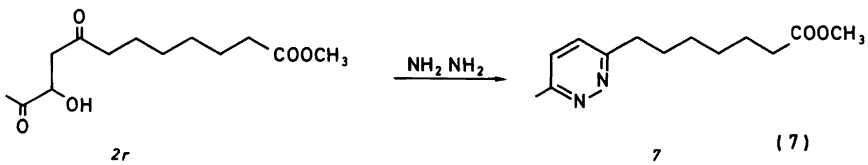
Pyridazines. Treatment of the hydroxydione *2b* obtained by Ti^{3+} reduction of *1b* with hydrazine gave 3,6-diethylpyridazine, *3*.[13] The same compound was formed in lower yield when the isoxazole was treated directly with hydrazine in refluxing ethanol for 6 h. *1b* was transformed to the nitrone *5* by methylation with dimethyl sulfate followed by treatment with triethylamine (5).

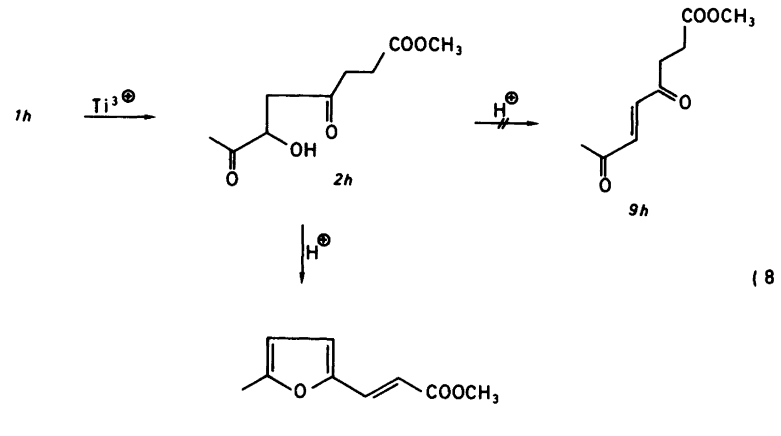
6-Ethyl-3-pyridazone *6* was similarly obtained from 3-ethyl-5-methoxycarbonyl-2-isoxazoline *1m* by refluxing with excess of hydrazine in toluene (6). The long-chain derivative *2r* gave the pyridazine *7* in a yield of 37 % (7).





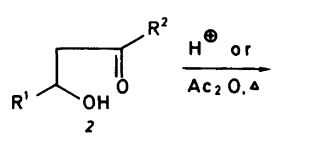






(8)

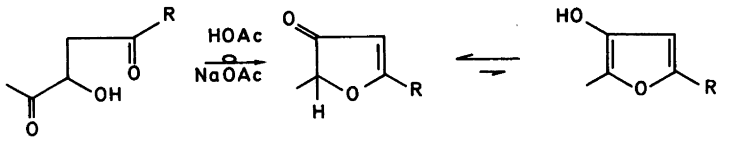
Furans, dihydrofuranones and acylation of olefins. In an attempt to prepare the endione derivative *9h* from *2h* by acid catalyzed elimination of water (*p*-TsOH in refluxing toluene), the furan derivative *8* was unexpectedly formed in a good yield (8). No endione *9h* was observed. This elimination method failed to produce any *9a,b,k—n* from *2a,b,k—n* nor were any furan derivatives obtained. Treatment of *9a,b,k* with hydrochloric acid or perchloric acid under various conditions did not lead to the endione structure. However, elimination of water from the phenyl derivatives, *2o,p* was carried out successfully in high yields by treatment with conc. hydrochloric acid in refluxing methanol. *9p* was obtained admixed with 9 % of the *cis* isomer. The 2-hydroxy esters *21m,n* eventually afforded the corresponding *β*-acylated acrylates by heating with catalytic amounts of conc. perchloric acid in chloroform to which a few drops of methanol had been added. Addition of methanol improved the yield considerably but minor amounts of 2-methoxy esters were obtained as side-products. A search in the literature revealed that elimination of water from 2-hydroxy-1,4-diones can be accomplished by acetylation and subsequent heating.[14] When this method was applied to our hydroxydiones it gave the wanted result (9) albeit in yields somewhat lower than stated earlier. *The procedures described constitute useful preparative methods for β-acylated acrylic esters, vinylketones and styrenes. In a wider context the present method and the complementary 1,3-dipolar addition of nitrile oxides to olefins*[15] *combined with reductive ring opening is a novel and general methods for hydroxyacylation of olefins.* A similar approach to acylation of olefins was recently published by Jäger *et al.*[16]



(9)

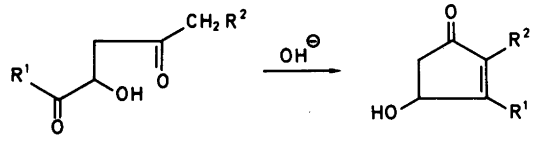
	R^1	R^2
2a, *9a*	CH_3CO	C_2H_5
2d, *9d*	$CH_3OOCCH=CH$	C_2H_5
2h, *9h*	CH_3CO	$(CH_2)_2COOCH_3$
2k, *9k*	CH_3CO	CH_3
2l, *9l*	CH_3OCO	CH_3
2m, *9m*	CH_3OCO	C_2H_5
2n, *9n*	CH_3OCO	C_5H_{11}
2o, *9o*	C_6H_5	CH_3
2p, *9p*	C_6H_5	C_2H_5
2r, *9r*	CH_3CO	$(CH_2)_6COOCH_3$

At this point in the investigation we came to inspect closer the ^1H NMR spectra of the crude hydroxydione *2a* from the Ti^{3+} reduction of 5-acetyl-3-ethyl-2-isoxazoline. They always contained a weak singlet at *ca.* 5.3 ppm which seemed to increase slightly in intensity when the reduction was run for a longer period of time. The peak at δ 5.3 was also visible in several other spectra of crude products from the Ti^{3+} reduction. Purification of *2a* on a preparative TLC plate afforded a small fraction, which gave a ^1H NMR spectrum consistent with *10a*. The lower homologue *10k* is described earlier[17,18] and was therefore synthesized from methyl vinylketone and nitroethane. Small amounts of *10k* were obtained as a side-product (*ca.* 10—15 %), by the Ti^{3+} reduction of *2k*. It turned out that the dihydro-3-furanones *10a,k,r* could be obtained in excellent yields by heating of *2a,k,r* in acetic acid in the presence of sodium



(10)

2a R = C_2H_5 *10a*
2k R = CH_3 *10k*
2r R = $(CH_2)_6COOCH_3$ *10r*



(11)

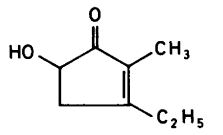
2a $R^1 = R^2 = CH_3$
2b $R^1 = C_2H_5$, $R^2 = CH_3$
2e $R^1 = CH_3$, $R^2 = CH_2CH=CH_2$

11a $R^1 = R^2 = CH_3$
11b $R^1 = C_2H_5$, $R^2 = CH_3$
11e $R^1 = CH_3$ $R^2 = CH_2CH=CH_2$
Allethrolone

acetate as catalyst (10). This novel route to dihydro-3-furanones resembles a procedure involving catalytic reduction of 5-isoxazolylcarbinols and subsequent acid cyclization.[19] According to the ^1H NMR the dihydro-3-furanones occur entirely in their oxo form.

Cyclopentenones, allethrolone, calythrone. 2-Cyclopentanones and pentanones are structural units contained in many important natural products such as rethrolones (insecticides) and prostaglandins (hormones). There are numerous methods for the construction of these structural moieties. One of them is based upon cyclization of 1,4-diketones, which now are available *via* our new route using silyl nitronates. Treatment of *2a,b,e* with dilute aqueous base gave the cyclopentenone derivatives *11a,b,e* (11). The diones can, in principle, cyclize in two directions but for *2a* and *2e* only the condensation involving the methylene group and not the methyl group was observed. Cyclization of *2b*, containing two methylene groups, leads chiefly to *11b* with the hydroxyl group in 4-position, but a small amount of the other isomer, *12*, could be isolated. The hydroxyl group of the major product is thus located in the same position as in natural rethrolones and prostaglandins. The cyclization of *2e* to *11e* completes the synthesis of the rethrolone analogue allethrolone *11e*. The spectral properties of *11e* were identical to those published earlier.[20,21] The hydroxyketone *2e* has been prepared earlier by another route and was cyclized by alkali to *11e*.[14]

11a was oxidized to the cyclopentenedione derivative *13* by treatment with manganese dioxide. *13* was earlier prepared by another route and acylated by isovaleric anhydride to calythrone, *14*.[22] Our route is thus a formal synthesis of this natural product.



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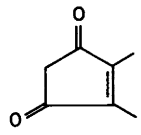

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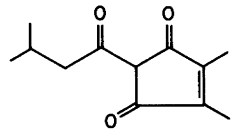
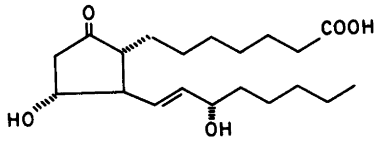


14

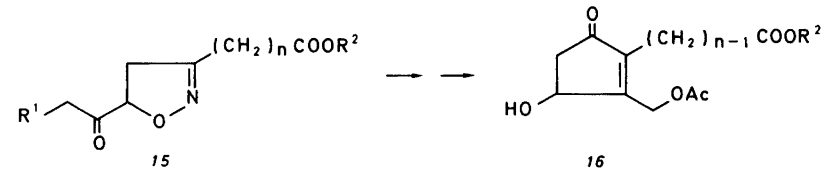
Calythrone

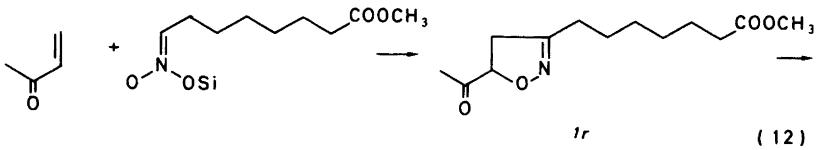
Prostanoids. The cyclopentenones produced by cyclization of the hydroxydiones have structural resemblances to the prostaglandins. If R^2 is a long fatty acid chain, R^1 an allylic alcohol derivative and the endocyclic double bond is reduced, we arrive at PGE_1. The obvious starting materials is therefore an ω-nitrocarboxylic acid for the upper

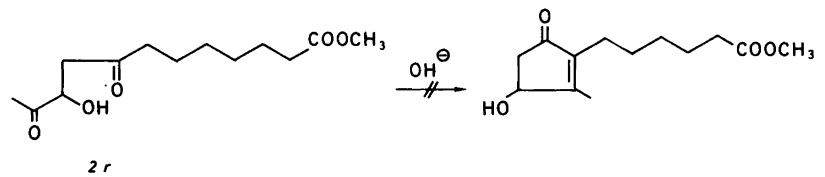


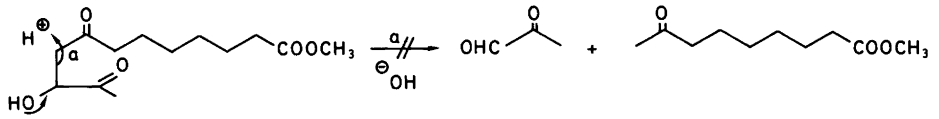
PGE_1

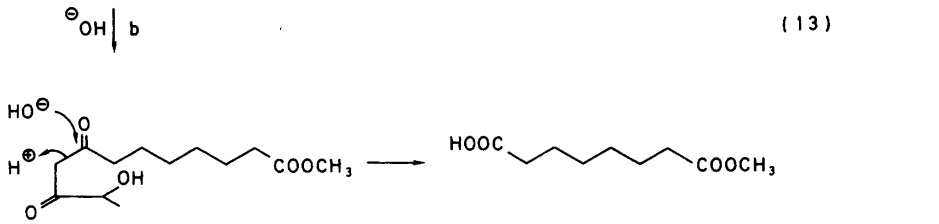
chain, which can be prepared by several methods, *e.g.* nitration of cyclic ketones,[9] and a suitably functionalized vinyl ketone for the lower chain, *e.g.* the easily available acetoxymethyl vinyl ketone.[8] These starting materials should produce the 2-isoxazoline *15*, which on reduction and cyclization could give the prostanoid *16* suited for further elaboration into natural prostaglandins. However, Ti^{3+} reduction of the model compound *1g* showed extensive elimination of the acetoxy function. It was, therefore, decided to start from methyl vinyl ketone and methyl 8-nitrooctanoate in our first attempt. They gave *1r* and subsequently *2r* in excellent yields (12) but the base catalyzed cyclization turned out to be difficult to accomplish despite a considerable variation of the reaction conditions.[14,23-31] The ^1H NMR spectrum of the reaction products showed no sign of the presence of $COCH_3$ or $C{=}C{-}CH_3$ functions, indicating that the starting material had vanished, that cyclization did not occur and that







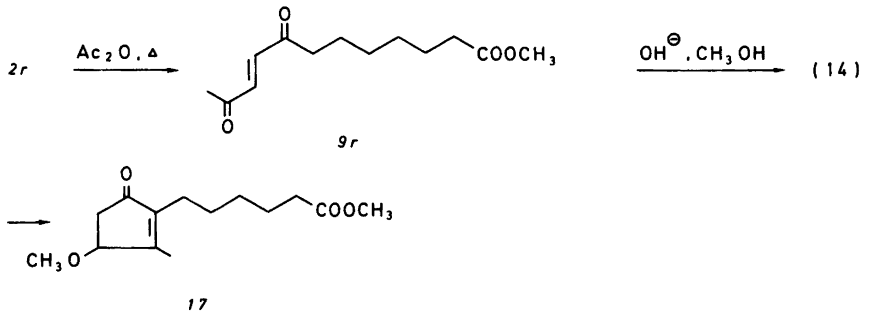




no retroaldol condensation to pyruvic aldehyde and methyl 8-ketononanoate (13a) occurred. A plausible explanation for the failure of the cyclization could be a base catalyzed ketoenol rearrangement followed by a retrocondensation to octadienoate and 3-hydroxy-2-butanone (13b).

It was reported that *trans*-2-en-1,4-diones are cyclized in basic methanol to 3-methoxycyclopentenones.[32,33] *9r* was therefore prepared from *2r* by the acetate procedure and treatment of *9r* with sodium hydroxide in aqueous methanol did indeed give the cyclopentenone *17* in a yield of 67 % (14). The cyclopentenone *18* was similarly prepared from *9a* in a yield of 83 %. The cyclization could be simplified considerably in that it turned out that the intermediate acetate of *2a* could be directly cyclized to *18* in high yield in basic methanol (15). Mechanistically the cyclization is interpreted as base catalyzed elimination of acetate followed by a reversible Michael addition of methanol to the double bond, deprotonation and finally cyclization. The methoxy derivative is evidently not prone to undergo rapid keto-enol rearrangement. Finally we seem to have access to a reliable cyclization method.

Cyano-hydroxylation and cyanation. 2-Isoxazolines with a proton at C^3 are cleaved by bases with formation of cyanohydroxy derivatives[34] (16).



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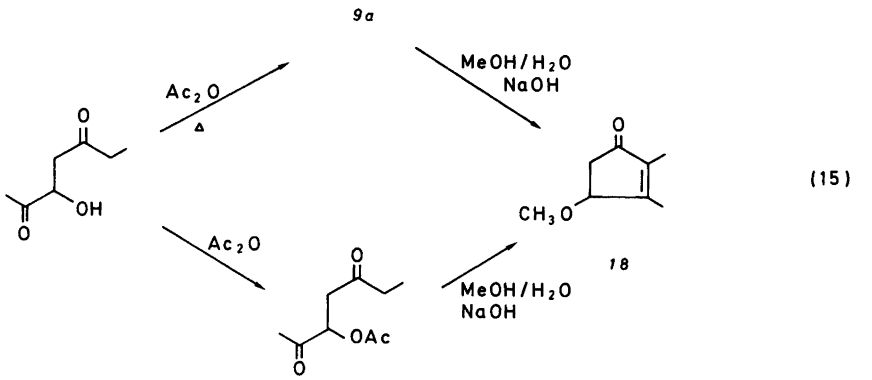
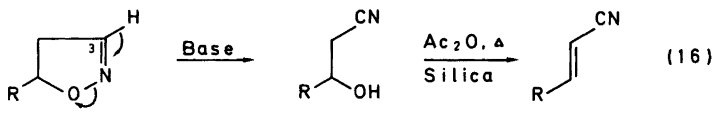


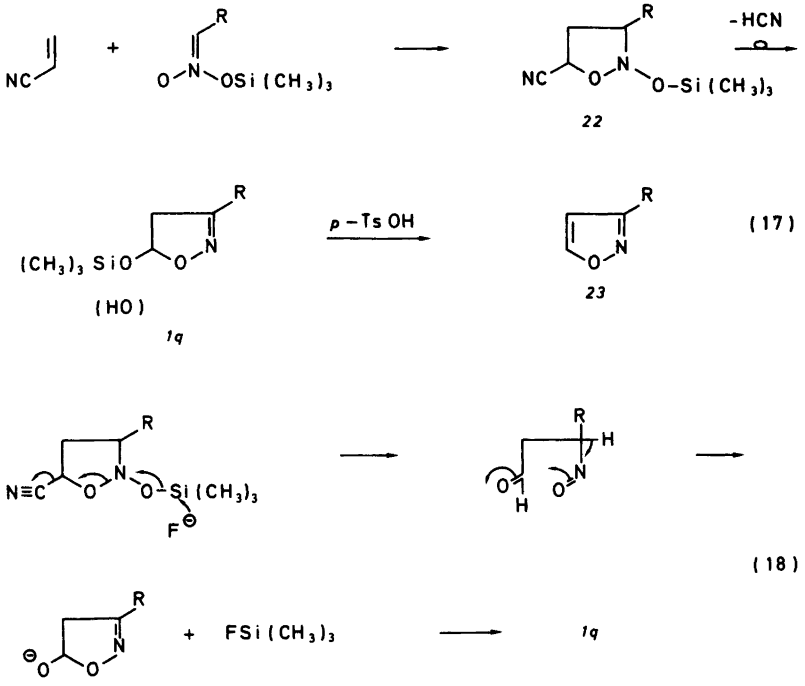
(15)



(16)

19a R = C_6H_5 *20a* *21a*

19b R = $COOCH_3$ *20b*



This finding was verified by treatment of 5-phenyl-2-isoxazoline *19a* with sodium methoxide in methanol and by treatment of 5-methoxycarbonyl-2-isoxazoline *19b* with triethylamine at room temperature. The cyano-hydroxylated products *20a,b* were obtained. Acetylation with acetic anhydride followed by thermolysis over basic alumina in refluxing toluene gave cinnamonitrile *21a* (96 %). The corresponding β-cyanoacrylic ester could not be obtained from the acetate of *20b* by this method.

Isoxazoles. The silyl nitronates from primary nitro compounds react rapidly with acrylonitrile to isoxazolidines, which in a fluoride promoted reaction rearrange to 5-silyloxy-2-isoxazolines[1,2] (17). The mechanism of this rearrangement is formulated in (18). Subsequent treatment with *p*-toluenesulfonic acid in refluxing benzene eliminates trimethylsilanol and 3-substituted isoxazoles are formed. This reaction was applied to 1-nitrohexane. The adduct *22*, R = pentyl, was formed in a yield of 93 % (crude). Treatment with potassium fluoride gave according to the ^{1}H NMR spectrum a mixture of the hydroxy and silyloxy derivatives, *1q*. Both components could be transformed into *23* in an overall yield of 33 % by treatment with *p*-TsOH in refluxing toluene. Preparation of isoxazoles by acid-catalyzed elimination of acetic acid and alcohol, respectively, from 5-acetoxy and 5-alkoxy-2-isoxazolines has been described earlier.[35]

EXPERIMENTAL

Acetoxymethyl vinyl ketone.[8] A mixture of 2-butyne-1,4-diol (1 mol, 86.0 g) and acetic anhydride (1.4 mol, 142 g) was heated carefully to 95–100 °C. The reaction was exothermic and the temperature could easily rise to 120–130 °C. The heating was continued for 2 h. After cooling the product was added dropwise to a mixture of mercuric oxide (4.0 g), conc. sulfuric acid (2.0 g) and acetic acid (40 ml). The reaction was exothermic and the temperature was kept at 50–60 °C by occasional cooling. An additional amount of mercuric oxide (2.0 g) was added and the stirring was continued for 0.5 h. The sulfuric acid was neutralized with sodium acetate (8 g) and the precipitate filtered off. Part of the acetic acid was evaporated *in vacuo* and methylene chloride (100 ml) and water (100 ml) were added. The excess of acetic acid was neutralized to pH 8 with solid sodium bicarbonate and the organic phase was separated. The aqueous phase was extracted once more with methylene chloride (25 ml) and the combined organic phases were

dried over sodium sulfate, evaporated and distilled *in vacuo*. The acetoxymethyl vinyl ketone distilled at 68–74 °C/1 mmHg (lit.[8] 70–71 °C/4 mmHg). It was immediately poured into ice-water, sodium bicarbonate was added until pH 8 and the mixture extracted twice with methylene chloride (75+25 ml). Occasionally the distilled vinyl ketone started to polymerize which could be observed by the formation of a water insoluble film. This film was removed by decanting the aqueous solution. The methylene chloride was dried over sodium sulfate and diluted to a known volume (150 ml). The concentration was determined by evaporation of the solvent from an aliquot. The vinyl ketone can be stored in this form for months in the refrigerator. The yield was *ca.* 70 %. ^{1}H NMR ($CDCl_3$): δ 2.16 (3 H, s), 4.87 (2 H, s), 5.88 (1 H, dd, *J* 4.4, 9 Hz), 6.3–6.4 (2H, m).

The *8-nitrooctanoates* were obtained by the literature methods.[9] Nitration of cyclooctanone with butyl nitrate gave a mixture of *2-nitrocyclooctanone*, bp. 73–74 °C/0.2 mmHg and *butyl 8-nitrooctanoate*, bp. 162 °C/0.6 mmHg. *Methyl 8-nitrooctanoate* was obtained practically quantitatively from the butyl ester (15 g) by treatment with 180 ml methanol containing 5 % dry hydrochloric acid for 20 h. The excess of methanol was evaporated *in vacuo*. The remainder was neutralized with aqueous potassium carbonate and extracted with methylene chloride. Evaporation of the solvent after drying with sodium sulfate gave pure methyl ester (98 %). ^{1}H NMR ($CDCl_3$): δ 1.2–2.4 (12 H, m), 3.64 (3 H, s), 4.32 (2 H, t, *J* 6.7 Hz). *2-Nitrocyclooctanone* (3 g) was also transformed into the methyl ester by treatment with potassium hydroxide (0.5 g) in methanol (50 ml) for 2 h at 65 °C. The excess of methanol was evaporated *in vacuo* and the remainder was neutralized with dilute hydrochloric acid. Extraction with methylene chloride gave 2.7 g *methyl 8-nitrooctanoate*.

The *methyl pentadieneoate* was obtained by esterification of crude pentadienoic acid[36] with dry methanol (5 % hydrogen chloride), bp. 38–40 °C/10 mmHg (lit.[37] 54 °C/24 mmHg). The yield was *ca.* 20 %.

Methyl 3-nitropropanoate was prepared according to the method of Leonard and Felley[38] and *5-nitro-1-pentene* and *6-nitro-1-hexene* were prepared from the corresponding bromides by the silver nitrite method.[39] p-*Tolyl nitromethylsulfone*[5] and *1-nitropent-2-en-4-one*[3] were prepared according to literature. Isopropanol was used as solvent instead of ethanol for the preparation of *2-dimethylamino-1-nitroethane*, mp. 97–101 °C (lit.[3] 104 °C). The yield was 62 %.

3-Ethyl-5-propionyl-2-isoxazoline, 1b, was prepared analogously to *1a*[2] from the trimethylsilyl ester of *aci*-nitropropane and ethyl vinyl ketone, bp. 77–80 °C/0.7 mmHg, 58 %. ^{1}H NMR ($CDCl_3$): δ 1.06 (3 H, t, *J* 7.3 Hz), 1.17 (3 H, t, *J* 7.3 Hz), 2.40 (2 H, qt, *J* 7.3 and 1.0 Hz), 2.69 (2 H, q, *J* 7.3 Hz), 3.20 (2 H, dt, 8.7 and 1.0 Hz), 4.90 (1 H, dd, *J* 8.1 and 9.6 Hz). IR (film): 1700 cm^{-1}. MS: 155 (M^+).

3-Ethyl-5-vinyl-2-isoxazoline, 1c, *and 3-ethyl-5-(2'-methoxycarbonylethenyl)-2-isoxazoline*, 1d, were synthesized from butadiene and methyl pentadienoate, respectively, and the trimethylsilyl ester of *aci*-nitropropane. The reactants were left standing for 2–3 days at room temperature in benzene and then treated with TsOH in the usual way. Distillation gave *1c*, 30 %, bp. 79–83 °C/11 mmHg. ^{1}H NMR ($CDCl_3$): δ 1.14 (3 H, t, *J* 7.5 Hz), 2.38 (2 H, qt, *J* 7.5 and 1 Hz), 2.69 and 3.08 (2 H, $ABXY_2$ system, *J* 16.9, 9.8, 8.7 and 1 Hz), 4.93 (1 H, dd, *J ca.* 9.3 and 6.1 Hz); *1d*, 31 %, bp. 108 °C/0.2 mmHg. ^{1}H NMR ($CDCl_3$): δ 1.15 (3 H, t, *J* 7.4 Hz), 2.38 (2 H, qt, *J* 7.4 and 1 Hz), 2.79 and 3.21 (2 H, $ABXY_2$ system, *J* 16.9, 10.4, 7.9 and 1 Hz), 3.72 (3 H, s), 5.13 (H, m), 6.11 (1 H, dd, *J* 15.8 and 1.3 Hz), 6.86 (1 H, dd, *J* 15.8 and 5.4 Hz).

3-(3'-Butenyl)-5-acetyl-2-isoxazoline, 1e. To 5-nitro-1-pentene (1.15 g, 0.01 mol), methyl vinyl ketone (0.70 g, 0.01 mol) and triethylamine (1.5 g, 0.015 mol) in benzene (25 ml), chlorotrimethylsilane (1.62 g, 0.015 mol) was added and then refluxed for 2 h. The solution was cooled, filtered and TsOH (200 mg) was added. After 1 h stirring the solution was washed with aqueous $NaHCO_3$, dried over anhydrous $CaCl_2$ and evaporated. The crude yield of *1e*, 1.69 g, was pure enough for the further reduction. ^{1}H NMR ($CDCl_3$): δ 2.27 (3 H, s), 2.3–2.5 (4 H, m), 3.14 (2 H, br. d, *J* 9 Hz), 4.82 (1 H, dd, *J* 10.0 and 7.6 Hz), 4.9–5.3 (2 H, m), 5.5–6.1 (1 H, m). IR (film): 1720 cm^{-1}.

3-(4'-Pentenyl)-5-acetyl-2-isoxazoline, 1f, was prepared analogously from 6-nitro-1-hexane, bp. 104–106 °C/0.9 mmHg in a yield of 60 %. ^{1}H NMR ($CDCl_3$): δ 1.4–2.2 (4 H, m), 2.26 (3 H, s), 2.37 (2 H, t, *J* 7.5), 3.14 (2 H, br. d, *J* 9 Hz), 4.82 (1 H, dd, *J* 10.0 and 7.6 Hz), 4.8–5.2 (2 H, m), 5.5–6.1 (1 H, m). IR (film): 1723 cm^{-1}.

3-Ethyl-5-acetoxyacetyl-2-isoxazoline, 1g. The mixture of the silyl ester of *aci*-nitropropane (30 g, 0.19 mol), acetoxymethyl vinyl ketone (23 g, 0.18 mol) and 0.2 ml triethylamine in methylene chloride (50 ml) was kept for 2 days at room temperature. TsOH (1 g) was added and the solution stirred for *ca.* 1 h. After usual work-up and distillation of the product *in vacuo*, 23 g of *1g*, 47 %, bp. 110–112 °C/0.2 mmHg, was obtained. The compound crystallized slowly in the flask, m.p. 40 °C. Found: C 54.27, H 6.68; calc. for $C_9H_{13}NO_4$: C 54.26, H 6.58. ^{1}H NMR ($CDCl_3$): δ 1.19 (3 H, t, *J* 7.3 Hz), 2.15 (3 H, s), 2.42 (2 H, q, *J* 7.3 Hz), 3.26 (2 H, d, *J* 9 Hz), 4.99 (2 H, s), 5.03 (1 H, dd, *J* 9.7 and 7.8 Hz).

3-(2'-Methoxycarbonylethyl)-5-acetyl-2-isoxazol-

ine, 1h, was prepared from the trimethylsilyl ester of methyl 3-*aci*-nitropropanoate[2] by condensation with methyl vinyl ketone (1.1 eqv.) in benzene at room temperature overnight and then refluxing the solution for 30 min. Trimethylsilanol was eliminated by addition of TsOH and the solution was washed with aqueous $NaHCO_3$, dried and evaporated. *1h* was obtained pure on distillation *in vacuo*, b.p. 120–122 °C/0.7 mmHg, 52 %. ^{1}H NMR ($CDCl_3$): δ 2.26 (3 H, s), 2.66 (4 H, s), 3.20 (2 H, d, *J* 9 Hz), 3.69 (3 H, s), 4.88 (1 H, dd, *J* 9.8 and 8.2 Hz).

1i. 1-Nitropent-2-en-4-one[3] (1.53 g, 0.012 mol), methyl acrylate (1.4 g, 0.016 mol) triethylamine (1.5 g, 0.015 mol) and trimethylchlorsilane were refluxed in benzene (10 ml) for 2 h and filtered. TsOH (0.5 g) was added and the reflux was continued for 0.5 h. Washing with water, drying over sodium sulfate and evaporation gave a crude material which according to the ^{1}H NMR spectrum contained a small amount of *1i*. Separation on a preparative TLC plate (SiO_2, $CHCl_3$) gave 5% of pure *1i* as an oil. ^{1}H NMR ($CDCl_3$): δ 2.35 (3 H, s), 3.39 (2 H, d, *J* 9 Hz), 3.80 (3 H, s), 5.16 (1 H, dd, *J* 10 and 9 Hz), 6.29 (1 H, d, *J* 16 Hz), 7.32 (1 H, d, *J* 16 Hz).

1j. When the preceding procedure was applied for *p*-tolyl nitromethyl sulfone *ca.* 10 % of pure oily *1j* was obtained from the preparative TLC plate (SiO_2/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.21 (3 H, s), 3.59 (2 H, d, *J* 9 Hz), 3.77 (3 H, s), 5.18 (1 H, dd, *J* 10 and 9 Hz), 7.36 (2 H, d, *J* 8 Hz), 7.82 (2 H, d, *J* 8 Hz). Two other products were also isolated in about 10 % yield, the structures of which according to the ^{1}H NMR spectra, proved to be the Michael addition product, *methyl 3-p-toluenesulfonyl-3-nitropropanoate* and p-*tolyl* p-*toluenethiosulfonate*.

1k was prepared in the same way as *1a*.[2] The yield was 76 %, b.p. 50–60 °C/0.1–0.2 mmHg. ^{1}H NMR ($CDCl_3$): δ 2.00 (3 H, s), 2.27 (3 H, s), 3.16 (2 H, d, *J* 9.2 Hz), 4.82 (1 H, dd, *J* 9.8 and 8.0 Hz).

1l,m,o,p were prepared according to literature.[1]

1n. *3-Pentyl-4-carbomethoxy-2-isoxazoline.* 1-Nitrohexane (0.50 g), triethylamine (0.58 g), trimethylchlorosilane (0.62 g) and methyl acrylate (0.46 g) were refluxed for 30 min in acetonitrile–benzene, 1:1 (8 ml). After cooling and filtration *p*-toluenesulfonic acid (100 mg) was added and the refluxing was continued for 1 h. The reaction mixture was washed with water (10 ml) and aqueous sodium bicarbonate (10 ml), dried and evaporated. Nearly pure *1n*, 0.62 g, 81 %, oil, was obtained. ^{1}H NMR ($CDCl_3$): δ 0.90 (3 H, t, *J* 7 Hz), 1.1–1.7 (6 H, m), 2.36 (2 H, t, *J* 7 Hz), 3.20 (2 H, d, *J* 6.8 Hz), 3.77 (3 H, s), 4.98 (1 H, t, *J* 6.8 Hz).

1q. *3-Pentyl-5-trimethylsilyloxy-2-isoxazoline and 3-pentylisoxazole*, 23. A mixture of 1-nitrohexane (5.0 g, 0.038 mol), triethylamine (5.8 g, 0.057 mol), trimethylchlorosilane (6.2 g, 0.057 mol) and acrylonitrile (2.8 g, 0.053 mol) in benzene–acetonitrile (1:1, 60 ml) was refluxed for 30 min. The solution was cooled, filtered, washed with ice-water, dried over sodium sulfate and evaporated. Potassium fluoride (1 g) was added to the crude product, *2-trimethylsilyloxy-3-pentyl-5-cyanoisoxazolidine*, (9.1 g, 93 %). The mixture was cooled in a water bath at 25 °C and stirred for 24 h. The flask is placed in a well-ventilated hood because hydrocyanic acid evolves during the reaction. Filtration gave 4.5 g of crude *1q*, which according to the ^{1}H NMR spectrum is admixed with the corresponding 5-hydroxy derivative. ^{1}H NMR ($CDCl_3$): δ 0.17 (9 H, s), 0.91 (3 H, t, *J* ~6 Hz), 1.1–1.8 (6 H, m), 2.42 (2 H, br. t, *J* ~7 Hz), 2.7–3.1 (2 H, m), 4.92 (1 H, br. s), 5.81 (1 H, dd, *J* 5.5 and 2.0 Hz). The mixture is dissolved in toluene (10 ml) and refluxed for 2 h with *p*-TsOH (1 g). The solution is filtered and washed with conc. aqueous sodium bicarbonate. The aqueous phase is extracted with chloroform and the combined organic phases are dried over sodium sulfate. Evaporation of the solvent and distillation *in vacuo* gave 1.8 g (33 %) of *23*, bp. 48–51 °C/0.12 mmHg. ^{1}H NMR ($CDCl_3$): δ 0.90 (3 H, t, *J* 5.5 Hz), 1.16–1.52 (4 H, m), 1.67 (2 H, br. t, *J* ~8 Hz), 2.70 (2 H, t, *J* 7.0 Hz), 6.18 (1 H, d, *J* 1.7 Hz), 8.29 (1 H, d, *J* 1.7 Hz).

3-(6'-Methoxycarbonyl)hexyl-5-acetyl-2-isoxazoline, 1r. A mixture of methyl 8-nitrooctanoate (8.12 g, 0.04 mol), triethylamine (9.0 g, 0.09 mol), trimethylchlorosilane (9.2 g, 0.084 mol) and methyl vinyl ketone (8.4 g, 0.12 mol) in acetonitrile–benzene (30+60 ml) was refluxed for 30 min then cooled and filtered. *p*-TsOH (2 g) was added to the filtrate that was refluxed for another hour. Methylene chloride (50 ml) was added and the solution extracted with a saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and evaporated. 10.7 g (*ca.* 100 % calc. on the nitroester) of crude liquid *1r* were obtained, sufficiently pure for further reactions. ^{1}H NMR ($CDCl_3$): δ 1.1–1.8 (8 H, m), 2.0–2.5 (4 H, m), 2.30 (3 H, s), 3.12 (2 H, d, *J* ~9 Hz), 3.65 (3 H, s), 4.81 (1 H, dd, *J* 10.0 and 7.2 Hz).

In another experiment butyl 8-nitrooctanoate was used instead of the methyl ester and the excess of triethylamine, trimethylchlorosilane and methyl vinyl ketone was kept at *ca.* 50 %. The crude yield of *1r* (butyl ester) was 80–85 %. ^{1}H NMR ($CDCl_3$): δ 0.96 (3 H, t, *J* ~6 Hz), 1.1–1.8 (12 H, m), 2.1–2.5 (4 H, m), 2.18 (3 H, s), 3.10 (2 H, d, *J* ~9 Hz), 4.3 (2 H, t, *J* 6.3), 4.80 (1 H, dd, *J* 9.8 and 7.8 Hz)

General procedure for reduction of 2-isoxazolines. The acid Ti^{3+} solution (Merck, 1 M) was treated with some powdered zink in order to ensure that it kept its full reducing power. It was neutralized with solid sodium bicarbonate to pH 3–4 and rapidly filtered or centrifued. The Ti^{3+} solution (2.3 mol) was added to a methanolic solution of the 2-

isoxazoline (1 mol) and the mixture was left standing under nitrogen for 3–4 days at room temperature with occasional stirring. Water was added and the product was extracted with methylene chloride in a continuous extractor to avoid emulsions. Drying and evaporation of solvent gave a crude hydroxyketone sufficiently pure for further reactions. The yield was *ca.* 80 % or better. If the reduction is carried out at *ca.* 0 °C, the reaction time has to be increased to about a week.

Reduction of 3-ethyl-5-acetyl-2-isoxazoline, 1a, *with* Ti^{3+} *and cyclization to* 11a. *1a* (1.40 g, 0.01 mol) in methanol (40 ml) was reduced with Ti^{3+} (1 M $TiCl_3$ solution, Merck, neutralized with solid $NaHCO_3$ to pH 3.5–4, 40 ml, 0.04 mol) under N_2 for 8 days at 5 °C. Water was added and the solution extracted with chloroform. Drying over anhydrous Na_2SO_4 and evaporation gave 1.1 g (80 %) of nearly pure 3-hydroxy-2,5-heptandione, *2a*, that was directly cyclized to *11a* with aqueous sodium hydroxide (1 M, 15 ml) for 24 h at 25 °C. The solution was saturated with sodium chloride and extracted with methylene chloride. Evaporation of the organic solvent gave *11a* (0.83 g, crude), bp. 106–108 °C/0.3 mmHg (0.25 g, 27 %, lit.[40] 108–110 °C/0.29 mmHg).

11a was obtained in a yield of 63 % when *2a* (200 mg) in methanol (1 ml) was cyclized with potassium carbonate (6 ml, 20 %, aqueous solution) for 20 h at 25 °C. Neutralization with hydrochloric acid and extraction with chloroform gave *11a* (110 mg, purified on TLC). ^{1}H NMR ($CDCl_3$) *2a:* δ 1.07 (3 H, t, *J* 7.2 Hz), 2.27 (3 H, s), 2.52 (2 H, q, *J* 7.2 Hz), 2.90 (2 H, m), 4.38 (1 H, dd, *J* 6.0 and 4.4 Hz). *11a:* δ 1.68 (3 H, s), 2.08 (3 H, s), 2.25 and 2.70 (2 H, ABX spectrum, *J* 18.4, 5.8 and 2.1 Hz), 4.1 (1 H, br. s), 4.71 (1 H, br. d, *J* 5.8 Hz).

The reduction time was reduced to 2.5 days by running the reaction at room temperature. 30 ml of Ti^{3+} solution was used for 1.4 g of *1a*. The yield was 82 %. The product contained *ca.* 10 % of *10a*.

Reduction of *1a* with acid Ti^{3+} solution at room temperature for 6 days under N_2 led to a mixture of *2a* (major) and *2,5-heptandione*. They could be separated by TLC. ^{1}H NMR of *2,5-heptandione* ($CDCl_3$): δ 1.07 (3 H, t, *J* 7.4 Hz), 2.19 (3 H, s), 2.50 (2 H, q, *J* 7.4 Hz), 2.70 (4 H, s). When the reduction was carried out in acid Ti^{3+} solution in the presence of Zn powder, *2,5-heptandione* became the major product.

2b. *4-Hydroxy-3,6-octandione.* ^{1}H NMR ($CDCl_3$): δ 1.05 (3 H, t, *J* 7.2 Hz), 1.10 (3 H, t, *J* 7.2 Hz), 2.52 (2 H, q, *J* 7.2 Hz), 2.64 (2 H, q, *J* 7.2 Hz), 2.9 (2 H, m), 4.43 (1 H, dd, *J* 6.2 and 4.8 Hz). IR (film): 1715 cm^{-1}. MS: 157 ($M^+ - 1$).

2c. *3-Hydroxy-1-hepten-5-one.* ^{1}H NMR ($CDCl_3$): δ 1.08 (3 H, t, *J* 7.3 Hz), 2.49 (2 H, q, *J* 7.3 Hz), 2.66 (2 H, d, *J* 6.1 Hz), *ca.* 3.0 (1 H, br. s), 4.60 (1 H, br. q, *J* 6 Hz), 5.0–5.5 (2 H, m), 5.9 (1 H, ddd, *J* 17.3 9.8 and 5.0 Hz).

2d. *Methyl 4-hydroxy-6-oxo-2-octenoate.* ^{1}H NMR ($CDCl_3$): δ 1.08 (3 H, t, *J* 7.2 Hz), 2.47 (2 H, q, *J* 7.2 Hz), 2.67 (2 H, d, *J* 6.5 Hz), 3.74 (3 H, s), 4.74 (1 H, m), 6.11 (1 H, dd, *J* 16.9 and 2 Hz), 6.86 (1 H, dd, 16.9 and 4.1 Hz).

2e. *7-Hydroxy-1-nonen-5,8-dione.* ^{1}H NMR ($CDCl_3$): δ 2.25 (3 H, s), 2.3–2.8 (4 H, m), 2.9 (2 H, br. d, *ca.* 5 Hz), 4.35 (1 H, br. t, *J ca.* 5 Hz), 4.7–5.3 (2 H, m), 5.4–6.1 (1 H, m).

2f. *8-Hydroxy-1-decen-6,9-dione.* ^{1}H NMR ($CDCl_3$): δ 1.4–2.3 (4 H, m), 2.25 (3 H, s), 2.49 (2 H, t, *J* 6.9 Hz), 2.9 (2 H, br. d, *J ca.* 5 Hz), 4.35 (1 H, dd, *J* 5.8 and 4.7 Hz), 4.75–5.25 (2 H, m), 5.4–6.1 (1 H, m).

Reduction of 1g. *1g* (0.5 g, 2.5 mmol) was reduced at 25 °C with 2.5 eqv. of Ti^{3+} in aqueous methanolic solution at pH 3.3 for 24 h. Extraction with methylene chloride gave 0.42 g an inseparable mixture of *2a*, *2g* and *1,3-dihydroxyheptan-2,5-dione*. When the reaction was carried out at 0 °C the relative amount of *2g* was slightly raised. *2g*, ^{1}H NMR ($CDCl_3$): δ 1.04 (3 H, t, *J* ~7 Hz), 2.13 (3 H, s), 2.49 (2 H, q, *J* ~7 Hz), 2.92 (2 H, d, *J* ~5.5 Hz), 3.87 (1 H, br. s), 4.51 (1 H, t, *J* ~5.5 Hz), 5.01 (2 H, s).

2h. *Methyl 6-hydroxy-4,7-dioxooctanoate.* ^{1}H NMR ($CDCl_3$): δ 2.26 (3 H, s), 2.50–2.75 (4 H, m), 2.91 (1 H, d, *J* 6 Hz), 2.92 (1 H, d, *J* 5.0), 3.65 (1 H, br. s), 3.68 (3 H, s), 4.36 (1 H, dd, *J* 6.0 and 5.0 Hz). The crude product was chromatographed on a silica column ($CHCl_3$, 2 % CH_3OH). The yield was 68 %.

2k. *3-Hydroxyhexan-2,5-dione.* ^{1}H NMR ($CDCl_3$): δ 2.21 (3 H, s), 2.24 (3 H, s), 2.90 (2 H, m), 3.8 (1 H, br. s), 4.32 (1 H, br. t, *J* 5 Hz). The yield was 82 %. The crude product contained *10k*, 16 %, which could be separated from *2k* by TLC.

2l. *Methyl 2-hydroxy-4-oxopentanoate.* The crude yield was 92 %. ^{1}H NMR ($CDCl_3$): δ 2.19 (3 H, s), 2.94 (2 H, d, *J* 5 Hz), 3.75 (3 H, s), 4.19 (1 H, br. s), 4.52 (1 H, t, *J* 5 Hz).

2m. *Methyl 2-hydroxy-4-oxohexanoate. 1m* (0.79 g, 0.005 mol) was dissolved in methanol (10 ml) and an aqueous solution of $TiCl_3$ (10 ml, 1 M, pH adjusted to 3.6) was added. The mixture was stirred for 3 days under N_2 at 25 °C. pH had changed to 1.6 after that time. The aqueous solution was extracted in a continuous extractor with chloroform. The organic solution gave after drying with Na_2SO_4 and evaporation practically pure *2m* (760 mg, 95 %). ^{1}H NMR ($CDCl_3$): δ 1.04 (3 H, t, *J* 7 Hz), 2.50 (2 H, q, *J* 7 Hz), 2.94 (2 H, d, *J* 5 Hz), 3.77 (3 H, s), 4.55 (1 H, t, 5 Hz), 4.74 (1 H, OH, br. s).

2n. *Methyl 2-hydroxy-4-oxononanoate.* Yield 49 %. ^{1}H NMR ($CDCl_3$): δ 0.89 (3 H, t, *J* 7 Hz), 1.1–1.8 (6 H, m), 2.43 (2 H, t, *J* 7 Hz), 2.90 (2 H, d, *J* 5 Hz),

3.74 (3 H, s), 4.0 (1 H, OH, br. s), 4.49 (1 H, t, *J* 5 Hz).

2o and *2p* were obtained in practically quantitative yields by reduction of *1o* and *1p* with aqueous methanolic Ti^{3+} solution (2.2 mol) for 2.5 days under nitrogen at 25 °C. The pH was adjusted to 3.8 with sodium bicarbonate at the start of the reduction. It decreased to 1.8 after 2 days. *2o* and *2p* were extracted with a continuous chloroform extractor to avoid emulsification. *2o*, ^{1}H NMR ($CDCl_3$): δ 2.10 (3 H, s), 2.75 (1 H, d, *J* 5.5 Hz), 2.77 (1 H, d, *J* 7.0 Hz), 3.6 (1 H, OH, br. s), 5.05 (1 H, dd, *J* 7.0 and 5.5 Hz), 7.0 – 7.4 (5 H, m). *2p*. ^{1}H NMR ($CDCl_3$): δ 1.02 (3 H, t, *J* 7.5 Hz), 2.41 (2 H, q, *J* 7.5 Hz), 2.76 (1 H, d, *J* 5.5 Hz), 2.78 (1 H, d, *J* 7.5 Hz), 3.4 (1 H, OH, br. s), 5.11 (1 H, dd, *J* 7.5 and 5.5 Hz), 7.3 (5 H, m). *2o* contained small amounts of *9o*. When the reduction is carried out at lower pH and for longer times the amount of *9o* increases. Some *4-phenylbutan-2-one* is formed. This compound arises by Ti^{3+} reduction of *9o*.

2r. *Methyl 10-hydroxy-8,11-dioxododecanoate* was prepared from *1r* (2.58 g, 0.01 mol) by reduction with Ti^{3+} solution (1 M, pH 3.6, 25 ml, 2.5 eqv.) in methanol (125 ml) and water (100 ml) under nitrogen at 25 °C for 3 days. Extraction with chloroform gave *2r* (2.34 g crude, 90 %) containing small amounts of *10r*. ^{1}H NMR ($CDCl_3$): δ 1.1 – 1.9 (8 H, m), 2.1 – 2.4 (4 H, m), 2.24 (3 H, s), 2.90 (2 H, d, *J* ~5 Hz), 3.63 (3 H, s), 4.34 (1 H, t, *J* ~5 Hz). The butyl ester was reduced with approximately the same yield but gave a mixture of butyl and methyl esters by transesterification.

Manganese dioxide oxidation of 11a *to 4,5-dimethyl-4-cyclopenten-1,3-dione*, 13. *11a* (120 mg) was dissolved in methylene chloride (10 ml) and stirred with manganese dioxide (commercial active, Merck, 400 mg) for 10 min at room temperature. Filtration and evaporation of the solvent gave *13* (oil, 50 mg), the spectral data of which agreed with those in the literature.[22] In another experiment with a less active MnO_2 the oxidation was run for 4 days. *13* was obtained in a yield of 87 %. ^{1}H NMR ($CDCl_3$): δ 2.00 (6 H, s), 2.83 (2 H, s).

Synthesis of allethrolone, 11e. Crude *2e* (1.2 g) from above was cyclized with sodium hydroxide (1 M, 20 ml aqueous methanol) at 5 °C for 18 h. The product, obtained on extraction with chloroform was purified by TLC (silica $CHCl_3$, 1 % MeOH). It gave *alletrolone 11e* (0.4 g). Its spectral properties agreed with data found in literature.[20,21] A minor band with a higher R_f value consisted of *deoxyallethrolone* arising from cyclization of *1-nonen-5,8-dione*, formed in minor quantities by the Ti^{3+} reduction. MS: 136 (M^+).

Base catalyzed cyclization of 2b (1.0 g) in methanol – water (1:4, 50 ml, 1 % NaOH) for 20 h at room temperature gave after work-up a crude product which was separated by preparative TLC into three components: *11b* (175 mg, oil, 20 %), *12* (25 mg, oil, 3 %) and a crystalline orange compound (20 mg) which was not investigated further. *11b*, ^{1}H NMR ($CDCl_3$): δ 1.15 (3 H, t, *J* 7.7 Hz), 1.69 (3 H, s), 2.51 (2 H, q, *J* 7.7 Hz), 2.25, 2.72 (2 H, ABX-spectrum, *J* 19.7, 6.0 and 2.1 Hz), 4.83 (1 H, br. d, *J ca.* 5 Hz). *12*, ^{1}H NMR ($CDCl_3$): δ 1.15 (3 H, t, *J* 7.7 Hz), 1.71 (3 H, s), 2.46 (2 H, q, *J* 7.7 Hz), *ca.* 2.5 (2 H, m), 4.43 (1 H, br. d, *J ca.* 5 Hz). MS: 140 (M^+).

3,6-Diethylpyridazine, 3. *2b* (200 mg, 0.0013 mol), hydrazine (150 mg, 0.0047 mol) and TsOH (50 mg), were refluxed in methanol (3 ml) for 3 h. Chloroform was added and the solution was washed with aqueous sodium bicarbonate (5 %) dried and evaporated. Preparative TLC (SiO_2, $CHCl_3$, 2 % CH_3OH) gave pure *3* (85 mg, 48 %). ^{1}H NMR ($CDCl_3$): δ 1.36 (6 H, t, *J* 7.5), 2.95 (4 H, q, *J* 7.5 Hz), 7.15 (2 H, s). MS: 137 ($M^+ + 1$). *3* was obtained in a yield of 9 % when *1b* (400 mg), hydrazine (200 mg) and TsOH (80 mg) were refluxed for 6 h in ethanol (6 ml). The work-up was carried out as above. The hydrazone and the hydrazine of *1b* were also detected in the reaction mixture.

6-Methylimino-4-octen-3-one N-*oxide*, 5. *1b* (300 mg, 0.0019 mol) was reacted with dimethyl sulfate (260 mg, 0.0021 mol) for one week at 25 °C. Triethylamine (200 mg, 0.002 mol) and chloroform were added and the mixture was refluxed for 2 h. The mixture was washed with water, evaporated and chromatographed on a TLC-plate (SiO_2, $CHCl_3$, 4 % CH_3OH). 60 mg of *5* (19 %) were obtained. ^{1}H NMR ($CDCl_3$): δ 1.10 (3 H, t, *J* 7.1 Hz), 1.15 (3 H, t, *J* 7.1 Hz), 2.66 (2 H, q, *J* 7.1 Hz), 2.70 (2 H, q, *J* 7.1 Hz), 3.98 (3 H, s), 6.42, 7.53 (AB-spectrum, 2 H, *J* 15 Hz). IR (film): 1690, 1670, 1590, 1300. MS: 169 (M^+).

6-Ethyl-3-pyridazone, 6. 5-Methoxycarbonyl-3-ethyl-2-isoxazoline (400 mg, 0.0025 mol) and hydrazine (200 mg, 0.0063 mol) were refluxed in toluene (6 ml) for 24 h. The toluene was evaporated and from the residue *6* (65 mg, oil, 21 %) was isolated. ^{1}H NMR ($(CD_3)_2CO$): δ 1.19 (3 H, t, *J* 7.5 Hz), 2.61 (2 H, q, *J* 7.5 Hz), 6.79, 7.25 (AB-spectrum, 2 H, *J* 10 Hz). IR (film): 1675 cm^{-1}. MS: 124 (M^+).

3-Methyl-6-(6'-methoxycarbonylhexyl)pyridazine, 7 was prepared by refluxing *2r* (150 mg, crude) with hydrazine (80 mg, 98 %) in methanol (1.5 ml) for 3 h. Evaporation of the solvent *in vacuo* and purification of the remainder on TLC (SiO_2, $CHCl_3$: $CH_3COOC_2H_5$; 4:1) gave *7* (50 mg, 36 %) as a light yellow liquid. ^{1}H NMR ($CDCl_3$): δ 1.0 – 2.0 (8 H, m), 2.30 (2 H, t, *J* 7.6 Hz), 2.66 (3 H, s), 2.93 (2 H, t, *J* 7.2 Hz), 3.64 (3 H, s), 7.18 (2 H, s).

Methyl 3-[2'-(5'-methylfuryl)] acrylate, 8. *2h* (250 mg, crude) and TsOH (30 mg) were refluxed in toluene (5 ml) for 3 h. The solution was washed with aqueous sodium bicarbonate and the solvent evaporated. Preparative TLC (SiO_2, $CHCl_3$, 2 % CH_3OH)

gave 7 (140 mg, oil, 62 %). ^{1}H NMR ($CDCl_3$): δ 2.36 (3 H, s), 3.78 (3 H, s), 6.10 (1 H, d, J 3.5 Hz), 6.25 (1 H, d, J 16 Hz), 6.53 (1 H, d, J 3.5 Hz), 7.39 (1 H, d, J 16 Hz).

2,5-Dioxo-trans-*heptene*, 9a, was obtained in a yield of *ca.* 50 % from *2a* together with 30–40 % of the acetate of *2a* by Schechter's method.[14]

Methyl 6-oxo-2,4-octadienoate, 9d, was obtained in practically quantitative yield (crude) by refluxing *2d* in benzene for 4 h with a catalytic amount of TsOH. Purified by preparative TLC it melted at 93–94 °C. ^{1}H NMR ($CDCl_3$): δ 1.13 (3 H, t, J 7.3 Hz), 2.64 (2 H, q, J 7.3 Hz), 3.79 (3 H, s), 6.0–6.6 (2 H, m), 7.1–7.5 (2 H, m). MS: 168 (M^+).

The *β-acylated methyl acrylates* 9l,m,n, were obtained in a yield of 70–90 % by refluxing *2l,m,n* (0.01 mol) in chloroform (24 ml), methanol (1.2 ml) and conc. perchloric acid (1.6 ml, 70 %) for 3 h. Ice-water (10 ml) is added and the organic phase is separated and evaporated. The crude *9l,m,n* obtained are contaminated by small amounts of the corresponding 2-methoxy derivatives formed by addition of methanol across the double bond. The ^{1}H NMR data of *9l,m,n* agree with the data given in the literature.[41] *9l* was obtained pure in a yield of 66 % by using the acetate procedure.[14]

trans-*1-Phenyl-1-buten-3-one*, 9o, *and* trans-*1-phenyl-1-penten-3-one*, 9p, were obtained in 90–95 % yields by keeping *2o,p* (0.01 mol) in methanol (30 ml) and conc. hydrochloric acid (5 ml) at 25 °C for 24 h. Most of the methanol is evaporated *in vacuo*, methylene chloride is added and the solution is washed with water and sodium bicarbonate. Evaporation of the solvent gives practically pure *9o,p*. ^{1}H NMR ($CDCl_3$) *9o*: δ 2.30 (3 H, s), 6.59 (1 H, d, J 16 Hz), 7.39 (1 H, d, J 16 Hz), 7.0–7.4 (5 H, m). *9p*: δ 1.14 (3 H, t, J 7.0 Hz), 2.66 (2 H, q, J 7.0 Hz), 6.69 (1 H, d, J 16 Hz), 7.2–7.6 (5 H, m), 7.51 (1 H, d, J 16 Hz). According to the NMR spectrum *9p* contained 9 % of the *cis*-isomer (J 13 Hz) which was separated by prep. TLC.

Methyl 8,11-dioxo-9-trans-*dodecanoate*, 9r. *2r* (1.2 g, 0.0046 mol) was acetylated with acetic anhydride (2.4 g) and sodium acetate (0.3 g) at 25 °C for 24 h and heated on an oil bath at 100 °C for 10 min. Chloroform (10 ml) was added and the solution extracted with aqueous sodium bicarbonate, dried and evaporated. *9r* (1.0 g) was obtained as a semi-solid which was recrystallized from methanol yielding 0.68 g, 68 %, of white crystals, m.p. 46–47 °C. ^{1}H NMR ($CDCl_3$): δ 1.1–1.9 (8 H, m), 2.3 (2 H, t, J ~6 Hz), 2.35 (3 H, s), 2.63 (2 H, t, J ~6 Hz), 3.65 (3 H, s), 6.77 (2 H, s). MS: 240 ($M^{\ominus}$).

General procedure for preparation of dihydro-3-furanones. The 2-hydroxy-1,4-dione, (*2a,k,r*, 3 mmol) is refluxed with sodium acetate (1.0 g) in acetic acid (10 ml) for 3 h. Chloroform (15 ml) is added and the solution extracted once with ice-water (15 ml) and once with conc. aqueous sodium bicarbonate. Evaporation of the solvent gives nearly pure dihydro-3-furanone in 80–90 % yield. The product can be further purified by Kugelrohr distillation or TLC (SiO_2, $CHCl_3$, 2 % CH_3OH). *10a*, b.p. 66–68 °C/15 mmHg, 49 %. ^{1}H NMR ($CDCl_3$): δ 1.44 (3 H, d, J 7.0 Hz), 2.44 (3 H, d, J 0.8 Hz), 4.50 (1 H, q, J 7.0 Hz), 5.43 (1 H, s). *10k*, b.p. 88 °C/15 mmHg, 51 %. ^{1}H NMR ($CDCl_3$): δ 1.16 (3 H, t, J 7.0 Hz), 1.42 (3 H, d, J 7.0 Hz), 2.51 (2 H, q, J 7.0 Hz), 4.45 (1 H, q, J 7.0 Hz), 5.36 (1 H, s). *10r*, 61 %, TLC, liquid. ^{1}H NMR ($CDCl_3$): δ 1.1–1.9 (8 H, m), 1.41 (3 H, d, J 7 Hz), 2.1–2.7 (4 H, m), 3.62 (3 H, s), 4.46 (1 H, q, J 7 Hz), 5.37 (1 H, s). MS: 240 (M^+).

2-(5'-Methoxycarbonylpentyl)-3-methyl-4-methoxy-2-cyclopentenone, 17. *9r* (120 mg, 0.50 mmol) was left standing for 48 h at 25 °C in methanol (15 ml) and aqueous sodium hydroxide (1 %, 2 ml). Ice-water was added and the solution acidified with dilute hydrochloric acid, and extracted with methylene chloride. Evaporation of the solvent and purification of the product on TLC (SiO_2, $CHCl_3$, 5 % ethyl acetate) gave *17*, liquid, 67 %. ^{1}H NMR ($CDCl_3$): δ 1.1–1.8 (6 H, m), 2.06 (3 H, s), 1.9–2.8 (6 H, m), 3.38 (3 H, s), 3.65 (3 H, s), 4.31 (1 H, m). MS: 254 (M^+).

4-Methoxy-2,3-dimethyl-2-cyclopentenone, 18, was prepared from *9a* as described for *17*. The yield was 83 %, liquid, TLC, (SiO_2, $CHCl_3$, 2 % CH_3OH). ^{1}H NMR ($CDCl_3$): δ 1.70 (3 H, s), 2.03 (3 H, s), 2.28 (1 H, dd, J 18.0 and 2.5 Hz), 2.61 (1 H, dd, J 18.0 and 5.5 Hz). 3.38 (3 H, s), 4.30 (1 H, m).

18 was also prepared directly from the acetate of *2a*. *2a* (400 mg, 2.8 mmol) was treated with acetic anhydride (800 mg) and sodium acetate (100 mg) for 24 h at 25 °C. Chloroform (5 ml) was added and the solution was washed with conc. aqueous sodium bicarbonate and evaporated. TLC (SiO_2, ethyl acetate–CH_2Cl_2, 2:1) gave 380 mg of the acetate (74 %) which was treated with a mixture of methanol (60 ml) and aqueous sodium hydroxide (8 ml, 1 %) for 2 days at 25 °C. The solution is neutralized with dilute hydrochloric acid and most of the methanol evaporated *in vacuo*. Extraction with methylene chloride gave *18* (250 mg, 87 %; TLC, SiO_2, $CHCl_3$, 2 % CH_3OH).

2-Phenyl-2-hydroxypropiononitrile, 20a. *19a*[1,2] (295 mg) was treated for 3 h at 25 °C in methanol (8 ml) with sodium methylate (100 mg). Methylene chloride and ice-water were added. The organic phase gave *20a* in practically quantitative yield. The spectroscopic data of *20a* agreed with the literature data.[34]

Methyl 2-hydroxy-3-cyanopropanoate, 20b, was obtained quantitatively from *19b*[1,2] by the method of Huisgen and Christl.[34]

The *acetates* of *20a* and *20b* were prepared by the method described for the acetate of *2a* above.

20a-Acetate, m.p. 97–99 °C, 97 %. ^{1}H NMR ($CDCl_3$): δ 2.10 (3 H, s), 2.81 (2 H, d, *J* 6.0 Hz), 5.90 (1 H, t, *J* 6.0 Hz), 7.30 (5 H, s). *20b*-Acetate, liquid, 86 %. ^{1}H NMR ($CDCl_3$): δ 2.17 (3 H, s), 2.93 (2 H, d, *J* 6.0 Hz), 3.77 (3 H, s), 5.27 (1 H, t, *J* 6.0 Hz).

Cinnamonitrile, 21a. *20a*-Acetate (320 mg, 1.7 mmol) was refluxed with Al_2O_3 (basic, Merck, 90 active I) in toluene (20 ml) for 3 h with stirring. Filtration and evaporation gave *21a* (210 mg, 96 %). ^{1}H NMR ($CDCl_3$): δ 5.82 (1 H, d, *J* 18 Hz), 7.32 (1 H, d, *J* 18 Hz), 7.37 (5 H, br. s).

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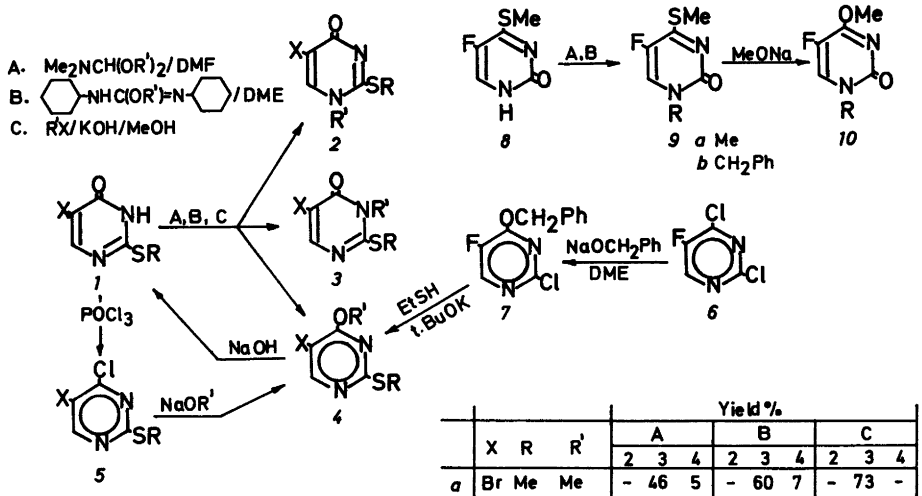
Alkylated 2- and 4-Thiouracils. Syntheses and HPLC Separations

MICHEL GACEK and KJELL UNDHEIM

Department of Chemistry, University of Oslo, Oslo 3, Norway

Alkylation of thiouracils using *N,N*-dimethylformamide acetals and *N,N'*-dicyclohexyl-*O*-alkylisoureas have been compared for yields and isomer formation with alkylations using alkyl halides. Isomer formation can be analyzed by TLC on RP-18 gel or the isomers are readily analyzed and separated by reverse-phase HPLC, the order of elution being the *N*(1)-, the *N*(3)- and the *O*-alkylated isomer.

From our studies of regioselective transformations of pyrimidines which eventually will lead us to selected target molecules for cytostatic studies,[1] we herein report on the use of less conventional reactants for alkylations of heterocycles, *viz. N,N*-dimethylformamide acetals[2,3] and *N,N'*-dicyclohexyl-*O*-alkylisoureas.[4,5] The thiated uracils used as substrates in these studies do in thermselves possess biological activities.[6,7] The alkylations with the above reactants were compared for yields and isomer formation with the conventional alkyl halide alkylation (Scheme 1). The alkylthiouracils *1* can react on either *N*(1) or *N*(3) or on the oxygen atom, whereas the thioether sulfur atom will not react under the conditions chosen for the reactions. Reference compounds for identification of *O*-alkylated isomers were prepared by selective nucleophilic displacement of the 4-substituent in suitably 2,4-disubstituted pyrimidines. Thus the 4-chloro substituent is the more reactive in 2,4-dichloro-5-fluoropyrimidine *6* and is replaced by sodium benzyl alcoholate to give *7;* the 2-chlorine substituent was subsequently replaced by sodium ethanethiolate to furnish *4b*. Alternatively, starting from a



	X	R	R'	Yield % A 2	A 3	A 4	B 2	B 3	B 4	C 2	C 3	C 4
a	Br	Me	Me	-	46	5	-	60	7	-	73	-
b	F	Et	$PhCH_2$	-	-	7	-	43	43	41	12	12
c	H	Me	Me	-	58	20	-	52	20	-	64	-
d	H	Me	$PhCH_2$	-	-	-	-	30	40	39	15	7

Scheme 1.

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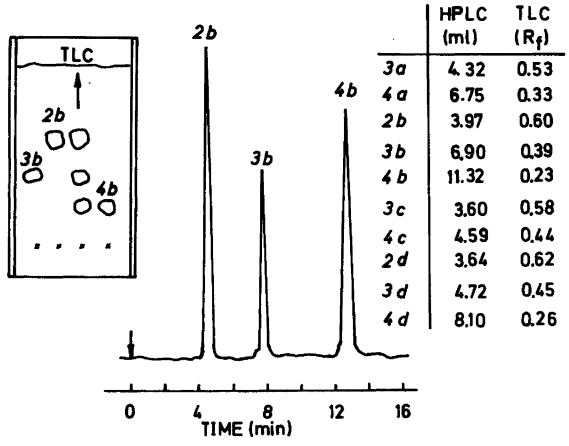
2-alkylthio-4-pyrimidinone *1*, the oxygen function is replaced by a chlorine substituent using phosphorous oxychloride *5*, and the latter reacted further with an alkoxide to yield *4*. The conditions of the reaction are decisive for thioether replacement; treatment of the 4-thiomethyl uracil *9* with sodium methoxide furnished the 1-alkyl-4-*O*-methyl uracil *10*.

The dimethylacetal of *N*,*N*-dimethylformamide in its reactions with uracils, thymines and nucleosides has been reported to give high yields of exclusively *N*-methylated products.[2,3] In its reactions with the thiouracils, however, a significant degree of *O*-alkylation was observed (*4a*, *4c*; Scheme 1). The dibenzyl acetal was much less reactive and the product isolated (7 %) from the reaction with the 5-fluorouracil was the *O*-benzylated isomer *4b* (Scheme 1). The *O*-alkyl isourea reagents, however, gave superior yields of products. The *N*(3)-methyl derivative (*3a*, *3c*) was the major product, and the *O*-methyl isomer (*4a*, *4c*) was the minor product from the reaction with the *O*-methyl isourea. From the *O*-benzylisourea reagent, *N*(3)- and *O*-alkylation were of similar importance. Dimethoxyethane is a good solvent for this reaction since the reactants and the products are soluble. In the comparative reactions using methyl iodide, exclusive *N*(3)-methylation (*3a*, *3c*) resulted, whereas benzyl bromide furnished all three isomers, the *N*(1)-isomer (*2b*, *2d*) being the major product, presumably because of steric effects. In the isomeric series of *1*, *i.e.* in the 2-pyrimidinone series, only one compound, *viz.* 4-methylthio-5-fluoropyrimidin-2-one *8* was studied. The latter has both nitrogen atoms directly attached to the oxo function; on treatment with the acetal and isourea reagents exclusively *N*(1)-alkylated products were obtained from the reaction (*9a*, *9b*). Similarly the reaction with methyl iodide gave only the *N*(1)-methylated isomer *9a*.[8]

Reverse-phase HPLC has been found very convenient and useful in the analyses and preparative separations of the various alkylated isomers (Fig. 1). Previously we have reported that *N*-substituted 2-pyrimidinones have shorter retention times than their *O*-substituted isomers.[1] The same pattern is retained in the thiouracil series. But more important is the finding that the *N*(1)- and *N*(3)-alkylated isomers are readily separable, the order of elution being the *N*(1)-, the *N*(3)- and the *O*-isomer (Fig. 1). Furthermore, TLC on RP-18 gel shows the same relative mobilities (Fig. 1).

In ^{1}H NMR the deshielding of *H*-6 appears to be different in the three series; δ 8.15–9.4 for the *O*-isomer, δ 7.7–8.0 for the *N*(3)-isomer and δ *ca.* 7.4 for the *N*(1)-isomer. A similar scale is observed for the benzyl protons; δ *ca.* 5.2 for the *N*(3)-isomer and δ 5.05 for the *N*(1)-isomer. The *CO*-absorption in IR also differs for the *N*(1)- and *N*(3)-isomers, being in the regions 1630–1660 cm^{-1}, and 1670–1690 cm^{-1}, respectively. The fragmentations of the isomers on mass spectrometry are similar and related to the behaviour of alkoxy-, alkylthio- and *N*-alkyluracils.[9–12]



	HPLC (ml)	TLC (R_f)
3a	4.32	0.53
4a	6.75	0.33
2b	3.97	0.60
3b	6.90	0.39
4b	11.32	0.23
3c	3.60	0.58
4c	4.59	0.44
2d	3.64	0.62
3d	4.72	0.45
4d	8.10	0.26

Fig. 1. HPLC retention volumes (ml) on μBondapak C_{18} column and TLC R_f-values on RP-18 (Merck) using 70 % MeCN aq.

EXPERIMENTAL

TLC was run on Merck Silica gel 60 F_{254} using (a) EtOAc or (b) iPr_2O, or on Merck RP-18 using 70 % MeCN in water. HPLC was run on a Waters HPLC system equipped with a UV monitor (260 mm) and a μBondapak C_{18} column (10 μm, 30 cm × 4 mm I.D.). The solvent was 70 % MeCN in water, and the flow rate was 0.9 ml/min. Preparative separations were carried out on Waters PrepLC/System 500 equipped with a PrepPak 500/C_{18} column.

The mass spectra were recorded on MM 70–70F VG Micromass spectrometer at 70 eV. The data are reported as MS [70 eV; m/z (% rel. int.)]

2-Methylthio-5-bromopyrimidin-4-one[13] 1a. Bromine (20 mmol) was added dropwise over 10 min. to a solution of 2-methylthiopyrimidin-4-one[13] (20 mmol) and NaBr (40 mmol) in dioxan (8 ml) and 1 M KOH (32 ml) at room temperature. The reaction mixture was heated at 70 °C for 1 h, cooled to room temperature and water (40 ml) added. The product was precipitated on acidification with acetic acid; yield 75 %, m.p. 245 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 2.53 (SMe), 8.16 (H–6). MS: 222/220 (99/100, M), 176/174 (25/27), 175/173 (26/21), 150/148 (8/15), 149/147 (17/24), 141 (62), 113 (27).

2-Ethylthio-5-fluoropyrimidin-4-one[14] 1b. A suspension of 2-ethylthio-4-benzyloxy-5-fluoropyrimidine (19 mmol) in 2 M NaOH (70 ml) was heated under reflux for 26 h. The resultant solution was concentrated to *ca.* half of its volume and acidified with acetic acid, which precipitated the product; yield 70%, m.p. 193–194 °C (H_2O). ^{1}H NMR (TFA): δ 1.56 and 3.50 (SEt), 8.03 (H–6). MS 174 (78, M), 159 (32), 146 (38), 141 (82), 130 (7), 114 (47), 88 (100), 87 (47).

Alkylation of 2-alkylthiopyrimidin-4-ones. Method A. A solution of the 2-alkylthiopyrimidin-4-one (6.5 mmol) and *N,N*-dimethylformamide dimethyl or dibenzyl acetal (15 mmol) in DMF (25 ml) was heated at 80 °C for 6 h. The reaction mixture was then evaporated, the residue was dissolved in the minimum volume of DMF and the solution applied onto a Waters PrepPak 500/C_{18} column which was eluted (100 ml/min) with 70 % MeCN aq. The order of elution was the *N*(1)-isomer, the *N*(3)-isomer and the *O*-isomer.

Method B. A solution of the 2-alkylthiopyrimidin-4-one (6.2 mmol) and *N,N'*-dicyclohexyl-*O*-methyl[15] or *O*-benzyl isourea[16] (7.5 mmol) in dimethoxyethane (60 ml) was heated under reflux for 70 min. The dicyclohexylurea formed was then filtered off, the filtrate evaporated to dryness and the residue chromatographed on Waters PrepPak 500/C_{18} column as above.

Method C: The alkyl halide (8.6 mmol) was added to a solution prepared from the 2-alkylthiopyrimidin-4-one (8.6 mmol) in 0.57 M methanolic KOH (15 ml), and the resultant solution heated under reflux for 3 h. The solvent was then removed at reduced pressure, the residue extracted with DMF and the DMF solution chromatographed as above.

1-Benzyl-2-ethylthio-5-fluoropyrimidin-4-one 2b. M.p. 118–119 °C (iPrOH). Anal. $C_{13}H_{13}FN_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 1.36 and 3.23 (Et), 5.05 (CH_2), 7.30 (Ph), 7.36 (H–6). IR (KBr): 1642 cm^{-1} (CO). MS: 264 (6, M), 236 (13), 235 (4), 203 (10), 173 (12), 148 (5), 91 (100).

1-Benzyl-2-methylthiopyrimidin-4-one 2d. M.p. 176–177 °C (iPrOH). Anal. $C_{12}H_{12}N_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.53 (SMe), 5.05 (CH_2), 5.91 (H–5), 7.28 (Ph), 7.39 (H–6). IR (KBr): 1630 cm^{-1} (CO). MS: 232 (25), 217 (7), 185 (9), 158 (12), 141 (9), 130 (6), 104 (8), 91 (100).

2-Methylthio-3-methyl-5-bromopyrimidin-4-one 3a. M.p. 130 °C (iPrOH). Anal. $C_6H_7BrN_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.56 (SMe), 3.56 (NMe), 8.00 (H–6). IR (KBr): 1681 (CO). MS: 236/234 (35/34, M), 221/219 (4/5), 190 (30), 189 (100), 188 (21), 187 (21), 160 (8), 155 (6), 118 (13).

2-Ethylthio-3-benzyl-5-fluoropyrimidin-4-one 3b. M.p. 46 °C (light petroleum). Anal. $C_{13}H_{13}FN_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 1.32 and 3.16 (Et), 5.28 (CH_2), 7.13 (Ph), 7.75 (H–6). IR (KBr): 1690 cm^{-1} (CO). MS: 264 (19, M), 236 (5), 235 (17), 203 (12), 176 (10), 173 (21), 130 (25), 118 (6).

2-Methylthio-3-methylpyrimidin-4-one[17] 3c. M.p. 126–127 °C (H_2O). ^{1}H NMR ($CDCl_3$): δ 2.56 (SMe), 3.50 (NMe), 6.13 (H–5), 7.66 (H–6). IR (KBr): 1670 cm^{-1} (CO). MS: 156 (32), 141 (7), 123 (6), 112 (9), 111 (100), 110 (25), 109 (68), 82 (20), 81 (29).

2-Methylthio-3-benzylpyrimidin-4-one 3d. M.p. 97 °C (iPrOH). Anal. $C_{12}H_{12}N_2OS$: C, H. ^{1}H NMR ($CHCl_3$): δ 2.50 (SMe), 5.26 (CH_2), 6.23 (H–5), 7.26 (Ph), 7.72 (H–6). IR (KBr): 1680 cm^{-1} (CO). MS: 232 (66), 217 (13), 199 (3), 185 (13), 158 (21), 148 (11), 141 (10), 112 (37).

2-Methylthio-4-methoxy-5-bromopyrimidine 4a. 2-Methylthio-4-chloro-5-bromopyrimidine[18] (11 mmol) was added gradually to methanolic 0.43 M NaOMe (30 ml) and the reaction mixture stirred at room temperature for 20 h. The solvent was then evaporated, the residue extracted with ether, the ether solution washed with water, and the dried ($MgSO_4$) solution evaporated, to leave the product; yield 91 %, m.p. 86 °C (light petroleum). Anal. $C_6H_7BrN_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.53 (SMe), 4.06 (OMe), 8.30 (H–6). MS: 236/234 (25/24, M), 235/233 (5/2), 221/219 (17/16), 191/189 (3/3), 190/188 (11/11), 149/147 (3/4), 118 (4), 109 (7), 32 (100).

2-Methylthio-4-methoxypyrimidine 4c was prepared as *4a* above from 2-methylthio-4-chloro-

pyrimidine[19] in 84 % yield, m.p. 35 °C (n-heptane). Anal. $C_6H_8N_2OS$: C, H. ^1H NMR ($CDCl_3$): δ 2.53 (SMe), 3.93 (OMe), 6.30 (H−5), 8.13 (H−6). MS: 156 (100, M), 141 (34), 125 (3), 111 (10), 110 (55), 109 (4), 95 (17), 82 (10).

2-Ethylthio-4-benzyloxy-5-fluoropyrimidine 4b. 2-Chloro-4-benzyloxy-5-fluoropyrimidine (12.3 mmol) was added to a mixture from ethanethiol (12.3 mmol) and t-BuOK (12.3 mmol) in dimethoxyethane (100 ml). The reaction mixture was heated under reflux for 2 h and then worked up as above; yield 89 %, b.p. 128−130 °C/0.01 mmHg, m.p. 36 °C. Anal. $C_{13}H_{13}FN_2OS$: C, H. ^1H NMR ($CDCl_3$): δ 1.37 and 3.10 (Et), 5.46 (CH_2), 7.38 (Ph), 8.13 (H−6). MS: 264 (4, M), 236 (2), 235 (4), 203 (3), 173 (12), 130 (3), 118 (3), 91 (100).

2-Methylthio-4-benzyloxypyrimidine 4d. 2-Methylthio-4-chloropyrimidine (29.3 mmol) was added to a mixture from benzyl alcohol (29.3 mmol) and t-BuOK (29.3 mmol) in dimethoxyethane (100 ml) and the mixture heated under reflux for 1 h. Work-up as above gave 73 % yield, b.p. 116−118 °C/0.01 mmHg. Anal. $C_{12}H_{12}N_2OS$: C, H. ^1H NMR ($CDCl_3$): δ 2.52 (SMe), 5.35 (CH_2), 6.37 (H−5), 7.30 (Ph), 8.13 (H−6). MS: 232 (28, M), 217 (3), 185 (5), 160 (7), 158 (5), 141 (7), 126 (18), 91 (100).

2-Chloro-4-benzyloxy-5-fluoropyrimidine 7. Sodium benzyl alcoholate (2.02 M) in benzyl alcohol (10 ml) was added dropwise to an ice-cold solution of 2,4-dichloro-5-fluoropyrimidine[20] (20.2 mmol) in dimethoxyethane (50 ml). After the addition was completed, the mixture was stirred at room temperature for 2 days. The solvent was evaporated, the residue extracted with ether, and the washed (H_2O) and dried ($MgSO_4$) solution evaporated to yield the product; yield 71 %, m.p. 109 °C (heptane). Anal. $C_{11}H_8ClFN_2O$: C, H. ^1H NMR ($CDCl_3$): δ 5.50 (CH_2), 7.40 (Ph), 8.10 (H−6).

1-Methyl-4-methylthio-5-fluoropyrimidin-2-one 9a[8] by alkylation of 4-methylthio-5-fluoropyrimidin-2-one;[21] yield 70 % by Method A and 82 % by Method B, m.p. 154 °C. MS: 174 (100, M), 173 (2), 159 (79), 144 (3), 141 (2), 129 (2), 118 (4), 116 (4), 100 (7).

1-Benzyl-4-methylthio-5-fluoropyrimidin-2-one 9b by alkylation of 4-methylthio-5-fluoropyrimidin-2-one; yield 48 % by Method A and 68 % by Method B, m.p. 177−170 °C (iPrOH) Anal. $C_{12}H_{11}FN_2OS$: C, H. ^1H NMR ($CDCl_3$): δ 2.56 (SMe), 5.00 (CH_2), 7.23 (H−6), 7.31 (Ph). IR (KBr): 1660 cm^{-1} (CO). MS: 250 (34, M), 249 (3), 235 (6), 203 (2), 192 (2), 144 (4), 91 (100).

1-Methyl-4-methoxy-5-fluoropyrimidin-2-one[22] 10a. A solution of 1-methyl-4-methylthio-5-fluoropyrimidin-2-one (3.3 mmol) in methanolic 0.26 M NaOMe (25 ml) was kept at room temperature for 24 h. The solvent was then evaporated, the residue extracted with chloroform, and the washed (H_2O) and dried ($MgSO_4$) chloroform solution evaporated to leave the product; yield 58 %. ^1H NMR ($CDCl_3$): δ 3.48 (NMe), 4.05 (OMe), 7.53 (H−6). MS: 158 (100, M), 157 (18), 143 (18), 130 (4), 129 (11), 128 (18), 127 (4), 114 (5), 102 (6), 101 (11), 100 (82).

1-Benzyl-4-methoxy-5-fluoropyrimidin-2-one 10b was prepared as *10a* above from 1-benzyl-4-methylthio-5-fluoropyrimidin-2-one in 51 % yield, m.p. 132−134 °C (EtOAc). Anal. $C_{12}H_{11}FN_2O_2$: C, H. ^1H NMR ($CDCl_3$): δ 4.01 (OMe), 5.00 (NCH_2), 7.30 (Ph), 7.36 (H−6). IR (KBr): 1645 cm^{-1} (CO). MS: 234 (27), 219 (8), 176 (11), 142 (2), 129 (2), 128 (10), 114 (2), 91 (100).

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The Torsional Barrier of the Dimethylamino Group in *N,N*-Dimethyltellurobenzamide. A Comparison with *N,N*-Dimethylbenzamide and its Thio and Seleno Analogues

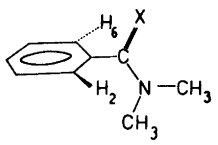
ULF BERG,[a] LARS HENRIKSEN,[b]* KNUD A. LERSTRUP[b] and JAN SANDSTRÖM[a]*

[a] Division of Organic Chemistry 3, Chemical Center, University of Lund, P.O.B. 740, S-220 07 Lund 7, Sweden and [b] Department of General and Organic Chemistry, University of Copenhagen, The H. C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen, Denmark

The torsional barrier of the dimethylamino group in *N,N*-dimethyltellurobenzamide has been determined by variable temperature ^{1}H NMR spectroscopy. The free energy barrier (80.5 kJ mol^{-1}) is slightly lower than in the seleno analogue and thus breaks the trend of increasing barriers for $PhCXNMe_2$ in the series X = O, S, Se.

The increasing trend is discussed in terms of second order perturbation theory and is related to the bonding parameter for the C=X π bond. The deviation of the telluroamide is ascribed to an increase in the ground state strain due to repulsion between the tellurium atom and the *Z*–*N*-methyl group. This interaction is reflected in an increased shielding of the *E*–*N*-methyl group caused by its deflection into a more strongly shielding region above the aromatic ring.

As *N,N*-dimethyltellurobenzamide (*1d*) has recently become available,[1] it was found of interest to measure the torsional barrier of its dimethylamino group in order to make a a comparison with *N,N*-dimethylbenzamide (*1a*), *N,N*-dimethylthiobenzamide (*1b*), and *N,N*-dimethylselenobenzamide (*1c*).



1a, X=O
b, X=S
c, X=Se
d, X=Te

EXPERIMENTAL

An NMR sample was prepared under nitrogen in degassed *o*-dichlorobenzene (ODC) and sealed under high vacuum. It was possible to record several exchange-broadened ^{1}H NMR spectra before the sample underwent violent decomposition at *ca.* 130 °C. Standard complete bandshape analysis of these spectra (all below coalescence), using a singlet signal in the solvent spectrum as a resolution standard,[2] gave a free energy barrier of 80.5 ± 0.5 kJ mol^{-1}, fairly independent of the temperature.

The chemical shift values given in Table 1 have been recorded at somewhat different concentrations and temperatures. However, unlike what is found for aliphatic amides and thioamides, the chemical shifts for the *N*-methyl protons in compounds *1a* to *1c* are fairly insensitive to these conditions, and the variations that may occur are too small to affect the conclusions reached in this communication.

The UPS data in Table 2 were recorded with a Perkin Elmer Model PS-18 photoelectron spectrometer, employing the He(*I*) 21.22 eV resonance line for ionization and the $^2P_{3/2}$ (12.13 eV) line of Xe and the $^2P_{3/2}$ (15.76 eV) line of Ar for calibration.

DISCUSSION

When comparing this barrier with those of the analogues, it seems advisable to use free energy barriers ($\Delta G^{\neq}$) measured in the same temperature region, rather than activation enthalpies ($\Delta H^{\neq}$), since good $\Delta H^{\neq}$ values are not available for all compounds *1*. Besides, these processes can be expected to have activation entropies close to zero.[3]

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Table 1. Torsional barriers and ^{1}H chemical shifts for the Me_2N groups in *1a* to *1d*.

Compound	X	$\Delta G^{\neq}$/kJ mol^{-1} [a]	Temp./K	δ_A [b]	δ_B	$\Delta\delta$	Ref.
1a	O	62.3	263	3.005	2.733	0.272	4
1b	S	77.0	365	3.325	2.793	0.532	5
1c	Se	80.8	360	3.433	2.733	0.700	6
1d	Te	80.5	370	3.516	2.568	0.948	This work

[a] Solvent ODC. [b] Downfield from TMS, in slow exchange limit.

The available $\Delta G^{\neq}$ and chemical shift values are found in Table 1.

The barrier differences between amides and thioamides have previously been discussed in relation to the interaction between the lone pair orbital on the nitrogen atom and the antibonding π orbital (π^*) in the C=X group.[7] When second order limited basis perturbation theory is employed on the interaction between a filled donor orbital and the lowest empty acceptor orbital (LUMO)*, the energy of interaction, ΔE, which in the present case approximates the torsional barrier, is given by eqn. (1). Here, H_{ij} is the Hamiltonian matrix element between

$$\Delta E = \frac{2H_{ij}^2}{\Delta\varepsilon_{ij}} \quad (1)$$

the interacting orbitals, and $\Delta\varepsilon_{ij}=\varepsilon_i-\varepsilon_j$ is the difference between their energies. If we assign ϕ_i to the donor orbital and ϕ_j to the acceptor orbital, we obtain eqn. (2) in the CNDO formalism,[8] where S_{NC} and S_{NX} are the pertinent overlap integrals and

$$H_{ij}=C_{jC}S_{NC}\beta^{\circ}_{NC}+C_{jX}S_{NX}\beta^{\circ}_{NX} \quad (2)$$

C_{jC} and C_{jX} are the LCAO coefficients for LUMO of the acceptor.

In Table 1, we find two results, which require explanation, *viz.* the steady increase in $\Delta G^{\neq}$ in the series *1a* to *1c*, and the drop from *1c* to *1d*.

The first trend can hardly be ascribed to H_{ij},[2] since the first term in eqn. (2) stays constant in the series and the contribution from the second term decreases from *1a* to *1c* (β°_{AB} is an empirical parameter, the absolute value of which decreases with decreasing electronegativity of A or B[8]). CNDO/2 calculations give much lower energy for the LUMO of CS than of CO, and it seems clear that the observed trend is due to energy gap control rather than to matrix element control.[9] Since the energy of the donor orbital, ε_i, is constant, changes in $\Delta\varepsilon_{ij}$ are due to changes in the LUMO energy of the acceptor.

Epiotis *et al.*[10] have shown by simple perturbation analysis that the LUMO energy of a C=X group and the HOMO*−LUMO energy gap are decreased, when X is chosen from successively lower levels in the same column of the Periodic Table. This is mainly explained by a decrease in the absolute value of the off-diagonal Hamiltonian matrix element in this direction. In the CNDO approximation, this parameter is given by eqn. (3).[8]

$$H_{C=X}=\beta^{\circ}_{C=X}S_{C=X} \quad (3a)$$

$$\beta^{\circ}_{C=X}=0.5(\beta^{\circ}_{C}+\beta^{\circ}_{X}) \quad (3b)$$

Calculation of the overlap integral for the π bond in C=X for X=S, Se and Te, using Slater type orbitals and standard bond lengths gave very similar results ($S\simeq 0.35$[11]). Even if this may partly be due to some deficiency in the second and third row Slater orbitals, it is likely that the effect is mainly due to a diminished absolute value of β°_{X}.

Ultraviolet photoelectron spectra (UPS) of *1b*−*1d* (Table 2) show three ionization events, 1, 2, and 5, which occur at progressively lower ionization potential (IP) when going down the column. Of these, 1 and 2 are undoubtedly due to either of the lone pair and highest π orbital, but beyond this no safe assignment can be made without a more through study. Ionizations 3 and 4 are assigned to π_3 and π_2 in the benzene ring, respectively, and 5 fits with the expectation for σ_{CX}. We can thus conclude that the HOMO energy increases in the series X=S, Se, Te, and according to the previous discussion the LUMO energy should decrease in the same succession. Therefore, the observed increase in barrier in the series *1a* to *1c* can be

* LUMO = lowest unoccupied molecular orbital.

* HOMO = highest occupied molecular orbital.

Table 2. Vertical IP's (in eV) below 11 eV for $PhCXNMe_2$.

X	1	2	3	4	5
S	7.70	8.27	9.26	9.5	10.93
Se	7.33	7.89	9.19	9.4	10.70
Te	6.80	7.50	9.20	9.35	10.5

rationalized by successive lowering of the electronegativity of the atom X in this series. It now remains to be explained why the barrier of *1d* does not fit into the series. A possible rationalization could be that steric strain in the ground state increases in the series *1a* to *1d* due to increasing interaction between the X atom and the *Z* methyl group. This interaction is probably quite weak in the thioamide, as judged from the *N,N*-diisopropylthioamides described in Ref. 12, where only a weak ground-state strain is found by molecular mechanics calculations. Parameters for similar calculations on seleno- and telluroamides are not available, but use of appropriate van der Waals radii [13] shows that, with unchanged geometry, a considerable interpenetration of the *Z*−*N*-methyl group and the X atom must occur in *1c* and even more so in *1d*. The concomitant repulsion must be partly released by an increase in the X=C−N angle, and this in turn leads to an increased twist between the thioamide group and the benzene ring due to stronger interaction with the ortho protons. Also on the side of the X atom, an interaction with the nearest ortho proton will occur. The dihedral angle between the thioamide group and the benzene ring is 63° in *1b*,[14] and an increase in this angle and in the X=C−N angle in *1c* and *1d* is revealed by the chemical shifts of the *E*−*N*-methyl protons (Table 1). While the *Z*−*N*-methyl is progressively less shielded in the series *1a* to *1d* due to the increasing magnetic anisotropy of the C=X group, the *E* protons in *1c* and *1d* experience an *increase* in shielding. This is well explained by the geometric changes discussed above, which move the *E* protons into successively more shielding regions with respect to the benzene ring.

The ring current effect may in fact be larger than shown by the shift values in Table 1, since in general the *E* protons appear at 0.2 to 0.5 ppm lower field in thioamides than in amides.[12] This effect, which is ascribed to a larger positive charge on the thiocarbonyl carbon atom, could well progress further in *1c* and *1d* and partly balance the effects of the changes in geometry.

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Rotational Barriers and Electronic Structures of Some 6,6-Diheterosubstituted Fulvenes

THOMAS OLSSON[a] and JAN SANDSTRÖM[b,*]

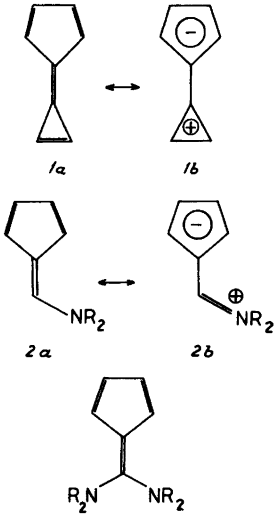
[a] Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, Fack, S-402 20 Göteborg, Sweden and [b] Division of Organic Chemistry 3, Chemical Center, P.O.B. 740, S-220 07 Lund 7, Sweden

The title fulvenes with N, O, or S as heteroatoms were prepared by reaction of trifluoromethylcyclopentadiene with 2-mercaptoethanol, 2-hydroxyethyl-methylamine, N-methyl-N'-phenylethylenediamine and N-benzyl-N'-isopropyltrimethylenediamine. The free energy barriers to rotation about the fulvene C-1=C-6 bond were studied by ^{1}H NMR bandshape technique and were found to be: with O, S as heteroatoms >105 kJ mol^{-1}, with N, O 75.4 kJ mol^{-1}, and with N, N 41 kJ mol^{-1} or <30 kJ mol^{-1}, with the higher value for the *N*-phenyl compound.

The electronic charges, the dipole moments, and the energies of the initial and transition states of three model compounds with N,S (*4a*), N,O (*4b*) and N,N (*4d*) as heteroatoms were calculated by the CNDO/2 method, employing limited geometry optimization.

The free energy barrier of the N,O-substituted fulvene was strongly dependent on solvent polarity, and the effect could be explained by the reaction field theory. The calculated rotational barriers were much higher than the experimental ones and the difference was shown to be far too great to be explained by neglect of solvation in the calculations.

The tendency of the cyclopentadiene ring to accept electrons from exocyclic groups has been the subject of interest for a long time. As an example, fulvene was at one time regarded as an aromatic compound,[1] supported among other things by the high dipole moment directed towards the aromatic ring that resulted from calculations of the Hückel type.[2] The demonstration by microwave spectroscopy that the dipole moment of fulvene is in fact only 0.49 D[3] has shown that fulvene cannot be classed as an aromatic hydrocarbon on any grounds. Nevertheless, fulvenes with donor substituents on the exocyclic carbon atom have in many instances been defined as more or less aromatic. Without taking a stand to this diffuse and controversial quality one can safely conclude that the cyclopentadienylidene ring interacts strongly with donor groups, thereby approaching a cyclopentadienide ion. Pertinent examples are the calicenes (*1*),[4] 6-aminofulvenes (*2*)[5] and 6,6-diaminofulvenes (*3*).[6,7] The charge delocalization and the development of the electronic structure of a cyclopentadienide ion are evidenced by large dipole moments,[4,6] equalized C–C bond lengths in the ring[8,9] and high barriers to rotation about the C–N bond in *2*.[10–12]



1a *1b* *2a* *2b* *3*

* Address correspondence to this author.

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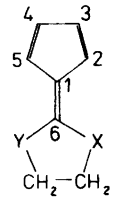
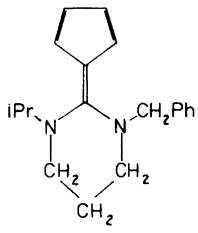


4a , X = O, Y = S

b , X = O, Y = NMe

c , X = PhN, Y = NMe

d , X = MeN, Y = NMe



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Compounds of types *1*[13] and *2*[11,12] have also shown exceptionally low barriers to rotation about the C-1 – C-6 double bond, which is readily explained by a good stabilization of the transition state, in which a cyclopentadienide ion is fully developed.

Our study was undertaken in order to investigate the delocalization of electrons and the barriers to rotation about the exocyclic double bond in some 6,6-dihetero-substituted fulvenes (*4a–4c*, *5*), in which the heteroatoms form part of a five- or six-membered saturated ring. The study has been performed by ^{1}H NMR bandshape analysis and by CNDO/2 calculations.

The stabilization of polar compounds in solution depends on the dipole moment of the solute and on the polarity and polarizability of the solvent. Since the dipole moment of the transition state to rotation about the exocyclic double bond is larger than that of the initial state, a study of the solvent effect on the rotational barrier has been performed with compound *4b*.

EXPERIMENTAL

Syntheses. 2-Cyclopentadienylidene-1-thia-3-oxacyclopentane (4a). A solution of trifluoromethylcyclopentadiene, $CpCF_3$,[14] (3 mmol) in ether, was added to a slurry of 0.6 g (9 mmol) potassium hydroxide in 350 mg 2-mercaptoethanol. After vigorous stirring for 30 min, the reaction mixture was washed with several portions of water. Cyclohexane was added and the solvent was evaporated until the product precipitated as bright yellow crystals, m.p. 79–81 °C. Yield 195 mg (43 %). NMR ($CDCl_3$, 270 MHz, 298 K): δ 6.54 (1 H, m), 6.41 (1 H, m), 6.31 (2 H, m), 4.64 (2 H, t) and 3.42 (2 H, t). UV (ethanol): λ_{max} 310 nm (sh) log ε 4.33, 320 nm log ε 4.43 and 332 nm (sh) log ε 4.30. MS (34 eV): *m/e* 152 (55 %, M^+) and 92 (100). Abs. mass 152.032; calc. for C_8H_8OS 152.030.

N-*Methyl-2-cyclopentadienylidene-1-aza-3-oxacyclopentane* (4b), was prepared as described for *4a* from 2-(hydroxyethyl)-methylamine, in 78 % yield as colourless crystals, m.p. 163–164 °C. NMR ($CDCl_3$, 270 MHz, 298 K): δ 6.74 (1 H, m), 6.65 (1 H, m), 6.40 (1 H, m), 6.33 (1 H, m), 4.52 (2 H, t), 3.82 (2 H, t) and 3.35 (3 H, s). UV (ethanol): λ_{max} 310 nm log ε 4.50. MS (34 eV): *m/e* 149 (100 %, M^+), 122 (13), 93 (48) and 92 (34). Abs. mass 149.083; calc. for $C_9H_{11}NO$ 149.084.

N-*Methyl*-N′-*phenyl-2-cyclopentadienylidene-1,3-diazacyclopentane* (4c), was prepared as described for *4a* from *N*-methyl-*N*′-phenyl-ethylenediamine, in 52 % yield as pale gray crystals, m.p. 148–150 °C. NMR ($CDCl_3$, 270 MHz, 298 K): δ 7.10–7.40 (5 H, m), 6.09 (4 H, s), 3.93 (2 H, t), 3.76 (2 H, t) and 3.40 (3 H, s). UV (ethanol): λ_{max} 266 nm log ε 4.08 and 333 nm log ε 4.24. MS (34 eV): *m/e* 224 (61 %, M^+), 223 (100), 207 (15), 107 (22) and 106 (23). Abs. mass 224.131; calc. for $C_{15}H_{17}N_2$ 224.131.

N-*Benzyl*-N′-*isopropyl-2-cyclopentadienylidene-1,3-diazacyclohexane* (5), was prepared as described for *4a* from *N*-benzyl-*N*′-isopropyl-trimethylenediamine, in 43 % yield, m.p. 132–135 °C. NMR ($CDCl_3$, 270 MHz, 298 K): δ 6.95–7.15 (5 H, m), 6.03 (2 H, m), 5.95 (2 H, m), 4.90 (1 H, septet), 4.71 (2 H, s), 3.03 (2 H, t), 2.99 (2 H, t), 1.75 (2 H, quintet) and 1.01 (6 H, d). UV (ethanol): λ_{max} 249 nm log ε 4.10, 267 nm log ε 4.08 and 332 nm log ε 4.22. MS (34 eV): *m/e* 280 (100 %, M^+), 279 (46), 265 (22), 238 (19), 237 (39), 189 (10), 146 (9), 98 (29) and 91 (39). Abs. mass 280.194; calc. for $C_{19}H_{24}N_2$ 280.194.

The variable temperature ^{1}H NMR spectra were recorded on *ca.* 0.5 M solutions in solvents given in Tables 1 and 2, on a JEOL Model MH-100 NMR spectrometer with standard variable temperature probe and temperature controller.

The rate constants for *4b* and *4c* were obtained by visual comparison of the experimental spectra with spectra calculated with the DNMR 3 program.[15] The slow exchange limit for *4c* is below –100 °C, and no well-resolved spectrum could be obtained because of extensive bandbroadening at this temperature. The spectra were therefore calculated with coupling constants that are averages of those derived by Mannschreck and Kölle[16] for 6-dimethylaminofulvene. Exchange-broadened ^{1}H NMR spectra for *4c* could be recorded over a 40° interval, but unfortunately, the sensitivity of the spectrum to changes in the rate constant was rather low in the region of fast exchange. This is because the chemical shifts of H-2 and H-5 ($\delta\nu$ = 90.8 Hz) are symmetrically disposed with respect to those of H-3 and H-4 ($\delta\nu$ = 22.5 Hz), and the averaged spectrum lacks completely AA'BB' structure and

Table 1. Rate constants and free energy barriers for *4c* in dichlorofluoromethane solution.[a]

T/K	k/s^{-1}	$\Delta G^{\neq}$/kJ mol^{-1}
175.0	4.0	40.1
189.2	22.5	40.7
193.8	33	41.1
197.7	55	41.2
201.7	75	41.5
211.3	200	41.8

[a] Spectral parameters (in Hz): ν_1 544.4, ν_2 575.9, ν_3 598.4, ν_4 635.2. J_{12} 4.57, J_{13} 1.64, J_{14} 2.10, J_{23} 2.48, J_{24} 1.64 and J_{34} 4.57.

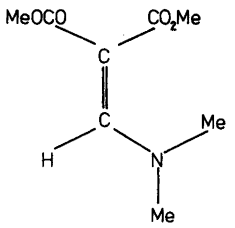
appears as a singlet in the fast exchange limit. Therefore, no attempt was made to calculate activation enthalpy or entropy, but the free activation energies recorded in Table 1 increase with temperature, indicating a negative activation entropy, as expected for this type of compound.[17]

The ^{1}H NMR spectrum of *4a* in DMSO-d_6 showed no effects of exchange below +150 °C, indicating a free energy barrier >105 kJ mol^{-1}, since a rate constant >1 s^{-1} should have caused an observable broadening.

The free energy barriers of *4b* in toluene-d_8, acetonitrile-d_3 and in two mixtures of these solvents are found in Table 2. No effects of slow rotation could be observed in the spectrum of *5* down to −130 °C.

The temperatures[18] and the transverse relaxation times[19] were measured as previously described, the NMe signal in the spectra of *4b* and *4c* serving as resolution standard.

The CNDO/2 calculations were performed by the standard program[20] (without *d*-orbitals for S) on the initial states (assumed planar) and the transition states (twisted 90° around the C-1−C-6 bond) of compounds *4a*, *4b* and *4d*. The latter structure was chosen as a model for *4c* in order to limit the computational work and also because the steric effects



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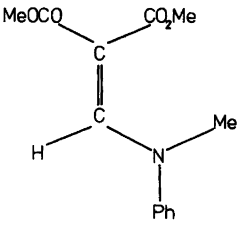

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in *4c* were not expected to be adequately treated in the CNDO/2 calculations. The barrier to rotation about the C-1−C-6 bond is certainly higher in *4c* than in *4d*. An upper limit to the difference of *ca.* 14 kJ mol^{-1} may be obtained by a comparison between compounds *6* and *7*,[21] but the difference is probably smaller since the steric hindrance to coplanarity is stronger in the initial state of *4c* than in that of *7*.

The geometry of the cyclopentadiene ring in the initial state was taken from an X-ray crystallographic study of 6-dimethylaminofulvene,[8] which differs very little from that of 6,6-bis(dimethylamino) fulvene.[9] This geometry was also first employed in the 90° twisted state, but a regular pentagon with a C−C bond length of 1.403 Å was found to give ca. 13 kJ mol^{-1} lower energy in *4b* and was employed throughout. The C-1−C-6, C-6−S, C-6−O and C-6−N bond lengths were optimized, and the final values are found in Table 3. The remaining bond lengths and angles in the rings containing the donor atoms were standard values and were not optimized, since they were not expected to undergo important changes during the rotational process. Only small changes in the ring angles at the heteroatoms (X, Y) were performed in order to keep the other bond lengths constant when the C-6−X (C-6−Y) bond lengths were varied. The calculated energies and dipole moments are found in Table 3.

Table 2. Solvent effects on the chemical shifts, rate constants and free energy barriers for *4b*.

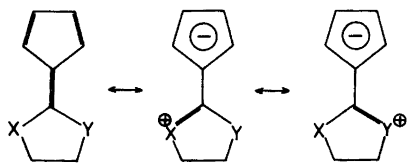
Solvent	Molar ratio	Chemical shift/Hz H-1	H-2	H-3	H-4	T/K	k/s^{-1}	$\Delta G^{\neq}$/kJ mol^{-1}
Toluene-d_8	–	697	660	~660	~660	362	100	75.4
Toluene-d_8 − acetonitrile-d_3	1.59:1	691	668	652	645	329	50	70.1
Toluene-d_8 − acetonitrile-d_3	0.81:1	685	668	646	637	315	38	67.8
Acetonitrile-d_3	–	658	668	626	613	281	17	62.0
		658	668	627	613	284	24	61.9
		658	669	628	613	288	35	62.0
		658	670	628	613	299	70	62.6

Table 3. Bond lengths, energies (E), rotational barriers (ΔE), and dipole moments, calculated by the CNDO/2 method.

Molecule	Optimized bond length/Å C-1–C-6	C-6–N	C-6–O	C-6–S	E/a.u.	ΔE/kJ mol^{-1}	μ/D
4a							
Initial state	1.390	–	1.360	1.755	−91.74154	173.6	5.24
Transition state	1.410	–	1.350	1.730	−91.67539		9.98
4b							
Initial state	1.375	1.375	1.360	–	−102.26196	125.9	6.38
Transition state	1.405	1.360	1.350	–	−102.21399		10.28
4d							
Initial state	1.390	1.380		–	−104.94954	95.8	6.15
Transition state	1.415	1.365		–	−104.91306		10.28

RESULTS AND DISCUSSION

The barriers to rotation about the C-1–C-6 bond in *4b*, *4c* and *5* are much lower than the barrier in a simple ethylene, which is 260 kJ mol^{-1} for 1,2-dideuterioethylene[22] and 251 kJ mol^{-1} for *cis*- to *trans*-2-butene.[23] The barrier differences are determined by the delocalization energies in the initial state (*8a*–*8c*), by the stabilizing interaction



8a *8b* *8c*

of the heteroatoms X and Y with the carbocation in the transition state (*9*), and by the delocalization energy in the cyclopentadienide ion. The first effect is barrier-raising and the second and third ones are barrier-lowering.

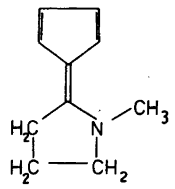
9

The energies of interaction of heteroatoms as electron donors with unsaturated groups and with carbocations have been much discussed in recent years, and PMO arguments supported by *ab initio* calculations show that the energy of interaction, ΔE, with a carbocation is much greater than with a double bond.[24] In the PMO model with neglect of overlap, ΔE is determined by the energy gap between the "lone pair" orbital of the donor (n_x) and the lowest empty orbital of the acceptor (π^*) and by the matrix element, H_{ij}, between these orbitals (eqn. (1)).[25] The variation in H_{ij} is normally much smaller than that in the energy gap, and ΔE is in

$$\Delta E \approx \frac{2H_{ij}^2}{E(\pi^*)-E(n_x)} \qquad (1)$$

most cases determined by the latter quantity. Thus the low barriers in compounds *4b*, *4c*, and *5* are explained by the good stabilization of the negative charge in the transition state in the cyclopentadienide ion and of the positive charge in the heteroatom-substituted carbocation. The order of these barriers and also the higher barrier in *4a* is determined by the order of n_X, n_Y energies, which fall off in the series X, Y = N, S, O as indicated by the vertical ionization potentials of Me_2NH (8.24 eV), MeSH (9.44 eV) and MeOH (10.85 eV).[26] Since π^* is always higher than n_x, the energy gap increases and ΔE decreases in the above sequence. The high barrier of *4c* as compared to *5* is probably largely due to the phenyl ring, which diminishes the donor capacity of the attached nitrogen atom in *4c*.

The free energy barrier of *4b*, 76 kJ mol^{-1} in toluene-d_8, is similar in magnitude to that of 6-methyl-6-dimethylaminofulvene, 73 kJ mol^{-1}, in deuteriochloroform[11] and that of the analogue *10*, 82 kJ mol^{-1}, in acetone.[12] This is in harmony with the fairly poor donor capacity of the oxygen atom. The barrier of *4b* is harder to reconcile with the observation by Downing *et al.*[11] that the analogue *11* shows an averaged (AA'BB') spectrum down to



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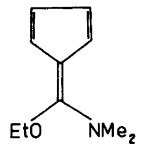
−60 °C. The explanation may be an accidental isochrony of H^2 with H^5 and of H^3 with H^4, rather than a low barrier, and it could be worth while to reinvestigate *11* in a variety of solvents, considering the strong solvent effect on the spectrum of *4b*.



11

Several authors[17,27-29] have observed that rotational barriers in push-pull ethylenes are successively lowered by increasing solvent polarity. This is in good agreement with the proposed mechanism for the rotation with a dipolar transition state that is more stabilized by a polar solvent than the less polar initial state. The influence of solvent polarity on the free energy barrier of *4b* is unusually large, (Table 2) with a lowering of 12 kJ mol^{-1} from toluene-d_8 to acetonitrile-d_3. This effect can be semiquantitatively treated by the reaction field model.[30] The reaction field is created by a point dipole in a polarizable medium, and its strength at the position of the dipole is given by eqn. (2). The direction of the field in this point is the same as that of the dipole, and the energy of interaction between dipole and field is given by eqn (3). In eqns. (2) and (3), ε is the dielectric constant

$$R = \frac{K(\varepsilon-1)}{2\varepsilon+1}\frac{\mu}{a^3} \qquad (2)$$

$$\Delta E = R\mu = \frac{K(\varepsilon-1)}{2\varepsilon+1}\frac{\mu^2}{a^3} \qquad (3)$$

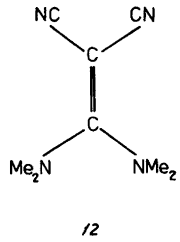
of the medium, μ is the dipole moment of the solute, and a is the radius of a cavity, assumed spherical, which contains the molecule. If a is given in Å, μ in D, and E in kJ mol^{-1}, K has the value 60.23.

For liquid compounds, a is calculated by eqn. (4), where M is the molecular weight, N is Avogadro's number, and ρ is the density of the pure liquid. For the compounds used in this study, the density in the

$$4\pi a^3/3 = M/N\rho \qquad (4)$$

liquid state is not known, but from comparisons with similar compounds, values between 1.0 and 1.1 seem reasonable for *4b* and *4d*. This gives $a = 3.77-3.89$ Å for *4b* and $3.88-4.00$ Å for *4d*.

The dipole moments of the initial and the transition states are also required in order to calculate the solvent effect on the rotational barrier. These moments are available from the CNDO/2 calculations. Previous experience shows that such calculations reproduce the experimental dipole moments of push-pull ethylenes reasonably well.[31] The calculated initial state moment for *4d* is 6.15 D, and it can be compared with that for 6,6-bis(dimethylamino)fulvene (*3*, R = CH_3), 5.4 D.[6] The moment for the latter compound is expected to be lower because of steric hindrance to coplanarity of the dimethylamino groups but this probably does not explain the whole discrepancy. The dipole moments for two similar compounds, *12* and *13*, are



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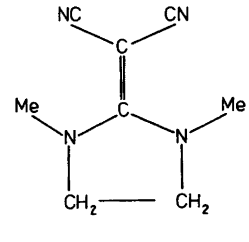

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7.84 D and 7.93 D, to be compared with the calculated values of 6.32 D and 7.86 D, respectively.[31]

The solvation energies for the initial state (ΔE_{is}) and the transition state (ΔE_{ts}) have been calculated by eqn. (3) for acetonitrile (ε36.2) and toluene (ε2.38). The solvent effect, $\delta\Delta E$, is given by eqn. (5). It depends greatly on the value chosen for a, and instead of fixing this value we have traced the

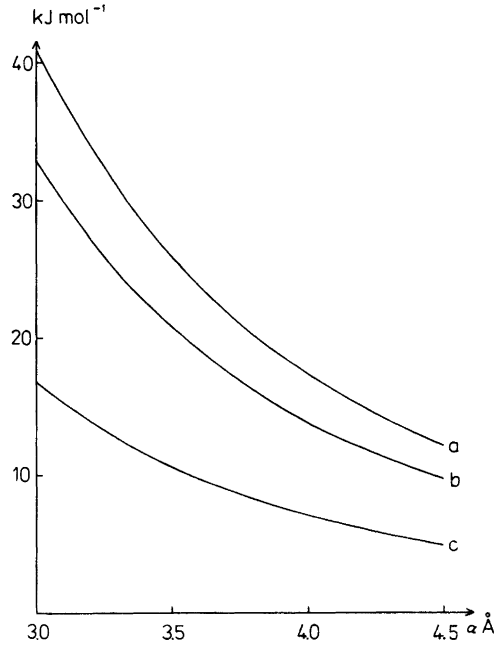

Fig. 1. Calculated difference in solvation energy between acetonitrile and toluene for *4b*. (a), $\mu_{is}=6.38$ D and $\mu_{ts}=10.84$ D; (b), $\mu_{is}=5.70$ D and $\mu_{ts}=9.70$ D; (c), Calculated solvation energy for *6* in dichloromethane for $\mu_{is}=4.30$ D and $\mu_{ts}=6.03$ D.

$$\delta\Delta E=(\Delta E_{ts}-\Delta E_{is})_{acetonitrile}-(\Delta E_{ts}-\Delta E_{is})_{toluene} \quad (5)$$

dependence of $\delta\Delta E$ on a in the range $a=3.0-4.5$ Å. The calculations were performed with the CNDO/2 dipole moments $\mu_{is}=6.38$ D and $\mu_{ts}=10.84$ D (Table 3), but also with the perhaps more realistic moments $\mu_{is}=5.70$ D and $\mu_{ts}=9.70$ D. These values were obtained by introducing a scaling factor for all the calculated moments, which gives *4c* the value 5.5 D, 0.1 D larger than that for *3*, $R=CH_3$ corresponding to the difference between *12* and *13*. The results are shown in Fig. 1.

It is obvious that the calculated solvent effect is somewhat larger than the experimental one of 12 kJ mol^{-1}. In view of the uncertainties in a and the dipole moments it is not very meaningful to discuss the reason for the discrepancy. In any case, the reaction field model accounts for the observed solvent effect in a semiquantitative way, and the large effect can be ascribed to an unusually large difference between μ_{ts} and μ_{is}.

The CNDO/2 calculations give the rotational barriers in the gaseous state, $E_{ts}-E_{is}$. The calculated barrier for *4b* is 125.9 kJ mol^{-1}, to be compared with the experimental free energy barrier of 76.0 kJ mol^{-1} (in toluene). The activation enthalpy is more appropriate for a comparison than the free energy barrier, but it is not known for *4b* though it is certainly lower than 76.0 kJ mol^{-1}. Shvo *et al.*[32] found the activation anthalpy to rotation around the C=C bond in *6* to be 34.7 kJ mol^{-1} in dichloromethane, to be compared with 145.6 kJ mol^{-1} calculated by the INDO method. The corresponding values for the rotation about the C–N bond were in much better agreement, 54.0 and 37.2 kJ mol^{-1}. The large difference between the experimental and calculated barriers to rotation about the C=C was mainly ascribed to the solvent effect, though no attempt was made to quantity this hypothesis. This can be made using the reaction field model. For *4c*, the increase in stabilization from gas phase to toluene solution is the same as the solvent effect from acetonitrile to toluene, since $(\varepsilon-1)/(2+1)$ for acetonitrile is by chance precisely twice the value for toluene. Clearly, the experimental value of 12 kJ mol^{-1} vastly underestimates the difference of 57 kJ mol^{-1} between the calculated and experimental barriers.

No experimental solvent effect is available for *6*, but an approximate calculation of the difference in stabilization energy between the gas phase and dichloromethane solution can be made by eqn. (3), using $\mu_{is}=4.30$ D and $\mu_{ts}=6.03$ D from the INDO calculations.[32] Values for the cavity radius a from 4.06 to 4.19 Å are obtained under the same assumptions as for *4b*. The $\delta\Delta E$ values are shown by the lowest curve in Fig. 1, and it is obvious that the solvent effect is quite small, and that the calculated rotational barrier for *6* is even more off the mark than that for *4b*. Thus, the discrepancies between experimental and calculated barriers cannot be ascribed to neglect of solvent stabilization but must be due to the approximations in the methods of calculation. All valence electron calculations with total neglect of differential overlap sometimes have given quite good agreement with experimental barriers,[33,34] but that has been when a group rotates that is small compared to the rest of the molecule, and the effect of the rotation is only a small perturbation of the total energy of the molecule. This is not the case with rotations about the C=C bond in compounds like *4b* and *6*, and calculation of barriers to such processes evidently requires more advanced methods.

Table 4. Calculated formal charges (π-electron charges in parentheses).

Molecule	q_1	q_2[a]	q_3[a]	q_6	q_N/q_S	q_O
4a						
Initial state	−0.1154	−0.0460	−0.0442	+0.3014	−0.1002	−0.2080
	(−0.2005)	(−0.0855)	(−0.0648)	(+0.1920)	(+0.2318)	(+0.1254)
Transition state	−0.1595	−0.0698	−0.0867	+0.4048	−0.0853	−0.1881
	(−0.3038)	(−0.1791)	(−1.4002)	(+0.3711)	(+0.3109)	(+0.1807)
4b						
Initial state	−0.1107	−0.0453	−0.0404	+0.3375	−0.1487	−0.2114
	(−0.1928)	(−0.0879)	(−0.0589)	(+0.1917)	(+0.2355)	(+0.1228)
Transition state	−0.1590	−0.0641	−0.0849	+0.4403	−0.1208	−0.1905
	(−0.3092)	(−0.1757)	(−0.1374)	(+0.3889)	(+0.3370)	(+0.1743)
4d						
Initial state	−0.1231	−0.0476	−0.0483	+0.2872	−0.1502	–
	(−0.2165)	(−0.0899)	(−0.0733)	(+0.1925)	(+0.2255)	–
Transition state	−0.1566	−0.0771	−0.0889	+0.3671	−0.1263	–
	(−0.2970)	(−0.1837)	(−0.1432)	(+0.3505)	(+0.2991)	–

[a] q_4 and q_3, like q_5 and q_2, are quite similar. X is the atom with lowest atomic number of X and Y.

The calculated barrier for *4d* is 95.8 kJ mol^{-1}. The experimental barrier is not known, but it is in all likelihood somewhat lower than that of *4c*, 42 kJ mol^{-1}, and higher than that of *5*, which has 30 kJ mol^{-1} as an estimated upper limit. A reasonable estimate for *4d* is 35 kJ mol^{-1} and it is evident that no realistic solvent effect can explain the difference of *ca.* 60 kJ mol^{-1} between the experimental and calculated barriers. However, even if the CNDO/2 calculations are unable to give good rotational barriers for these compounds, some comfort can be taken from the fact that the barriers of *4a*, *4b* and *4d* fall in the right order, and that the calculated difference between *4b* and *4d*, 37.4 kJ mol^{-1}, does not differ much from the estimated difference between their free energy barriers of *ca.* 40 kJ mol^{-1}.

The calculated charge distributions for the initial and transition states are shown in Table 4. The effects are similar to those found in previous calculations on push−pull ethylenes.[31] The donor atoms (N,O,S) lose π-electrons but are more than compensated through their inductive attraction for σ-electrons. The electron density on C−1 is high and that on C−6 is low with respect to both π- and σ-electrons. The cyclopentadiene ring attracts electrons in the initial state, but the polarization is much increased in the transition state.

The charge density on C−1 and C−6 is reflected in the chemical shifts of the corresponding ^{13}C resonances. While C−2 to C−5 fall in the range of δ113−120, the C−1 resonance falls at *ca.* δ 100 and that of C−6 at *ca.* δ 160, in agreement with data from other push−pull ethylenes.[35]

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Photooxidation with Simultaneous Reduction of Hydroperoxides with Tetrabutylammonium Borohydride. Synthesis of Perillenal from Myrcene *

P. BÆCKSTRÖM, S. OKECHA, N. DE SILVA, D. WIJEKOON and T. NORIN

Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden

The synthetic routes to 2-methyl-5-(3-furyl)-2-pentenal, perillenal (*1*), starting from 2-methyl-6-methylene-2,7-octadiene, myrcene (*2*), are described. Myrcene (*2*) was either photooxidized to a mixture of the allylic alcohols *3* and *4* or converted to the aldehyde *11* by oxidation with selenium dioxide followed by chromium trioxide dipyridine in acetic acid. The alcohols *3* and *4* and the aldehyde *11* were cyclized with singlet oxygen to the endoperoxides *5*, *6*, and *12*, respectively. The endoperoxides were converted to the furans *7*, *8*, and *1* by treatment with Fe(II). The secondary allylic furan *8* was converted to perillenal (*1*) by a one-step reaction involving an allylic rearrangement and an oxidation with pyridinium chlorochromate in the presence of *p*-toluenesulfonic acid in dichloromethane. A method for photooxidation and simultaneous reduction of hydroperoxides with tetrabutylammonium borohydride is presented.

The furanoid monoterpene *E*-2-methyl-5-(3-furyl)-2-pentenal (*1*), for which the name perillenal was adopted, has been shown to be the main volatile component of glands excised from males and females of the pine sawfly *Neodiprion sertifer* (Geoff.).[1] The identification was confirmed by comparison of spectral (MS) and chromatographic (GC) data with those of a synthetic sample prepared[1] according to a method described by Thomas,[2] who used compound *1* as an intermediate for the synthesis of some furanoid terpenes.

In order to investigate the biological significance of perillenal *1* by means of electrophysiological measurements and field tests, there is a need for alternative and simple synthetic routes to this compound.

DISCUSSION AND RESULTS

The preparative utility of the reactions of singlet oxygen with olefins was already emphasized by Schenk and his co-workers in their pioneering work on sensitized photooxygenations.* Singlet oxygen reacts rapidly with tri- and tetrasubstituted double bonds and an allylic hydrogen in an ene-type reaction to give allylic hydroperoxides. With cyclic 1,3-dienes a facile Diels-Alder type reaction takes place. Although the formation of endoperoxides from open-chain 1,3-dienes and singlet oxygen is very slow due to conformational factors Kondo *et al.*[4] have recently shown that endoperoxide formation from 2-substituted butadienes is synthetically feasible. This is due to the slow rate of the competing ene-type reaction with mono- and disubstituted double bonds.[4-6] The endoperoxides can easily be converted to 3-substituted furans, *e.g.* by treatment with Fe(II) as shown by Herz,[5] thus providing attractive possibilities for the synthesis of perillenal (*1*), starting from the readily available 2-methyl-6-methylene-2,7-octadiene, myrcene (*2*).

Two different synthetic routes were followed. The first route, outlined in the upper part of Scheme 1, began with photooxidation of myrcene itself.[4b,4c,8] Due to the much greater reactivity of the trisubstituted double bond, a mixture of allylic

* Part of this work was presented at the 8th Conference on Isoprenoids, Toruń, September 1979.

* For comprehensive reviews of the reaction of singlet oxygen see Ref. 3.

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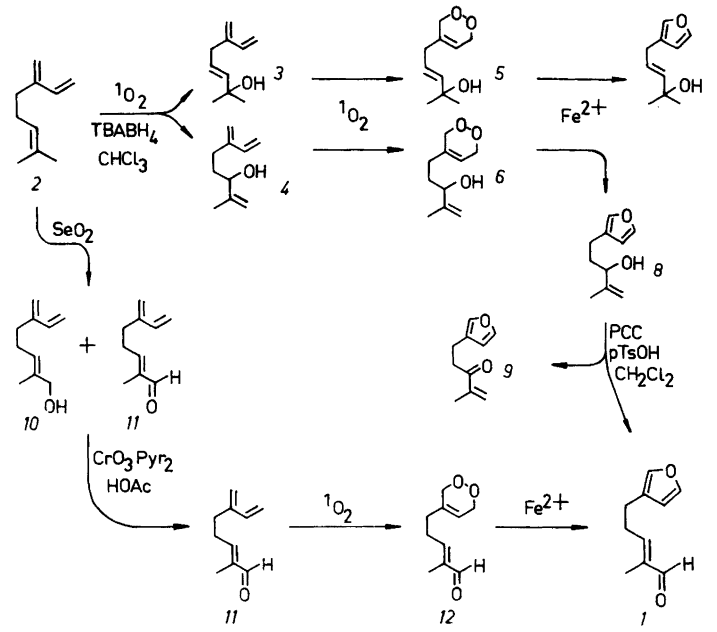

Scheme 1.

hydroperoxides was rapidly formed. In our procedure the hydroperoxides were continuously reduced during irradiation (*vide infra*) to the corresponding alcohols *3* and *4*.* Extended irradiation gave a mixture of the endoperoxides *5* and *6*. The mixture of *5* and *6* was converted to the furans *7* and *8* with Fe^{2+} according to Herz, and the products separated by column chromatography. A slight modification of Herz's procedure was introduced for the conversion of the endoperoxides. The solvent mixture (water – furan) was changed to water – acetone in order to avoid radical-induced reactions of furan.

The reaction sequence according to Scheme I could also be performed using a step-wise photooxidation procedure in which the alcohols *3* and *4* were first prepared and separated. The alcohol *4* was then subjected to a prolonged photooxidation followed directly by a transformation of the endoperoxide *6* to the furan alcohol *8*. Such a step-wise procedure is convenient since fewer by-products are formed and the separation of the reaction products can easily be performed by preparative chromatography. Furthermore, the alcohol *3*, which is a by-product, is a useful starting material for a syhthesis of ipsdienol, a phermone component of the spruce bark beetle (*Ips typographus* L.).[12]

The necessary operations to obtain perillenal (*1*) from compound *8* consisted of an allylic rearrangement and an oxidation. This was accomplished in one step** by treating *8* with pyridinium chlorochromate (PCC)[14] in the presence of *p*-toluensulphonic acid (pTsOH) in dichloromethane. In this reaction a minor amount (< 20%) of ketone *9* was also formed. Although the isolated yield of pure *1* (*E*:*Z* > 98:2) was only ≅ 35% this method was preferred to alternative multistep sequences. When PCC in dichloromethane was used without addition of pTsOH for the treatment of *8*, ketone *9* was formed as the major product together with trace amounts of *1*.

A more direct route to perillenal (*1*), outlined in the lower part of Scheme 1 has also been investigated. In this sequence the aldehyde was introduced prior to cyclization of the diene system in order to avoid the attack of singlet oxygen on the

*The tertiary alcohol *3* has been identified as a constituent of the frass of *Ips paraconfusus* (Lanier)[9] and of guts of *Ips amitinus* (Eichh.).[10] It is also a plant constituent.[11] To our knowledge, *4* has not been described as a natural product although the corresponding ketone has been isolated from *Ledum palustre* (L.).[11b]

**For an earlier example of the same type of reaction see Ref. 13.

trisubstituted double bond. This was done by oxidizing myrcene (*2*) with selenium dioxide[15] which gave a mixture of the alcohol *10* and the aldehyde *11*. The mixture was subjected to oxidation with chromium trioxide dipyridine in acetic acid[16] to transform the alcohol *10* to aldehyde *11*.

The aldehyde *11* could be photooxidized to the endoperoxide *12*, which on treatment with Fe^{2+} gave perillenal (*1*), albeit the over-all yield of *1* ($E:Z \cong 3.5:1$) from *11* was only 6%.

Simultaneous oxidation and reduction. In connection with this work we have developed a method for continuous reduction of hydroperoxides as they are formed in photooxidation reactions. In this way, the corresponding alcohols can be isolated without a build-up of potentially hazardous compounds. The substrate, *i.e.* myrcene, was photooxidized in the presence of tetrabutylammonium (TBA) borohydride, which proved to be surprisingly stable towards oxygen. Rose bengal was made soluble in chloroform by the formation of ion pairs with TBA ions.[17] A slight molar excess was added in portions to ensure proper reduction. Although relatively large amounts of TBA ions were added in this process, removal did not constitute a problem. Stripping off the chloroform, followed by the addition of diethyl ether and a concentrated solution of potassium iodide in water caused precipitation of easily filtered TBA iodide.

The use of TBA borohydride provided an additional benefit. Initial attempts to photooxidize myrcene dissolved in various solvents, with light >320 nm led to serious bleaching of rose bengal. This problem was obviated by the method described above.

EXPERIMENTAL

UV measurements were obtained using a Beckman DU instrument connected to Optilab Multiblank 171 and Multilog 802 units. NMR spectra were recorded at 200 MHz in $CDCl_3$ with TMS as internal standard using a Bruker model WP 200 unless specified. A Finnigan model 4021 connected to an INCOS data system was used to record GC-MS spectra which are reported as stored in the INCOS MS-library. Analytical GLC was performed on a PYE GCV instrument with an FID detector connected to an integrator (Spectra Physics Minigrator). Merck 60 silica gel 0.040–0.063 mm, dry packed in 2.54 cm i.d. columns was used for liquid chromatography. The solvent, light petroleum b.p. 40–60 °C with stepwise increased amounts of EtOAc, was delivered by a metering pump at a rate of 100 ml/min. B.p.'s are uncorrected. Irradiations were carried out in a Rayonet reactor equipped with 16 RPR 350 nm lamps or by using a Wisconsin Black Box[18] (WBB) fitted with a 1000 W AH-6-B high pressure mercury arc employing a filter combination of 2 cm concentrated water solution of copper sulfate and 1 mm soft glass to cut off light <320 nm. Stock solutions of solubilized rose bengal were made by mixing 1.50 g rose bengal and 0.97 g TBA bromide per liter chloroform followed by filtration to remove insoluble residue.

Tetrabutylammonium (TBA) borohydride. This reagent was prepared according to Brändström.[19] A suspension of 340 g (1.0 mol) of TBA hydrogen sulfate and 50 g (1.25 mol) of sodium hydroxide in 250 ml of water were mixed and cooled to room temperature. Dichloromethane, 500 ml, and 40 g (1.1 mol) of sodium borohydride in 100 ml of water were added and the mixture was agitated a few minutes. Precipitated sodium sulfate was removed by filtration using glass wool. The *upper* dichloromethane layer was separated and the aqueous layer extracted with 250 ml of dichloromethane (lower layer). After drying with anhydrous K_2CO_3 and filtration, 250 ml of toluene were added and the solvents were removed by reduced pressure and by heating <50 °C. The crystals were washed with ethyl ether and recrystallized from ethyl acetate, m.p. 126 °C.

Exploratory irradiation of 2-methyl-6-methylene-2,7-octadiene, myrcene (2). To a 0.1 M solution of myrcene dissolved in the stock solution of rose bengal in chloroform was added tetradecane as internal standard and an excess of TBA borohydride. Irradiations were carried out in the Rayonet reactor with a constant flow of oxygen bubbling through the solution. Before GLC monitoring (2 m, 4 mm, i.d., 10% Carbowax 20 M, 160 °C) withdrawn samples were filtered through basic alumina and eluted with ether. These investigations showed that a maximum yield of 38% of tertiary alcohol *3* and 36% secondary alcohol *4* could be obtained at 95% conversion of myrcene (*2*).

2-Methyl-6-methylene-3,7-octadiene-2-ol (3) and 2-methyl-6-methylene-1,7-octadiene-3-ol (4). Myrcene (*2*), 10 g (0.074 mol), was dissolved in 750 ml of the stock solution of rose bengal in chloroform and was irradiated in the WBB with a constant flow of oxygen bubbling through the solution. At 0, 30, 60 and 90 min of irradiation, portions of 10, 5, 2.5 and 2.5 g, in all 20 g (0.078 mol), of TBA borohydride were added. After 2 h of irradiation the chloroform was removed under reduced pressure (<0.1 Torr). To the viscous residue was added 20 g (0.120 mol) of potassium iodide in 25 ml of water and 250 ml of ethyl ether. The mixture was then stirred for 1 h. The resulting crystals were separated by filtration and

washed with ether. The combined red ethereal layer was separated from the aqueous phase and dried with $MgSO_4$. After removal of the drying agent, 50 g of basic alumina were added and the ether was removed under reduced pressure. The red dry powder was poured on top of a column of 70 cm of silica gel with a top layer of 3 cm of basic alumina. After wetting the column with light petroleum (300 ml) the compounds were eluted by pumping 600 ml each of 0, 1.25, 2.5, 5 and 10 % of ethyl acetate in light petroleum. Elution was continued with 20 % ethyl acetate in light petroleum. Of the two alcohols, the secondary alcohol *4* had the smaller retention volume. The yield of the reaction varied with the quality of the myrcene used. The best isolated yields obtained were 4.1 g of *3* (36 %) and 3.3 g of *4* (27 %). The analytical samples were further purified by distillation. Alcohol *3*, b.p. 41 °C/0.3 Torr; n_D^{23} 1.4835; MS: *m/e* (rel. int.) 137 (1.2), 134 (3.5), 119 (6.2), 95 (5.1), 93 (7.4), 91 (11.0), 85 (10.5), 81 (10.2), 80 (7.9), 79 (13.7), 77 (7.0), 67 (7.1), 59 (26.9), 55 (15.4), 53 (11.0), 43 (100), 41 (25.2); NMR: δ 6.35 (1H, dd, $J = 17.6$ and 10.7 Hz, $=CH-C=$), 5.67 (2H, apparent s, CH $=CH$), 5.22 (1H, d, $J = 17.6$ Hz, $-HC=CH_2$, *trans*), 5.05 (1H, d, $J = 10.6$ Hz, $-HC=CH_2$, *cis*), 5.03 (1H, $=CH_2$), 4.99 (1H, $=CH_2$), 2.92 (2H, 5.9 Hz br., CH_2), ~1.71 (1H, br., OH), 1.29 (6H, s, gem. CH_3). Alcohol *4*, b.p. 46 °C/0.2 Torr; n_D^{23} 1.4820, lit.[4c] b.p. 85 °C/11 Torr; MS: *m/e* (rel. int.) 137 (3.3), 123 (4.4), 119 (4.2), 109 (5.1), 96 (6.7), 93 (10.4), 91 (10.5), 84 (21.7), 83 (9.2), 81 (11.8), 79 (21.0), 71 (27.5), 69 (28.3), 68 (19.0), 67 (29.8), 57 (11.1), 55 (24.5), 53 (27.1), 43 (64.7), 41 (100); NMR: δ 6.39 (1H, dd, $J = 17.7$ and 10.7 Hz, $=CH-C=$), 5.26 (1H, d, $J = 17.7$ Hz, $-CH=CH_2$, *trans*), 5.07 (1H, d, partially obscured, $J = 10.7$, $-CH=CH_2$, *cis*), 5.04 (2H, 3 Hz br. s, conj. $=CH_2$), 4.98 (1H, 3.7 Hz br., $=CH_2$), 4.87 (1H, 3 Hz br., $=CH_2$), 4.11 (1H, apparent t, $J \sim 6.5$ Hz, $HO-CH$), 2.3–2.2 (2H, m, $=-CH_2-$), 1.8–1.6 (2H, m, partially obscured, $HO-CH-CH_2-$), 1.74 (3H, t, $J = 1.2$ Hz, $-CH_3$), ~1.6 (1H, OH).

2-Methyl-5-(3-furyl)-1-penten-3-ol (8). The secondary alcohol *4*, 6.727 g (44.26 mmol), was dissolved in 750 ml of the stock solution of rose bengal in chloroform and irradiated in the WBB for 24 h. The solvent was removed under reduced pressure with heating <40 °C. The last traces of solvent were removed at a pressure <1 Torr. The residue was dissolved in 150 ml of acetone and 12 g of $FeSO_4 \cdot 7H_2O$ in 300 ml of water were added. The mixture was stirred for 2 h. Most of the acetone was removed under reduced pressure before extraction with two portions of ethyl ether. The organic layer was washed with water and dried with $MgSO_4$. Filtration and solvent removal gave a red oil which was chromatographed on silica gel, yielding 1.342 g of starting material and 3.574 g of compound *9* (61 % of nonrecovered starting material). The analytical sample was further purified by distillation. B.p. 80 °C/0.3 Torr; n_D^{22} 1.4895; MS: *m/e* (rel. int.) 166 $M^{\cdot+}$ (1.8), 151 (2.8), 149 (2.0), 148 (17.4), 133 (5.2), 123 (3.8), 119 (3.6), 108 (3.8), 105 (3.6), 95 (22.8), 94 (11.4), 91 (4.5), 83 (7.7), 82 (100), 81 (66.8), 72 (9.9), 71 (23.5), 67 (13.9), 57 (9.6), 54 (8.7), 53 (25.3), 43 (41.5), 41 (60.3); NMR: δ 7.36 (1H, furan, α-pos.), 7.24 (1H, furan, α-pos.), 6.29 (1H, furan, β-pos.), 4.97 (1H, $=CH_2$), 4.87 (1H, $=CH_2$), 4.10 (1H, apparent t, $J \cong 6$ Hz, $HO-C-H$), 2.52–2.45 (2H, m, fur.$-CH_2-$), 1.86–1.78 (2H, m, $HO-C-CH_2-$), 1.74 (3H, s, $=-CH_3$), $\cong$1.5 (1H, OH).

2-Methyl-5-(3-furyl)-3-penten-2-ol (7) and compound 8 from prolonged irradiation of myrcene (2). Starting with 10 g (0.074 mol of myrcene (*2*), the same procedure was followed as for the synthesis of *3* and *4* but irradiation was extended to 60 h. Silica gel chromatography gave 1.54 g of the two alcohols *3* and *4* and 1.90 g (14 %) of the endeperoxides *5* and *6*, which were collected in one fraction. The endoperoxides *5* and *6*, 1.90 g (0.010 mol) dissolved in 50 ml of acetone, were mixed with 4.59 g (0.017 mol) of $FeSO_4 \cdot 7H_2O$ dissolved in 75 ml of water and stirred for 2 h. The reaction mixture was worked up as described for the preparation of compound *6*. The isolated yield of furans from endoperoxides was 1.44 g (87 %). The furan alcohols *7* and *8* were separated by silica gel chromatography and further purified by bulb-to-bulb distillation. For NMR data of *6* see Ref. 4c. Compound *7* exhibits the following spectral data: MS: *m/e* (rel. int.) 166 $M^{\cdot+}$ (1.6), 151 (9.7), 149 (1.1), 148 (3.5), 133 (2.2), 123 (1.4), 109 (2.3), 105 (2.3), 95 (3.7), 94 (2.1), 91 (3.4), 85 (17.5), 82 (9.4), 81 (8.7), 79 (6.8), 77 (6.4), 72 (4.0), 59 (15.6), 57 (5.4), 55 (9.3), 53 (8.0), 43 (100), 41 (11.9); NMR: δ 7.36 (1 H, furan, α-pos.), 7.22 (1H, furan, α-pos.), 6.26 (1H, furan, β-pos.), 5.76–5.71 (2H, m, $HC=CH$), 3.16 (2H, d, $J = 4.2$ Hz, CH_2), ~1.5 (1H, br. s, OH), 1.32 (6H, s, gem., CH_3).

E-2-Methyl-5-(3-furyl)-2-pentenal, perillenal (1) and 2-methyl-5-(3-furyl)-1-penten-3-one (9). The secondary allylic furan alcohol *8* (2.490 g, 0.015 mol) was added to a stirred suspension of PCC[14] (6.465 g, 0.030 mol) and *p*-toluenesulfonic acid monohydrate (8.55 g, 0.045 mol) in 1500 ml of dichloromethane. The reaction mixture darkened rapidly. After 1 h, 100 ml of water and 200 ml of saturated sodium chloride solution were added and the layers separated. The dichloromethane layer was treated twice more in the same way and dried with $MgSO_4$. Silica gel chromatography gave 0.188 g (7.3 %) of ketone *9* and 0.858 g (35 %) of perillenal (*1*), with an E:Z ratio >98:2 as determined by GC (PYE GCV equipped with a capillary inlet system described in Ref. 20, 25 m i.d. 0.2 mm SE 30, 70–150 °C, 6 °C/min). Compound *1* was further purified by distillation. B.p. 76–77 °C/0.3 Torr; n_D^{23} 1.5060. Perillenal (*1*) exhibits the following spectral data: MS: *m/e* (rel. int.) 164 $M^{\cdot+}$ (4.6), 149 (0.9), 146 (1.2), 136 (1.9), 135

(1.1), 107 (3.7), 94 (1.7), 91 (1.4), 82 (11.0), 81 (100), 77 (1.3), 65 (1.3), 55 (2.3), 54 (1.9), 53 (24.4), 51 (4.3), 41 (5.6), 40 (21.5); NMR: δ 9.69 (1H, CHO), 7.37 (1H, furan, α-pos.), 7.26 (1H, furan, α-pos.), 6.53 – 6.46 (1H, m, =CH), 6.28 (1H, furan, β-pos.), 2.64 – 2.57 (4H, m, CH_2-CH_2), 1.73 (3H, s, CH_3). For a 60 MHz spectrum of an *E*- and *Z*-mixture of *1* recorded in CCl_4 see Ref. 2. Compound *9* exhibits the following spectral data: MS: *m/e* (rel. int.) 164 $M^{\cdot+}$ (11.0), 149 (1.5), 136 (1.3), 135 (2.6), 122 (1.0), 121 (2.2), 108 (1.3), 96 (5.0), 95 (61.9), 94 (8.9), 91 (2.7), 82 (3.6), 81 (37.4), 69 (23.2), 67 (10.0), 65 (5.0), 55 (5.0), 53 (13.2), 41 (100), 40 (6.4); NMR: δ 7.34 (1H, furan, α-pos.), 7.25 (1H, furan, α-pos.), 6.27 (1H, furan, β-pos.), 5.96 (1H, $=CH_2$), 5.77 (1H, $=CH_2$), 2.99 – 2.91 (2H, m, part of aa'bb'syst.), 2.80 – 2.72 (2H, m, part of aa'bb'syst.), 1.89 (3H, $-CH_3$).

2-Methyl-6-methylene-2,7-octadienal (11). Myrcene (*2*) was oxidized with selenium dioxide according to Delaby and Dupin[15] which gave a mixture of alcohol *10* and aldehyde *11*. The mixture was distilled and the fraction b.p. 80 – 105 °C/1 – 5 Torr was subjected to oxidation with chromium trioxide dipyridine in acetic acid prepared according to Stensiö and Wachtmeister.[16] To 350 ml of a 1 M solution of the complex, cooled in an ice-bath, was added 10 g of the alcohol aldehyde mixture during 5 min. After additional 10 min the reaction mixture was poured into 500 ml of water and extracted with ether. The ether layer was washed with water and aqueous sodium carbonate. Drying with $MgSO_4$ and work-up gave 6.5 g of yellow oil which was distilled to yield aldehyde *11* b.p. 45 – 55 °C/1 Torr. MS: *m/e* (rel. int.) 150 $M^{\cdot+}$ (4.8), 149 (1.1), 135 (5.4), 121 (4.1), 117 (2.9), 107 (4.0), 105 (3.4), 95 (6.0), 93 (44.5), 92 (31.1), 91 (23.1), 82 (8.4), 79 (24.1), 77 (13.5), 67 (21.6), 65 (15.5), 55 (27.8), 54 (10.2), 53 (22.5), 43 (13.9), 41 (100); NMR 60 MHz ($CDCl_3$): δ 9.40 (1H, s, CHO), 6.40 (1H, dd, J = 17.0 and 10.5 Hz, =CH – C =), 6.42 (1H, m, $=CH-CH_2$), 5.20 (1H, d, J = 17.0 Hz, $=CH_2$), 5.08 (1H, d, J = 10.5 Hz, $=CH_2$), 5.01 (2H, br. s, $=CH_2$), 2.55 – 2.20 (2H, m, CH_2-CH_2), 1.70 (3H, s, CH_3).

Perillenal (1) from photooxidation of 2-methyl-6-methylene-2,7-octadienal (11). Aldehyde *11* (1 g) was added to a solution of rose bengal (0.509 g) and TBA bromide (0.070 g) in 100 ml of a mixture of methanol (5%) in dichloromethane. The solution was irradiated in the Rayonet reactor for 19 h with a continuous flow of oxygen bubbling through the reaction mixture. Occasionally some rose bengal solution was added to make up for the bleaching of the sensitizer that occurred. The solvent was removed under reduced pressure and the residue was dissolved in 20 ml of THF. A slight excess of ferrous sulfate dissolved in 40 ml of water was added. The reaction mixture was left overnight. Water (300 ml) was then added and the reaction mixture was worked up by extraction with ethyl ether. The ethereal phase was dried with $MgSO_4$. The solvent was removed under reduced pressure (10 mm) with heating < 25 °C to yield 0.740 g of red oil. Silica gel chromatography using 10% ether in light petroleum as the eluent gave 0.059 g, 5% of an *E*:*Z* mixture (85:15) of perillenal (*1*).

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Tobacco Chemistry. 54.* (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-Epoxy-2,6-cembradiene-4,12-diol, a New Constituent of Greek Tobacco

INGER WAHLBERG,[a] DAN BEHR,[a] ANN-MARIE EKLUND,[a] TOSHIAKI NISHIDA,[a] CURT R. ENZELL[a] and JAN-ERIC BERG[b]

[a] Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62 Stockholm, Sweden and [b] Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

A new diterpenoid has been isolated from Greek tobacco and is formulated as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*) by spectroscopic methods, synthesis and X-ray analysis of its *4R*-epimer (*12*). The biogenesis of diol *1* is discussed on the basis of results obtained from epoxidation and rearrangement reactions.

The cembranic diterpenoids isolated from the cuticular wax of the leaf of certain 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 (*2*, *3*), whereas *i.a.* a series of 8,11-epoxy bridged cembranoids, all having an 8*R*,11*S*-stereochemistry, *e.g.* *4*–*7*, is present in a minor amount.[2] We now report the isolation of the first 8*S*,11*R*-epoxy bridged cembranoid from sun-cured Greek tobacco.

RESULTS

The new compound (*1*), $C_{20}H_{34}O_3$, gave a ^{13}C NMR spectrum containing signals due to five methyl, five sp^3 methylene, three sp^3 methine carbon atoms, of which one was oxygen-carrying, three fully substituted oxygen-carrying sp^3 carbon atoms and four sp^2 methine carbon atoms, *i.e.* two disubstituted double bonds. Since the ^1H NMR spectrum displayed two three-proton doublets at δ 0.86 and 0.90 and since the IR spectrum had bands at 1375 and 1390 cm^{-1}, two of the methyl groups were deduced to form part of an isopropyl group. The remaining three methyl groups, giving rise to singlets at δ 1.03, 1.29 and 1.31, are evidently linked to fully substituted oxygen-carrying carbon atoms.

It followed from the characteristic chemical shift values of two of the signals in the ^{13}C NMR spectrum, δ 88.6 (d) and 82.6 (s), and the presence of a signal at δ 4.03 (t) in the ^1H NMR spectrum that one of the oxygen atoms is present as an ether group extending from a methine to a fully substituted carbon atom. The remaining two oxygen atoms are accommodated by tertiary hydroxyl groups (OH-absorption in the IR spectrum). These results indicated that diol *1* is a carbomonocyclic diterpenoid and a cembranic structure seemed most plausible from a biogenetic point of view.

Additional structural information, which reinforced this alternative, was provided by the ^1H and ^{13}C NMR spectra. Thus, spin decoupling and spin simulation experiments allowed the allocation of the double bonds, both of which were found to have *E*-configurations ($^3J = 16$ and 15.5 Hz), to partial structures A and B. These were suggested to be included in diol *1* as the C-1 to C-8 portion of an 8,11-epoxy-2*E*,6*E*-cembradiene-4,12-diol structure by a comparison, which showed that fourteen signals in the ^{13}C NMR spectrum of diol *1* were of appropriate multiplicities and had chemical shift values close to those observed for the C-1 to C-5, C-8, C-11, C-12 and C-15 to C-20 signals for (1*S*,2*E*,4*S*,-6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*4*).[3]

A clue to the chiralities at C-4 and C-8 was obtained by a comparison, which included the ^{13}C

*For Part 53 see Ref. 1.

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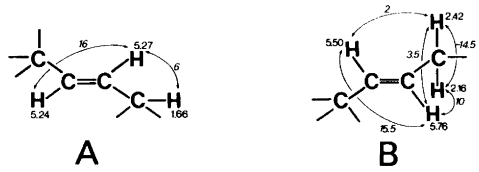
Table 1. Carbon-13 chemical shifts and assignments for compounds *1*, *4* – *7*, *9*, *11* and *12*.[a]

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	51.0	129.7	138.3[b]	73.7	45.8	126.5	138.5[b]	82.6	32.5	24.4	88.6	73.7	39.5	27.0	32.3	20.9	20.1	30.4	28.9	22.4
4	50.9	129.4	138.2	73.6	45.6	122.6	139.6	82.9	35.1	27.0	88.9	74.1	36.7	28.5	32.3	20.7	20.0	30.1	29.2	22.1
5	50.9	132.2	137.4	73.3	46.7	121.5	140.1	83.0	34.6	25.7	89.0	74.2	36.0	28.5	31.9	20.7	20.4	24.8	29.1	21.8
6	51.4	130.1	138.3	73.4	45.2	123.1	140.0	83.5	34.0	25.4	87.5	74.8	36.2	26.8	32.4	20.9	19.8	30.1	29.5	24.8
7	51.4	132.7	137.5	73.3	46.1	122.5	140.2	83.5	34.0	24.7	87.5	74.8	35.7	26.9	32.4	20.8	19.9	24.7	29.5	24.7
9	46.3	129.4	137.5	73.2	46.9	124.2	140.2	73.2	35.9	23.7	63.7	61.4	37.7	28.2	33.8	20.5	19.1	30.9	30.9	15.6
11	46.8	130.5	138.3	73.1	47.2	124.2	138.8	72.0	36.0	23.8	64.4	61.3	38.4	27.2	33.3	20.1	19.4	27.4	26.7	15.6
12	50.9	132.4	138.0	73.2	46.4	125.7	139.2	82.5	32.6	24.4	88.4	73.7	39.2	26.8	32.3	20.8	20.1	25.5	28.7	22.2

[a] δ-Values in $CDCl_3$ relative to TMS. [b] Assignment may be reversed.



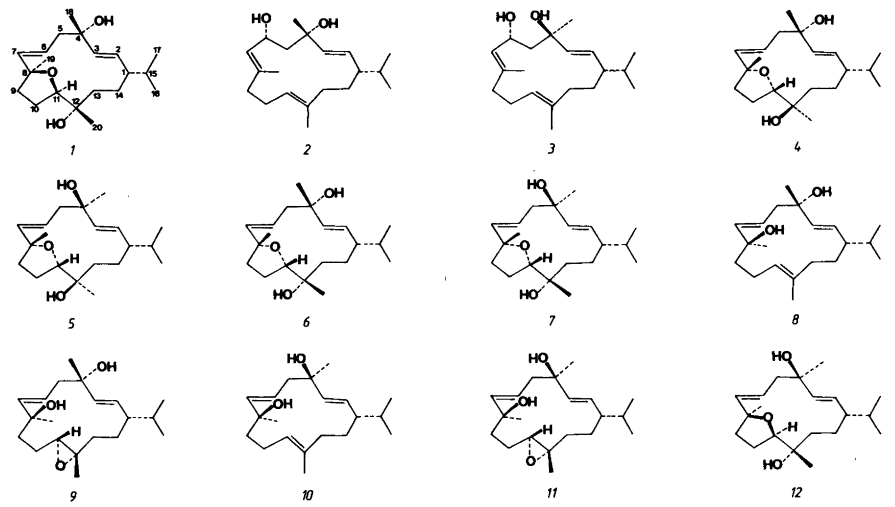
A B

Partial structures A and B. Chemical shift values (δ) are in Roman; coupling constants (Hz) in italic.

NMR spectra of diols *1* and *4* and the (1*S*,2*E*,4*R*,6*E*,-8*R*,11*S*,12*R*)-,[3] (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*S*)-[3] and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diols[4] (*5* – *7*). Thus, the chemical shift values of the C-2 and C-18 signals, δ 129.7 and 30.4, respectively, are only consistent with a 4*S*-configuration in diol *1*,[5] and the significantly different shielding of C-6 in diol *1* and in the 8*R*,11*S*-epoxy bridged compounds *4* – *7*, δ 126.5 as against δ 121.5 – 123.1, suggested that the configuration at C-8 is *S*.

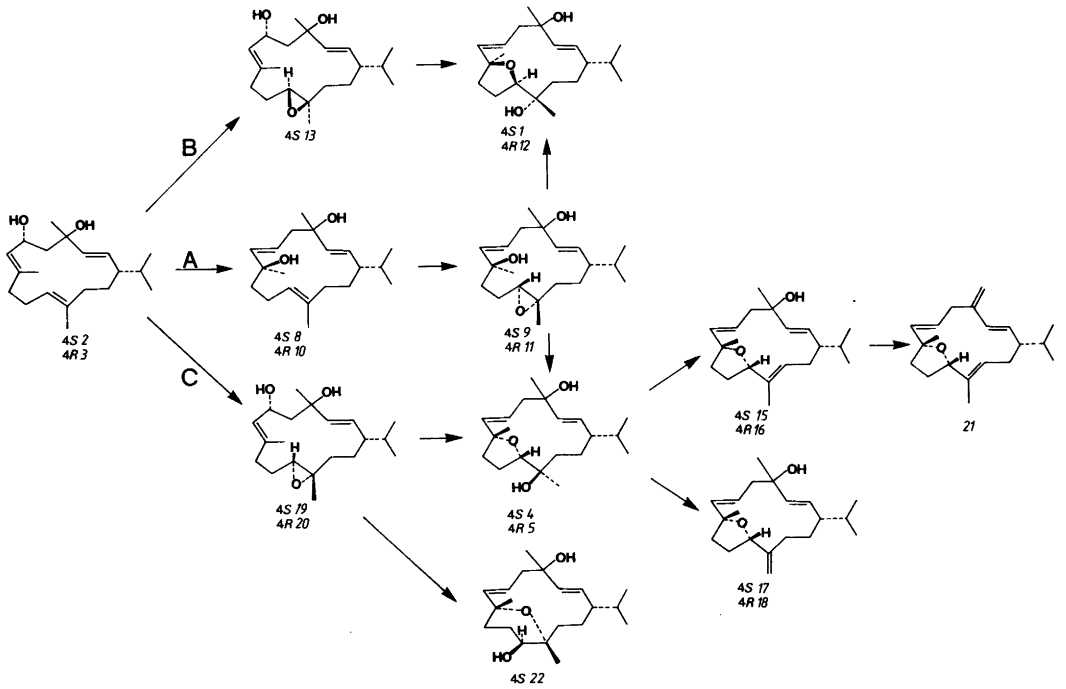
With this information at hand structural elucidation was sought by synthesis. Thus, (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*8*), available through acid-induced rearrangement of the 4*S*,6*R*-diol (*2*)[6] was reacted with *m*-chloroperbenzoic acid to afford an 11,12-epoxide (*9*), whose ^1H NMR spectrum displayed the signal due to H-11 as a doublet of doublets at δ 3.12. By analogy with the formation of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*) from the 4*R*,8*S*-diol (*10*),[6] epoxide *9* was assigned an 11*S*,12*S*-stereochemistry. On treatment with weakly acidified chloroform it underwent a facile conversion to a product, which proved to be identical in all respects to the new tobacco constituent (*1*). Since the mechanism involved is most likely an S_N2 type of epoxide opening at the secondary C-11 by attack of the hydroxyl group at C-8, (for other alternatives, *cf.* below) the new compound was tentatively formulated as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*).

Under similar conditions epoxide *11* was converted to a diol (*12*), which in contrast to diol *1*, was amenable to X-ray analysis. Diol *12* formed orthorhombic crystals of space group $P2_12_12_1$. The crystal data, obtained on a computer-controlled Philips PW 1100 diffractometer, were $a=17.843$, $b=10.655$ and $c=10.283$ Å, $Z=4$. The present *R*-value including anisotropic thermal parameters for all non-hydrogen atoms is 0.111, location of the hydrogen atoms and further refinement being under way.[7] A stereoscopic view, which summarizes the X-



ray results and demonstrates that diol *12* is (1*S*,2*E*,-4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol, is shown in Fig. 1. Since the ^{13}C NMR spectrum of diol *12* with the exception of shift differences for the C-2 and C-18 signals reflecting the configurational differences at C-4 was virtually superimposable on that of diol *1*, the latter is conclusively identified as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol.

Biogenesis. The new tobacco constituent (*1*) is the only 8,11-epoxy bridged cembranoid encountered so far, which has an 8*S*,11*R*-stereochemistry. It may arise in tobacco by a route (A in Scheme 1) similar to the synthetic one described above. This



Scheme 1. Probable biogenesis of the 8*S*,11*R*-, 8*R*,11*S*- and 8*R*,12*R*-epoxy bridged tobacco constituents.

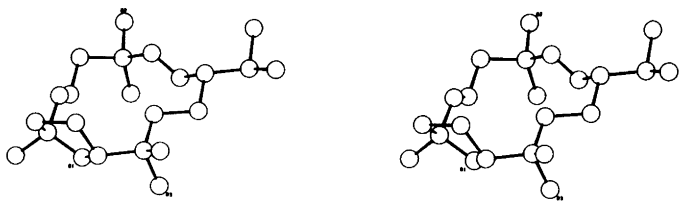
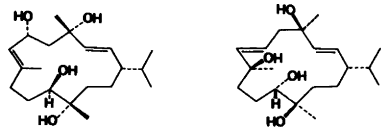


Fig. 1. Stereoscopic view of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*12*).

view is reinforced by the fact that the precursor, *i.e.* the 4*S*,8*S*-diol *8*, has been found present in tobacco.[8]

Another route (B) to compound *1* would involve (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*13*) as an intermediate and proceed *via* an *anti*-opening of the epoxide group with formation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (*14*). The latter would undergo protonation of the hydroxyl group at C-6, migration of the 7,8 double bond and an attack of the 11-hydroxyl group at C-8. In consistence with this view, epoxide *13*, which is a minor product obtained upon epoxidation of the 4*S*,6*R*-diol *2*[4] and as yet not encountered in tobacco, yielded compound *1* on treatment with dilute H_2SO_4 in dioxane–water. It should be noted that pathway B is analogous to pathway C, which describes the generation of the 8*R*,11*S*-epoxy bridged tobacco constituents (*4*, *5* and *15*–*18*) *via* acid-induced rearrangements of the (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,-11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diols (*19*, *20*) (*cf.* Scheme 1, which also includes plausible modes of formation of compounds *21* and *22*).[4]



14 *23*

Experimental support for the existence of a pathway between the 4,8*S*-diols *8* and *10* and the 8*R*,11*S*-epoxy bridged compounds was provided by the fact that treatment of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*) with dilute H_2SO_4 in dioxane–water afforded, besides *12*, also (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*16*). Its generation evidently involves hydroxylation of C-12 and a proton-induced loss of the hydroxyl group at C-8. Whether this reaction occurs in one step initiated by protonation of the hydroxyl group at C-8 or takes place *via* an initial *anti*-opening of the epoxide group with formation of an intermediate tetrol (*23*) is presently unclear.

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. 9 were used.

Isolation. Column chromatography over silica gel of fraction A3[10] obtained from an extract of 295 kg of sun-cured Greek *Nicotiana tabacum* L. followed by HPLC using columns packed with Partisil/PAC, μ-Bondapak/C_{18} and μ-Bondapak/CN gave 3.7 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*), which had m.p. 130–132 °C, $[\alpha]_D$ +88° (*c* 0.36, $CHCl_3$) (Found: $M^{\cdot+}$ 322.2498, Calc. for $C_{20}H_{34}O_3$: 322.2507); IR ($CHCl_3$) bands at 3605, 3570, 3450, 1390 and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.86 (d, J = 6.4 Hz)/0.90 (d, J = 6.4 Hz) (H-16/H-17), 1.03 (s, H-19), 1.29 (s)/1.31 (s) (H-18/H-20), 2.16 (dd, J = 10 and −14.5 Hz, H-5a), 2.42 (ddd, J = 2, 3.5 and −14.5 Hz, H-5b), 4.03 (t, J = 6.5 Hz, H-11), 5.24 (d, J = 16 Hz, H-3), 5.27 (dd, J = 16 and 6 Hz, H-2), 5.50 (dd, J = 2 and 15.5Hz, H-7) and 5.76 (ddd, J = 3.5, 10 and 15.5 Hz, H-6); MS[*m*/*z* (%, composition)]: 322 (M, 1), 304 (17, $C_{20}H_{32}O_2$), 286 (3, $C_{20}H_{30}O$), 261 (10), 260 (6, $C_{18}H_{28}O$), 243 (7, $C_{17}H_{23}O$ and $C_{18}H_{27}$), 227 (10, $C_{13}H_{23}O_3$), 217 (6, $C_{15}H_{21}O$), 206 (14, $C_{14}H_{22}O$), 177 (27, $C_{12}H_{17}O$), 159 (23, $C_{12}H_{15}$), 135 (25, $C_{10}H_{15}$ and $C_9H_{11}O$), 121 (34, C_9H_{13} and C_8H_9O), 109 (33, C_8H_{13} and C_7H_9O), 93 (32, C_7H_9), 81 (40, C_5H_5O), 71 (46), 55 (28) and 43 (100).

Preparation of (1S,2E,4S,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (9). To a cooled (0 °C) solution of 9.4 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*8*)[6] and 15.4 mg of sodium acetate in 4 ml of chloroform was added 5.9 mg of *m*-chloroperbenzoic acid. The

reaction mixture was kept at 0 °C for 15 min. Work-up and separation by HPLC using a column packed with μ-Bondapak/CN gave 2.1 mg of(1*S*,2*E*,4*S*,6*E*,-8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*9*), which was an oil and had $[\alpha]_D$ +24° (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3420, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (d, J=6.8 Hz)/ 0.86 (d, J=6.6 Hz) (H-16/H-17), 1.18 (s, H-20), 1.30 (s)/1.33 (s) (H-18/H-19), 2.2–2.5 (m, H-5a, H-5b), 3.12 (dd, J=1.5 and 10.5 Hz, H-11), 5.40 (dd, J=8 and 16 Hz, H-2), 5.47 (d, J=16 Hz, H-3), 5.60 (d, J=15.2 Hz, H-7) and 5.80 (ddd, J=5.7, 8.2 and 15.2 Hz, H-6); MS [*m/z* (%)]: 304 (M-18, 5), 286 (10), 268 (5), 243 (7), 225 (6), 215 (4), 145 (18), 123 (23), 109 (23), 95 (33), 81 (50), 69 (24), 55 (30) and 43 (100).

Treatment of (1S,2E,4S,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (9) with acid. A solution of 13 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*9*) in 1 ml of chloroform was acidified by adding 1 ml of chloroform, which was saturated with aqueous hydrochloric acid. The reaction mixture was kept at room temperature for 5.5 h. Work-up and chromatography over silica gel yielded 7.5 mg of (1*S*,2*E*,4*S*,-6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*), which was identical (m.p., $[\alpha]_D$, IR, ^1H NMR and MS) to the naturally occurring compound.

Treatment of (1S,2E,4R,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (11) with acid. I. A solution of 4.0 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*)[6] in 1 ml of chloroform was acidified by adding 1 ml of chloroform, which was saturated with aqueous hydrochloric acid. The reaction mixture was kept at room temperature for 2.5 h. Work-up and chromatography over silica gel furnished 2.2 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*12*), which had m.p. 167–169 °C, $[\alpha]_D$ +69° (*c* 0.28, $CHCl_3$) (Found: M-18 $^+$ 304.2426. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$) bands at 3600 and 3440 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (d, J=6.7 Hz)/0.90 (d, J=6.7 Hz) (H-16/H-17), 1.03 (s, H-19), 1.30 (s)/1.43 (s) (H-18/H-20), 2.27 (dd, J=8 and −13.6 Hz, H-5a), 2.43 (dd, J=3.5 and −13.6 Hz, H-5b), 4.01 (t, J=6.5 Hz, H-11), 5.16 (dd, J=7.5 and 15.5 Hz, H-2), 5.33 (d, J=15.5 Hz, H-3), 5.47 (ddd, J=3.5, 8 and 15.7 Hz, H-6) and 5.56 (d, J=15.7 Hz, H-7); MS [*m/z* (%)]: 304 (M-18, 8), 286 (4), 261(3), 243 (5), 227 (3), 217 (4), 177 (13). 159 (42), 133 (23), 121 (33), 109 (22), 93 (43), 81 (40), 71 (38), 55 (42) and 43 (100).

II. A solution of 33.7 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,-12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*) in 8 ml of dioxane-H_2O (3:1) and 0.5 ml of aqueous H_2SO_4 (5%) was stirred at room temperature for 5.5 h. Work-up and chromatography over silica gel gave 8.8 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*12*) and 1.3 mg of (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratriene-4-ol (*16*), which has m.p. 55–56 °C and was identified by comparison of its IR, ^1H NMR and mass spectra with those of an authentic sample.[4]

Treatment of (1S,2E,4S,6R,7E,11R,12R)-11,12-epoxy-2,7-cembradiene-4,6-diol (13) with acid. A solution of 30 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*13*)[4] in 10 ml of dioxane-H_2O (2:1) and 0.5 ml of aqueous H_2SO_4 (5%) was kept at room temperature for 2.5 h. Work-up and separation by column chromatography over silica gel followed by HPLC using a column packed with μ-Bondapak/CN yielded 0.2 mg of(1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (1), whose ^1H NMR and mass spectra were identical to those of the naturally occurring compound (*1*), as well as a series of unidentified products.

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On the Mechanism of the Hydroxylation of the Thianthrene Cation Radical in Acetonitrile and Dichloromethane

OLE HAMMERICH[a] and VERNON D. PARKER[b]

[a] Department of General and Organic Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark and [b] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The rate law for the reaction between thianthrene cation radical ($Th^{\cdot+}$) and water in dichloromethane containing trifluoroacetic acid (TFA) resembles that established earlier for the reactions of $Th^{\cdot+}$ with anisole or phenol (i) and suggests the complex reaction schemes (ii)–(iv). The inhibition of the reaction by TFA is attributed to the deactivation of water. Earlier kinetic data reported for the reaction in acetonitrile are re-examined and found to be consistent with a similar mechanism.

$$\text{Rate} = k_{app}[Th^{\cdot+}]^2[H_2O]/(\text{constant} + [Th])[TFA]^2 \quad \text{(i)}$$

$$Th^{\cdot+} + H_2O \rightleftarrows Th^{\cdot+}/H_2O \quad \text{(ii)}$$

$$Th^{\cdot+}/H_2O + Th^{\cdot+} \rightleftarrows Th^{2+}/H_2O + Th \quad \text{(iii)}$$

$$Th^{2+}/H_2O \rightarrow \text{products} \quad \text{(iv)}$$

In previous publications[1,2] it has been shown that the reactions of the thianthrene cation radical ($Th^{\cdot+}$) with nucleophiles can be described by the general reaction scheme (1)–(3). The features of

$$Th^{\cdot+} + Nu \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Th^{\cdot+}/Nu \quad (1)$$

$$Th^{\cdot+}/Nu + Th^{\cdot+} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Th^{2+}/Nu + Th \quad (2)$$

$$Th^{2+}/Nu \xrightarrow{k_3} \text{products} \quad (3)$$

the kinetic data which support this mechanism are the appearance of a constant and [Th] in the denominator of the rate law. Mechanism (1) to (3) can then be described by rate law (4) which is consistent with the observed kinetics.

$$\text{Rate} = 2\,k_3K_1K_2[Th^{\cdot+}]^2[Nu]/(k_3/k_{-2} + [Th]) \quad (4)$$

A more recent kinetic study on the mechanism of the reaction of $Th^{\cdot+}$ with water in acetonitrile led to the formulation of rate law (5) and mechanism (6)–(9).[3] This mechanism has been considered

$$\text{Rate} = 2\,k_8K_6^2K_7[Th^{\cdot+}]^2[H_2O]^3/[H_3O^+] \quad (5)$$

$$Th^{\cdot+} + H_2O \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} Th(OH_2)^{\cdot+} \quad (6)$$

$$Th(OH_2)^{\cdot+} + H_2O \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} Th(OH)^{\cdot} + H_3O^+ \quad (7)$$

$$Th(OH)^{\cdot} + Th(OH_2)^{\cdot+} \xrightarrow{k_8} Th + Th(OH)^+ + H_2O \quad (8)$$

$$Th(OH)^+ + H_2O \xrightarrow{\text{fast}} ThO + H_3O^+ \quad (9)$$

to be established in a recent review article.[4] The essential difference between mechanism (6)–(9) and that observed in the presence of other nucleophiles, (1)–(3), is that the primary electrophilic reaction which involves bond formation between the thianthrene and nucleophile moieties involves the cation radical, eqn. (6), in the one case and the dication, eqn. (3) in the other. The third order dependence on $[H_2O]$ is the feature of the rate data for the reaction in acetonitrile that diverges most from all of the other kinetic data available for the reactions of $Th^{\cdot+}$ with nucleophiles. An unsavory

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feature of this mechanism is that in order for rate law (5) to hold, equilibrium (7) must be displaced strongly to the left, otherwise H_3O^+ would not significantly alter the concentration of $Th(OH)^{\cdot}$. Furthermore, in order that $[H_2O]$ influence the effective concentration of $Th(OH_2)^{\cdot +}$, equilibrium (6) must be displaced strongly to the left as well. However, the mechanism requires that $Th(OH_2)^{\cdot +}$ be the oxidant even though $Th^{\cdot +}$ will be present in much higher concentration. The uncomplexed cation radical would be expected to be a better oxidant than $Th(OH_2)^{\cdot +}$ which suggests that eqn. (8) in the mechanism will be insignificant in comparison to reaction (10). When this is taken into

$$Th(OH)^{\cdot} + Th^{\cdot +} \xrightarrow{k_{10}} Th(OH)^+ + Th \qquad (10)$$

account the rate law becomes (11). It is therefore our opinion that the mechanism proposed by Evans

$$\text{Rate} = 2\, k_{10} K_6 K_7 [Th^{\cdot +}]^2 [H_2O]^2 / [H_3O^+] \qquad (11)$$

and Blount[3] is inconsistent with the kinetic data presented and further work is required to establish the mechanism of the hydroxylation of thianthrene cation radical.

RESULTS AND DISCUSSION

Kinetics of complex ion radical reactions. For the general case of the reaction of an ion radical (A) with B, a species capable of complexing with A, the intermediate complex A/B will generally not be distinguishable from A by the kinetic measurement technique. The overall reaction, eqns. (12)–(13),

$$A + B \underset{}{\overset{K_{12}}{\rightleftharpoons}} A/B \qquad (12)$$

$$A/B \xrightarrow{k_{13}} \text{products} \qquad (13)$$

can give rise to either first order, mixed order, or second order kinetics depending upon the magnitude of K_{12}. The situation can be even more complicated if reaction (12) cannot be considered to be in equilibrium. If K_{12} is large the rate law is (14)

$$\text{Rate} = -d[A/B]/dt = k_{13}[A/B] \qquad (14)$$

and providing that B is in excess, as is usually the case, the rate is independent of the concentration of B. On the other hand, if K_{12} is small, rate law (15) is valid. The intermediate case where K_{12} is

$$\text{Rate} = -d[A]/dt = k_{13} K_{12} [A]/[B] \qquad (15)$$

approximately unity is described by eqn. (16) which takes into account the monitoring of two different

$$\text{Rate} = -d[A]/dt - d[A/B]/dt \qquad (16)$$

species giving rise to non-integral reaction orders and is difficult to use in the evaluation of kinetic data. Thus, the two useful cases are those giving either first or second order kinetics.

The analysis of Evans and Blount which gave rise to rate law (5) involved the implicit assumption that $Th^{\cdot +}$ does not participate in reaction (8) which implies that the mechanism should be of the first general case where K_{12}, K_6 in the specific case, is large. However, they did not then use the proper rate expression which should have been $-d[Th(OH_2)]^{\cdot +}/dt$ instead of $-d[Th^{\cdot +}]/dt$. The overall result then was to assign a reaction order of 3 instead of the correct value, either 2 when K_6 is small or 1 when K_6 is large, for water in mechanisms (6)–(9).

The kinetics of the reaction of thianthrene cation radical with water in dichloromethane–trifluoroacetic acid. In the presence of excess Th, water, and

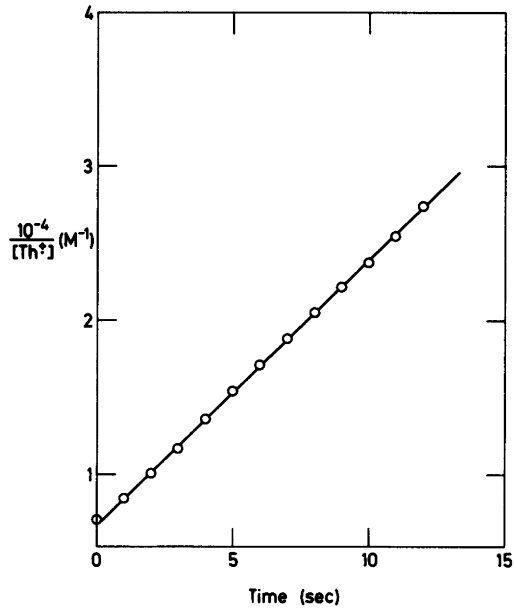

Fig. 1. Second order rate plot for the hydroxylation of thianthrene cation radical in dichloromethane containing thianthrene (0.00548 M), TFA (0.0275 M) and water (0.0484 M).

Table 1. The dependence of the apparent second order rate constant on the thianthrene concentration.[a]

Run	$[Th]_o \times 10^3$ (M)	$k_{obs} \times 10^{-4}$ (M^{-1} s^{-1})	$1/k_{obs} \times 10^4$ (M s)
1	1.01	1.38	0.72
2	2.73	0.984	1.02
3	4.99	0.523	1.91
4	7.27	0.413	2.42
5	9.92	0.282	3.55

[a] Reactions carried out in dichloromethane containing Bu_4NBF_4 (0.1 M), TFA (0.0144 M) and water (0.0484 M).

trifluoroacetic acid (TFA), the decay in $Th^{\cdot+}$ follows a second order rate law. This is demonstrated by the data summarized in Figure 1 which were measured over approximately two half-lives of $Th^{\cdot+}$. The effect of the initial thianthrene concentration $[Th]_o$ on the observed second order rate constants (k_{obs}) is illustrated by the data in Table 1. Correlation of $(k_{obs})^{-1}$ *vs.* $[Th]_o$ resulted in eqn. (17) with a correlation coefficient of 0.993 which

$$(k_{obs})^{-1} = 3.18 \times 10^{-2}[Th]_o + 2.8 \times 10^{-5} \quad (17)$$

results in eqn. (18) after inversion. The dependence

$$k_{obs} = 31.4/([Th]_o + 8.8 \times 10^{-4}) \quad (18)$$

of k_{obs} on the water concentration is shown by the data summarized in Table 2 at two different [TFA]. At both acid concentrations the calculated apparent third order rate constants were independent of $[H_2O]$ indicating a reaction order of 1.0 in water. The effect on the apparent rate constant of [TFA] is shown by the data in Table 3. The calculated rate constants in the last column assume a reaction order of -2 in TFA. The observed value, 5.18 $(\pm 0.15) \times 10^3$ M/s, indicates a good fit to this relationship.

The mechanism of the hydroxylation of thianthrene cation radical. A mechanism consistent with the kinetic data is given by eqns. (19)–(22) which result in rate law (23). Reaction (19) indicates that the

$$xH_2O + yTFA \overset{K_{19}}{\rightleftharpoons} \text{complex} \quad (19)$$

Table 2. The effect of the water concentration on the apparent second order rate constant.[a]

Run	$[Th]_o \times 10^3$ (M)	$[TFA]_o \times 10^2$ (M)	$[H_2O]_o \times 10^2$ (M)	$k_{obs} \times 10^{-3}$ (M^{-1} s^{-1})	$k_{calc} \times 10^{-3}$[b] (M^{-1} s^{-1})
13	0.98	2.75	2.06	1.79	3.94
14	0.99	2.75	4.84	5.00	3.92
11	5.31	7.99	4.84	0.191	0.137
15	5.18	7.99	7.62	0.343	0.140
16	4.98	7.99	10.40	0.493	0.145

[a] In dichloromethane containing Bu_4NBF_4 (0.1 M). [b] Corrected for variations in the thianthrene concentration.

Table 3. The effect of trifluoroacetic acid concentration on the apparent second order rate constant.[a]

Run	$[Th]_o \times 10^3$ (M)	$[TFA]_o \times 10^2$ (M)	$k_{obs} \times 10^{-4}$ (M^{-1} s^{-1})	$k_{calc} \times 10^{-4}$[b] (M^{-1} s^{-1})
3	4.99	1.44	0.523	0.532
6	5.48	2.75	0.167	0.490
7	5.21	2.75	0.108	0.512
8	5.06	5.37	0.0597	0.526
9	5.00	5.37	0.0277	0.531
10	5.04	6.68	0.0236	0.527
11	5.31	7.99	0.0191	0.504
12	5.08	7.99	0.0105	0.524

[a] In dichloromethane containing Bu_4NBF_4 (0.1 M) and water (0.0484 M). [b] Corrected for variations in the thianthrene concentration.

$$Th^{\cdot+} + H_2O \underset{k_{-20}}{\overset{k_{20}}{\rightleftharpoons}} Th^{\cdot+}/H_2O \quad (20)$$

$$Th^{\cdot+}/H_2O + Th^{\cdot+} \underset{k_{-21}}{\overset{k_{21}}{\rightleftharpoons}} Th^{2+}/H_2O + Th \quad (21)$$

$$Th^{2+}/H_2O \xrightarrow{k_{22}} ThO + 2H^+ \quad (22)$$

$$-d[Th^{\cdot+}]/dt = 2k_{22}K_{21}K_{20}[Th^{\cdot+}]^2[H_2O]/(k_{22}/k_{-21} + [Th]) \quad (23)$$

function of TFA is to deactivate the water. Since protic equilibria can be complicated with a number of species involved, we do not wish to speculate on the detailed nature of the deactivation and (19) is only meant to signify a deactivation giving the required order in TFA. Mechanisms (20) to (22) and rate law (23) are identical to those found for reactions of both anisole[1] and phenol[2] with $Th^{\cdot+}$ which were established some years ago. A noteworthy point is the implication of a reaction order of 1 for water in this reaction. Water is surely the strongest base in the system. If proton transfer occurred before the rate determining step, a reaction order of 2 is expected for water. This implies that reaction (22) involves the formation of the S–O bond followed by rapid proton transfer.

The mechanism of the reactions of cation radicals with nucleophiles is frequently referred to as the half-regeneration mechanism.[3,4,7] The half-regeneration term arises from the fact that the ion radical is an odd electron species and after reaction, regardless of the mechanism, with a nucleophile a second equivalent of the cation radical is necessary to achieve a stable oxidation state. What our kinetic studies have established[1,2,5,6] is that the cation radicals are the reactive species and that they initially form a complex with the nucleophiles in which formal bonding has not taken place. The second order in cation radical then arises because electron transfer to give the dication is necessary before irreversible bond formation occurs. For want of a better term, we have called this the "complexation mechanism".

EXPERIMENTAL

Dichloromethane was reagent grade and passed through a column of neutral alumina (Woelm W 200) immediately before use. Trifluoroacetic acid was Fluka (*Purum* grade) and used as obtained.

The cell used both for the kinetic study and for the preparation of the cation radical solutions was a cylindrical, round-bottom, jacketed container with openings for the auxiliary electrode compartment, reference electrode, inert gas supply, thermometer, and a Beckman rotating disk electrode. The temperature was controlled with tap water at 13.0 ± 0.5 °C. The cation radical solutions were prepared by partial oxidation of solutions of thianthrene in the solvent systems. Oxidations were carried out at constant current (12.5 mA) at a large area platinum gauze electrode. Current was passed for a time calculated to give the desired concentration of cation radical. The concentration of the cation radical was determined exactly after oxidation by the magnitude of the limiting current at the rotating disk electrode. The limiting current was followed as a function of time for several minutes, and no significant decrease in cation radical concentration was observed before adding water. The procedure used for carrying out the kinetic runs has previously been described.[1,2,8]

The kinetic experiments were carried out during the period 1972–1973 and the data were abstracted from the doctoral thesis of O.H.

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Ion Radical Cleavage Reactions. III. The Cleavage of Halide Ion from Mono- and Dihalobenzophenone Anion Radicals

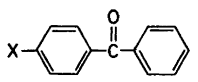
BJØRG AALSTAD and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

Benzophenone anion radicals substituted in the 4- or 4,4'-positions with either chlorine or bromine undergo cleavage in acetonitrile or *N,N*-dimethylformamide to generate the corresponding aryl radicals. Cleavage of the monochloro-substituted anion radical is 6–7 times faster than that of the corresponding disubstituted anion radical and bromo-substituted anion radicals cleave about 10^3 times faster than the corresponding chloro derivatives. A very small solvent effect was found with the reactions being somewhat slower in acetonitrile. Arrhenius activation energies are very similar for mono- and disubstituted anion radicals but about 8 kcal/mol greater for chloro substitution than for the corresponding bromo-substituted anion radicals. Entropies of activation were observed to be close to zero for the chloro-substituted anion radicals but of the order of −(12 −20) cal/K mol for the bromo derivatives. The more negative values of $\Delta S^{\ddagger}_{298}$ in the latter case were interpreted to indicate that bond cleavage is much further advanced in the transition state in the case of bromo-substituted anion radicals.

In other papers in this series, the cleavage reactions of halo-substituted anthracene [1] and nitrobenzene [2] anion radicals have been examined. The two reaction series were observed to be similar in that the bromo derivatives were in both cases $>10^3$ times as reactive as the corresponding chloro-substituted anion radicals. However, the two differed sharply in the role of the entropy of activation in determining the reaction rates. In the case of the nitrobenzene anion radicals, $\Delta S^{\ddagger}_{298}$ was small and positive regardless of the nature or the location of the halogen substituent. In contrast $\Delta S^{\ddagger}_{298}$ for the cleavage of 9-bromo- or 9,10-dibromoanthracene anion radicals was of the order of 21 cal/K mol more negative than for the corresponding chloro-substituted anion radicals. The large negative entropies of activation for the bromoanthracene anion radicals was taken to indicate a high degree of bond cleavage in the transition state. Thus, the position of the transition state in that series of reactants is very much dependent upon the nature of the halogen substituent. On the other hand, the near independence of $\Delta S^{\ddagger}_{298}$ for the reactions of nitrobenzene anion radicals on the substituent must indicate that the position of the transition state along the reaction coordinate does not vary significantly with the nature of the substituent. The differences in the two reaction series can then be a reflection of the differences in charge distributions in the anion radicals. The nitrobenzene anion radicals have highly polarized structures with most of the charge concentrated on the heteroatoms while the anthracene anion radicals apparently have a more dispersed charge distribution. However, it is still not clear why the structures of the transition states for the cleavage reactions in the latter series are so strongly dependent upon the leaving group.

The 4- and 4,4'-substituted benzophenones, structures *1* and *2*, would be expected to form anion radicals with charge distributions intermediate between those of the highly polarized nitrobenzenes and the more dispersed charge encountered in the anthracenes. Another interesting feature of the structures of these anion radicals as compared to the



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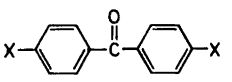

2

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anthracenes is that in this case the disubstituted anion radicals have substituents in two different rings which are not in complete conjugation because of interactions between the 2- and 2'-hydrogen atoms which cause the two rings to lie in different planes.

The cleavage reactions of mono-halobenzophenones anion radicals have been studied intensively by Savéant and co-workers.[3-5] The reaction pathway has been convincingly demonstrated to be a simple unimolecular cleavage reaction to yield the reactive aryl radical as in eqn. (1). The aryl radical can then undergo further electron transfer (2) or abstract hydrogen atoms from the solvent (3). The conditions under which the two competitive reactions (2) and (3) operate have been analyzed.[6] Since the cleavage reaction (1) is rate-determining, the kinetics and activation parameters are expected to be a direct reflection of this microscopic step as in the case of the haloanthracene anion radicals.

1 *2*

$$Ar-X^{\cdot -} \xrightarrow{k_1} Ar^{\cdot} + X^{-} \quad (1)$$

$$Ar^{\cdot} + ArX^{\cdot -} \rightarrow Ar^{-} + ArX \quad (2)$$

$$Ar^{\cdot} + S-H \rightarrow ArH + S^{\cdot} \quad (3)$$

With regard to our interest in these reactions, *i.e.* the activation parameters and the nature of the transition states, some very interesting observations were made in the previous studies. The reactions were reported to be from 30 to 80 times faster in *N,N*-dimethylformamide (DMF) than in acetonitrile (AN).[3] Although activation parameters were not determined, the rate constants at -10 and $+20$ °C correspond to an energy of activation for both 3-bromo- and 4-bromobenzophenone anion radicals in DMF equal to about 21 kcal/mol, an unusually high value for the rapid reactions and requires $\Delta S^{\ddagger}_{298}$ of from 20 to 31 cal/K mol. On the other hand, data for measurements in liquid ammonia[5] for the cleavage of 4-bromobenzophenone anion radical indicate that E_a in that solvent is about 13 kcal/mol and $\Delta S^{\ddagger}_{298}$ is 2 cal/K mol. It should be pointed out that the rate constants of previous studies[3,5] were measured by ordinary cyclic voltammetry and large error limits were given. This, of course, is a good reason for not reporting activation parameters and the values quoted here must be considered with that in mind. However, the data did serve to stimulate our curiousity and a reinvestigation appeared necessary.

We chose to investigate mono- and disubstituted anion radicals of *1* and *2* for two reasons. As in the case of the anthracene anion radical cleavage reactions, we anticipated that the dibromo derivative would be less reactive than 4-bromobenzophenone anion radical and thus make the rate data more reliable. Also, the effect of the second halo substituent on the activation parameters was expected to shed some light on the nature of the transition states for the cleavage reactions. There have been no previous reports of data for the cleavage reactions of the anion radicals of *2*.

EXPERIMENTAL

The monohalobenzophenones were reagent grade and were recrystallized before use. The dihalobenzophenones were generously provided by Dr. Torkil Holm.

Solvents containing electrolyte, Bu_4NBF_4 (0.1 M), were passed through a column of neutral alumina (Woelm W 200, neutral, super grade 1) before adding the substrate and carrying out kinetic studies.

The temperature was controlled either by a Haake cryostat (<0 °C) or by suitable water baths maintained at constant temperature over the relatively short measurement times by virtue of the large heat capacity of the baths.

The cells, reference electrodes, working electrodes, and the data retrieval system have been described in recent papers from this laboratory.[7,8]

RESULTS

Kinetic Measurements. The kinetics of the cleavage reactions were studied by derivative cyclic voltammetry[9] using procedures recently described.[10] Difficulty was encountered in evaluating rate constants for the bromo-substituted anion radicals because the oxidation peak due to the anion radical of the dehalogenated product overlapped with that for the primary intermediate. This evidently was also the source of much of the error in rate constants previously reported for 4-bromobenzophenone anion radical cleavage. We found that the problem could be minimized by evaluating rate constants after little reaction had

taken place. The procedure used was to determine $v_{3/4}$, *i.e.* the voltage sweep rate necessary for the derivative peak ratio, R_i', to equal 0.750. Rate constants could then be evaluated from eqn. (4)

$$k = 515.6\ v_{3/4}/T \quad (4)$$

determined from theoretical data. Rate constants evaluated from $v_{3/4}$ were found to be within experimental error of those calculated from eqn. (5)

$$k = 1444\ v_{1/2}/T \quad (5)$$

using $v_{1/2}$, as is usually done,[10] when the substrate was 4,4'-dichlorobenzophenone. A disadvantage of this procedure for rapid reactions is that the voltage sweep rate required to evaluate the rate constant is almost 3 times greater when (4) is used than when $v_{1/2}$ is determined. On the other hand, the data are very much more reliable when the effect of the interfering reactions is minimized.

Error analysis. In general, the values of R_i' used in the evaluation of rate constants were based on sufficient data so that the mean values were 0.750 $\pm$0.002 which corresponds to about a 1 % error in k. Thus the error in the measurement of R_i' is nearly negligible. In order to get an estimate of the error from all sources we can consider the data in Table 1. The maximum deviation of rate constants determined during the reduction of 4,4'-dichlorobenzophenone for duplicate runs was observed to be about 2 % at 0.50 mM and less than 1 % at the higher concentrations. However, rate constants evaluated at a substrate concentration of 0.50 mM were about 10 % lower than those obtained at 2.00 mM. The reason for this deviation does not appear to be measurement precision but rather some other complicating factors. The measurements involving the chloro-substituted derivatives are more reliable than those on the bromo derivatives because of the lower temperatures and higher sweep rates required for the latter. All comparisons of rate constants were made at the same substrate concentration, 2.00 mM. Under these conditions k is believed to be reproducible to $\pm$1 % for the chloro-substituted anion radicals and about $\pm$10 % for the corresponding bromo derivatives.

Reaction order analysis. The criterion for a first order reaction of the anion radical generated during cyclic voltammetric reduction of the substrate is that the voltage sweep rate necessary for a particular value of the derivative peak ratio should be independent of the substrate concentration.[10] The data in Table 1 are for the reduction of 4,4'-dichlorobenzophenone in DMF for the case where $v_{1/2}$ was measured as a function of substrate concentration. The value of $v_{1/2}$ increased by about 12 % in going from 0.50 mM to 2.00 mM. As pointed out in the previous paragraph, the variation observed is clearly larger than experimental error. The data suggest that either there is a minor competing second order reaction or that oxidation of products is beginning to interfere at the higher concentrations. In any case the complication is not very severe.

Table 1. Reaction order analysis of the cleavage of 4,4'-dichlorobenzophenone anion radical in DMF.[a]

C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	k/s^{-1}
0.50	1.107	5.45
0.50	1.078	5.30
1.00	1.128	5.55
1.00	1.138	5.60
2.00	1.232	6.06
2.00	1.231	6.06

[a] Measurements by derivative cyclic voltammetry in solvent containing Bu_4NBF_4 (0.1 M) at a mercury electrode at 22 °C. The duplicate runs at each substrate concentration were on separately prepared solutions. The difference in switching and reversible potentials, $E_{sw} - E_{rev}$ was 200 mV in all cases.

Table 2. Kinetic data for the cleavage of halobenzophenone anion radicals in DMF.[a]

Substituents	T/K	$v_{\frac{1}{2}}$[b]/V s^{-1}	k/s^{-1}
4-Chloro	296.2	8.67	42.3
4-Chloro	304.4	19.0	90.1
4-Chloro	314.2	42.2	194
4,4'-Dichloro	295.5	1.20	5.84
4,4'-Dichloro	304.4	2.80	13.3
4,4'-Dichloro	312.4	5.71	26.4
4,4'-Dichloro	319.6	8.70	39.3
4,4'-Dibromo	234.8	220[c]	483
4,4'-Dibromo	229.9	160[c]	359
4,4'-Dibromo	224.9	110[c]	252

[a] Conditions as in Table 1. [b] The voltage sweep rate necessary for $R_i' = 0.500$. [c] The voltage sweep rate necessary for $R_i' = 0.750$, $v_{3/4}$, was used in these analyses due to the interference of further reaction products at higher conversions.

Table 3. Kinetic data for the cleavage of halo-benzophenone anion radicals in acetonitrile.[a]

Substituents	T/K	$v_{3/4}$[b]/V s^{-1}	k/s^{-1}
4-Chloro	294.9	14.2	24.8
4-Chloro	304.0	31.0	52.5
4-Chloro	311.0	69.7	115.5
4-Chloro	321.9	151.9	243.3
4,4'-Dichloro	293.5	2.20	3.87
4,4'-Dichloro	304.9	6.32	10.7
4,4'-Dichloro	313.7	14.0	23.0
4,4'-Dichloro	320.3	25.6	41.2
4-Bromo	225.0	109	250
4-Bromo	229.3	180	405
4-Bromo	233.3	235	520
4-Bromo	243.8	490	1037
4,4'-Dibromo	233.5	72.0	159
4,4'-Dibromo	238.4	109	236
4,4'-Dibromo	243.4	163	346

[a] Conditions as in Table 1. [b] Defined in Table 2.

Rate constants for the cleavage reactions. Kinetic data for the cleavage reactions in DMF and AN are summarized in Tables 2 and 3, respectively. Rate data are not reported for 4-bromobenzophenone anion radical in DMF since $v_{3/4}$ of the order of 500 V s^{-1} or more were necessary at the low temperatures and the data did not appear to be of sufficiently high quality for the determination of activation parameters. In DMF, $v_{1/2}$ was evaluated for the chloro-substituted anion radicals and $v_{3/4}$ was used for the analysis of the data for the cleavage of 4,4'-dibromobenzophenone anion radical. In AN, $v_{3/4}$ was the basis for all of the rate constant determinations.

Activation parameters for the cleavage reactions The energy of activation for the cleavage of the chloro-substituted anion radicals was very nearly the same in all cases, independent of the solvent. The mean of the four values, 16.2 ± 0.4, suggests that the value for the 4-chloro derivative in DMF is on the low side. If the mean E_a is used in the calculation of $\Delta S^{\ddagger}_{298}$ in that case the value goes from -0.4 to $+1.7$ cal/K mol. However, this is probably extending the expected precision too far. The error in E_a is estimated to be of the order of ± 0.5 kcal/mol and that for $\Delta S^{\ddagger}_{298}$ is then of the order of ± 1 cal/K mol for the reactions involving cleavage of chloride ion. Thus, $\Delta S^{\ddagger}_{298}$ appears to be from 1 to 3 cal/K mol more negative for the dichloro-substituted anion radical than for the monochloro derivative.

On the other hand, the data for the cleavage reactions of the bromo-substituted anion radicals are clearly of lower quality. In this case E_a was observed to be equal to 8.0 ± 1.0 kcal/mol. If this value is used in the calculation of the entropy of activation in all three cases, values of -13.8 for 4-bromobenzophenone anion radical and -15.2 or -15.1 cal/K mol are found for 4,4'-dibromobenzophenone anion radical. This suggests that the E_a values measured in this case are within experimental error of being the same and that the $\Delta S^{\ddagger}_{298}$ is more negative for bromo-substituted *2* than for *1* anion radicals. Furthermore, it appears that the cleavage of bromide ion is accompanied by an entropy of activation of the order of 14 cal/K mol more negative than the corresponding cleavage of chloride ion.

DISCUSSION

Contrary to expectations based on previous work,[3,5] we find a very small solvent effect on the cleavage reactions. An increase of less than 50 % in

Table 4. Activation parameters for the cleavage of halo-benzophenone anion radicals.[a]

Substituents	Solvent	E_a/kcal/mol	k_{298}/s^{-1}	$\Delta S^{\ddagger}_{298}$[b]	Correlation coefficient
4-Chloro	AN	16.3	33.5	1.0	−0.9968
4-Chloro	DMF	15.6	51.2	−0.4	−0.9996
4,4'-Dichloro	AN	16.4	5.94	−2.0	−0.9998
4,4'-Dichloro	DMF	16.3	7.53	−1.7	−1.0000
4-Bromo	AN	8.0	21700	−13.8	−0.9942
4,4'-Dibromo	AN	9.0	10900	−11.7	−1.0000
4,4'-Dibromo	DMF	6.9	11300	−18.9	−0.9995

[a] Arrhenius correlation of data from Tables 2 and 3. [b] In cal/K mol.

the rate constants for the cleavage of chloride ion was observed in going from DMF to AN. In fact, if we compare the rate constant we obtain at −40 °C for the cleavage of bromide from 4-bromobenzophenone anion radical in AN with that reported for the same reaction in liquid ammonia,[5] we find that the values, 520 and 590 s^{-1} are surely within experimental error of being the same. We conclude that the large differences observed earlier were most likely due to the fact that the products of the reaction interfere severely with analysis. The interference is especially severe when peak current ratios are evaluated after considerable reaction has occurred. This is normally the way cyclic voltammetric data are evaluated and in this case the analysis must be very uncertain.

The activation energies that we find for the cleavage of bromide ion from benzophenone anion radicals, 8.0±1.0 kcal/mol, are also much lower than those predicted from previous work in any of the solvents. This again, can be attributed to precision problems.

We do find that the activation parameters for the cleavage of halobenzophenone anion radicals are intermediate between those found for the anthracene series and the nitrobenzene series. However, the trends in the data resemble those found for the haloanthracene anion radicals. For the anthracene series, E_a was about 11 kcal/mol lower for the cleavage of bromide ion, ΔS^*_{298} was about 21 cal/K mol more negative and cleavage from dihalo derivatives was accompanied by about 4 cal/K mol more negative ΔS^*_{298}. The corresponding differences appear to be 8 kcal/mol, −14 cal/K mol and −2 cal/K mol, respectively, for the cleavage of halide ion from benzophenone anion radicals.

Because of the very close similarity in the reactions considered here to those of the haloantracene anion radicals,[1] the data reported serve to reinforce the conclusions drawn from the previous study. The standard entropy of formation of ion radicals of aromatic compounds has been observed to be small in acetonitrile, usually less than 10 cal/K mol, and negative in the case of anion radicals.[11] Values of −46.7 and −43.3 cal/K mol have been given for chloride and bromide, respectively, in DMF.[12] Thus, the entropy of reaction for the cleavage of halide ion from a haloaromatic anion radical would be expected to be of the order of −40 cal/K mol in DMF and similar in AN. If cleavage of the carbon–halogen bond is essentially complete in the transition state the entropy of activation is expected to be large and negative approaching about −40 cal/K mol in the limit. On the other hand, the observation of a positive or small entropy of activation implies that bond cleavage is not very far advanced in the transition state. Thus, the entropy of activation serves as a good measure of the position of the transition state along the reaction coordinate in these reactions.

As in the case of the cleavage of haloanthracene anion radicals, a nearly zero entropy of activation is observed for the cleavage of the chlorobenzophenone anion radicals in both DMF and AN. This implies that there is not very much reorganization of the solvent in the transition state as compared to the anion radical. The logical interpretation then is that an early transition state is involved in this case. On the other hand, activation entropies ranging from −12 to −19 were found for the corresponding reactions where bromide is the leaving group. The larger negative entropies of activation in this case imply that there is considerable solvent reorganization upon going to the transition state. The latter means that bond cleavage is rather far advanced.

We conclude that halobenzophenone anion radicals resemble those of the haloanthracenes quite closely with respect to cleavage reactions. The reason for the apparently large differences in the structures of the transition states for chloride and bromide cleavage is still not apparent. It is hoped that further work in related systems will shed light on this perplexing question.

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Studies on Electrolytic Substitution Reactions. XX.* The Anodic Acetoxylation of Fluorene – A Mechanistic Study

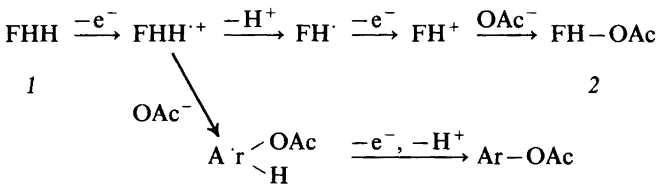
LENNART EBERSON** and ANDREW WEBBER***

Division of Organic Chemistry 3, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund 7, Sweden

A re-investigation of the very ineffective anodic acetoxylation of fluorene, using 9,9-dideuteriofluorene or non-deuterated fluorene in acetic acid or perdeuterioacetic acid, respectively, disproves a previously proposed cyclic mechanism. It is found that anode passivation occurs in tetrabutyl tetrafluoroborate–acetic acid solutions. In acetate-containing media nuclear and side-chain substitution do occur but do not account for all of the reacted fluorene. Thus, it is concluded that another, as yet unidentified, oxidation process competes with acetoxylation. The nature of this reaction is briefly discussed.

The anodic acetoxylation of fluorene (FHH, *1*) is expected to yield nuclear, as opposed to side-chain, substituted acetates due to the anti-aromatic nature of the cation intermediate FH^+ shown in Scheme 1.[1] In media where acetate ion is not present, nuclear substitution is known to be inhibited[2] as the cation radical, $FHH^{\cdot+}$, loses a proton instead of undergoing attack by the nucleophilic acetate ion. In the case of fluorene and other aromatics possessing a side-chain, the proton loss is expected to occur at the benzylic site to give ultimately, in this particular case, 9-acetoxyfluorene (*2*). An earlier paper in this series[1] showed that these expectations are borne out to some degree. In 0.1 M Bu_4NBF_4 – acetic acid only 9-acetoxyfluorene is formed (0.5 % current yield), whereas both nuclear and side-chain acetates are produced when fluorene is oxidized in 1 M NaOAc–acetic acid. In the latter case the ratio of nuclear to side-chain substitution is indeed high as compared to "normal" alkylbenzenes. However, the total current yield is only 15 % for all of the acetates combined. To explain this the mechanism depicted in Scheme 2 was proposed, whereby the fluorenyl radical, $FH^{\cdot}$, abstracts a hydrogen atom from the medium to regenerate fluorene. The anti-aromatic nature of the fluorenyl cation, FH^+, was thought to be responsible for favouring hydrogen atom abstraction over further oxidation of the radical.

We decided to test this mechanism by investigating the anodic oxidation of 9,9-dideuteriofluorene (FDD) in acetic acid and of fluorene



Scheme 1.

*Part XIX See Blum, Z. and Nyberg, K. *Acta Chem. Scand. B 35* (1981) 743.

**Author to whom correspondence should be addressed.

***Present address: Chemistry Department, State University of New York at Buffalo, Buffalo, N.Y. 14214, U.S.A.

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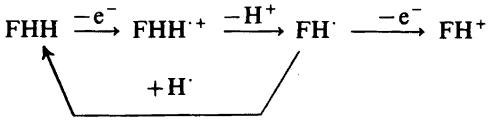


Scheme 2.

(FHH) in perdeuterioacetic acid, using sodium acetate (perdeuterioacetate) or tetrabutylammonium tetrafluoroborate as supporting electrolyte. The mechanism predicts that the substrate should undergo hydrogen–deuterium exchange at the position of proton loss from the radical cation $FHH^{\cdot+}$, *i.e.*, the benzylic 9-position.

RESULTS AND DISCUSSION

Oxidation of fluorene in 1 M sodium acetate–acetic acid. Cyclic voltammetry at a platinum bead electrode gave an irreversible wave with a peak potential of +1.70 V *vs.* s.c.e.. Comparison of the peak height with that of ferrocene suggested that the process was a two-electron oxidation. However, caution should be exercised when comparing such dissimilar electrode processes. Further electroanalytical investigation in this medium was not attempted and preparative scale electrolyses with subsequent product determination were used in this study.

FDD was oxidized at both constant potential (+1.7 V *vs.* s.c.e.) and constant current (5 mA cm^{-2}). Table 1 gives some typical examples of the product distribution. Concentrations from 7 to 200 mM were employed. The product distribution appears to be independent of concentration, current density and whether constant current or controlled potential conditions were used. The mixture was worked-up and analyzed by GLC/MS and ^1H NMR spectroscopy. Note that when the internal standard was added directly to the crude electrolyte, GLC analysis gave yields identical to those determined by addition of the standard to the ethereal extract of the electrolyte. Thus, it is ascertained that none of the products listed in Table 1 are lost in the work-up procedure.

It was thought possible that dimeric products could be formed from either the relatively stable fluorenyl radicals or the cation radicals. Dimerization of fluorenyl radicals has been reported,[3] albeit in non-nucleophilic media. However, the medium used here is too nucleophilic to allow formation of 9,9′-bifluorenyl or other dimeric products. Oxidation of fluorene to fluorenone was also considered a possibility and small amounts of fluorenone were indeed found in the product mixture. The major products detected were nuclear and, to a lesser extent, side-chain acetates, the identities of which were confirmed by ^1H NMR, comparison of their retention times with authentic samples and GLC/MS (the latter of which distinguishes between nuclear and side-chain acetates when the substrate is FDD but not FHH). The exact identity of the isomer(s) "1,3,4-acetoxyfluorene" is not known. The ratio of nuclear to side-chain acetoxylation is high, as previously reported.[1]

We also note, as before,[1] that the total current yield of identified products is low, around 20 %,

Table 1. Typical product distributions from the anodic oxidation of fluorene[a] (FHH or FDD).

Substrate (conc./mM)	Electrolyte	Charge passed/F mol^{-1}	Recovered fluorene/%	Yield/%[h] F=O	9-OAc	2-OAc	1,3,4-OAc	Material not accounted for/%
FDD (200)[b]	CH_3COOH/CH_3COONa	2.0	69[f]	≈0.1	0.2	16	1.9	12.8
FDD (94)[c]	CH_3COOH/CH_3COONa	2.0	64[g]	0.6	0.2	17	2.0	16.2
FHH (8.2)[c]	CD_3COOD/CD_3COONa	1.0	73[g]	6.0	0.5	5.9	0.6	14.0
FHH (7.0)[c,e]	CD_3COOD/CD_3COONa	1.0	73[g]	6.8	1.6	3.4	–	15.2
FDD (38)[d]	CH_3COOD/Bu_4NBF_4	2.0	77[g]	–	–	–	–	23
FHH (36)[d]	CD_3COOD/Bu_4NBF_4	2.0	67[f]	4.8	0.9	2.9	–	24.4

[a] Undivided cell, Pt electrodes (1 cm^2 each). [b] Constant current electrolysis (c.c.e.) at 5 mA cm^{-2}. [c] C.p.e. at 1.7 V *vs.* s.c.e. [d] C.c.e. at 0.5 mA cm^{-2}. [e] Divided cell. [f] Yield determined by analysis of the ethereal extract of the electrolyte. [g] Yield determined by analysis of the crude electrolyte. [h] F=O is fluorenone, 9-OAc is 9-acetoxyfluorene, *etc.* Yields are based on the amount of starting fluorene.

which now at least partly can be ascribed to the fact that *ca.* 15 % of the starting material cannot be accounted for in the analysis. Isotope analysis of the recovered fluorene (GLC/MS and ^{1}H NMR) showed that no H – D exchange had occurred, even in experiments in which charges up to 8 F mol^{-1} has been passed through the electrolyte. Thus the cyclic mechanism proposed earlier[1] (Scheme 2) is refuted. This mechanism in principle can only account for low current yields (*i.e.* inefficient oxidation) but of course not for missing material. Oxidation of FDD in a divided cell gave results virtually identical to those discussed above where an undivided cell was used. Thus the possibility of some cathodic mechanism being responsible for the lost material is excluded.

Nondeuterated fluorene, FHH, was also oxidized in 1 M $NaOOCD_3 - CD_3COOD$ (Table 1). Product analysis again confirmed that no H – D exchange had occurred and the yields of the products were similar to those from the oxidation of FDD in nondeuterated medium. Due to the weaker C – H bond at the side-chain position, it was expected that higher yields of fluorenone and 9-acetoxyfluorene would be found and this is in fact observed (see Table 1).

Oxidation of fluorene in 0.1 M Bu_4NBF_4-acetic acid. FDD was oxidized at both constant current (current densities from 0.3 to 10 mA cm^{-2}) and controlled potential (initially +1.6 V, then +1.8 V *vs.* s.c.e.) until 2 F mol^{-1} had been passed. In every case the anode became coated with a black film. In the controlled potential experiments this resulted in a rapid initial decrease in current and continual switching of the potentials of the counter and working electrodes only partly alleviated this problem. Removal and cleaning of the anode temporarily restored the current to its original value. Concentrations of FDD ranging from 20 to 170 mM were used. In every case GLC/MS and ^{1}H NMR examination of the electrolysis product showed that the isotopic composition of the fluorene was unchanged, no FDH or FHH being present. The ^{1}H NMR analysis gave a "clean" spectrum of FDD with no other signals present. No acetates, fluorenone, bifluorenyl or bifluorenylidene could be detected by GLC. However, only about 75 % of the substrate, FDD, was recovered at the end of the electrolysis. Control experiments in the absence of FDD showed that the blackening and associated anode passivation only occurred when FDD was present. We believe that this film may be either the results of polymerization of a fluorene radical species ($FDD^{\cdot+}$ or $FD^{\cdot}$) or of attack of such a species on the substrate and/or supporting electrolyte and that the formation of the film probably accounts for the missing material.

Oxidation of FHH at constant current in 0.1 M $Bu_4NBF_4 - CD_3COOD$ gave similar results (*i.e.*, no H – D exchange). However, fluorenone, 9-acetoxyfluorene and 2-acetoxyfluorene were formed in low yields (Table 1). That neither fluorenone nor 9-acetoxyfluorene were detected when FDD was oxidized can presumably be attributed to the stronger C – D bond at the 9-position.

Further evidence of electrode passivation was supplied by cyclic voltammetry of fluorene (4.5×10^{-3} M) in 0.1 M Bu_4NBF_4 – acetic acid at a platinum bead electrode. An irreversible peak with a peak potential of +1.61 V *vs.* s.c.e. was observed. Repetitive cycling of the electrode potential resulted in a markedly greater than normal drop in peak current, shifting of the peak to less anodic potentials and a crossing-over of the anodic and cathodic branches at +2.0 V *vs.* s.c.e.. Furthermore, subsequent stirring of the solution and resweeping did not fully restore the peak current to the original value. Removal and cleaning of the electrode did however accomplish this. No such effects were observed in sodium acetate – acetic acid solutions. The peak height was virtually the same as that for the same concentration of fluorene in 1 M NaOAc – acetic acid.

From the above-mentioned results we conclude that the cyclic mechanism proposed earlier[1] (Scheme 2) to account for the low current yields of acetates is incorrect. One can support this conclusion by an estimate of the energy of activation for hydrogen abstraction by $FH^{\cdot}$ on a C – H bond of acetic acid. Using the benzyl radical as a model for $FH^{\cdot}$ (necessitated by the lack of data for the $FH^{\cdot}$ system) the bond energy – bond order (BEBO)[4] and equibonding[5] method both give $E_a = ca.$ 20 kcal mol^{-1} for attack on $H - CH_2COOH$.[6] To compare, the much more reactive methyl radical has an experimental E_a of 10.2 kcal mol^{-1} for the same process,[7] and it is known that anodically generated methyl radical just barely can effect hydrogen abstraction from $H - CH_2COOH$.[8]

In Bu_4NBF_4-acetic acid media electrode passivation occurs and the oxidation is inhibited. It is probable that the film formation accounts for part or all of the lost fluorene in this medium. In NaOAc-acetic solutions nuclear acetoxylation is favoured

over side-chain acetoxylation.[2] However, this process is in competition with another, as yet unidentified, process. Our results suggest that the product(s) of this/these other reaction(s) are water-soluble, non-volatile or thermally unstable (since no unidentified peaks were observed in the GLC analysis). The formation of carboxylic acids or aldehydes is considered a possibility, although no direct evidence for their formation is available at present.

MATERIALS AND METHODS

Calculations. A computer program was written to calculate the final isotopic distribution of FHH, FDH and FDD after passage of a known amount of charge, based on the mechanism proposed in Scheme 2. Two cases were considered; oxidation of FHH in deuterated medium and oxidation of FDD in non-deuterated medium. Scheme 3 shows the system for the oxidation of FDD in non-deuterated medium. The program is based upon the following assumptions: The oxidation has a current efficiency of 100 %. The probability of electron transfer to each fluorene species is solely dependent on their relative concentrations. Abstraction of a deuterium atom from non-deuterated medium and of a hydrogen atom from deuterated medium is negligible. The relative probability of loss of D^+, as opposed to loss of H^+, from the radical cation $FDH^{\cdot+}$ is estimated[9] to be 0.2. Note that each step in Scheme 3 involves oxidation to a radical cation, loss of a proton (or deuterium ion) and subsequent abstraction of a hydrogen atom. Thus, passage of 2 F mol^{-1} through a solution of FDD in non-deuterated medium is predicted to give a product ratio for FDD:FDH:FHH of 14:67:20, whereas the same ratio for oxidation of FHH in deuterated medium is 53:33:14. Using values for the probability of loss of D^+ as opposed to H^+ of 0.15 or 0.25 gave results that differed only slightly from those above. Even if the process were only, *e.g.*, 20 % efficient, any significant H−D exchange would easily have been detected.

Calculations according to the BEBO and equibonding method were performed as described earlier.[6]

EXPERIMENTAL

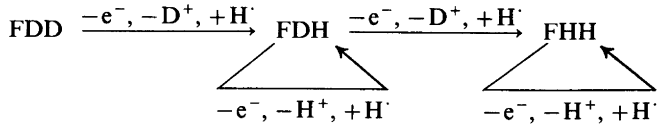
Equipment and materials. The cyclic voltammetry equipment has been described previously.[10] Controlled potential and constant current electrolyses were carried out using an AMEL 552 potentiostat/-galvanostat in conjunction with a coulometer built in this Department. GLC analyses were performed using either a Hewlett Packard model 3380A or 5830A gas chromatograph.

Fluorene (B.D.H., England) was recrystallized from methanol prior to use. Fluorene-9,9-d_2 and fluorene-9-*d* were prepared according to published procedures,[11,12] the latter being shown (MS, ^{1}H NMR) to contain 69.5 % FDH and 30.5 % FDD.

GLC examination of all three fluorene samples showed that they contained less than 0.1 % fluorenone or acetoxyfluorenes. All compounds gave satisfactory mass and ^{1}H NMR spectra. The relative abundancies of the peaks at *m/e* values M−2, M−1, M+1 and M+2 were found to be independent of whether the direct or GLC inlet was employed. A simple computer program was written to calculate the distribution of FHH, FDH and FDD from the relative abundancies of the peaks at *m/e* 166, 167 and 168. ^{1}H NMR spectroscopy was also used and gave results in agreement with the MS-method. However, analysis of a known mixture of FDD and FHH indicated that the MS method was more accurate (error $< \pm 1$ %). It was established that FDD did not undergo any H−D exchange when stirred at room temperature in 0.5 KOAc−acetic acid for 48 h. The limits of detection of the various products by GLC was typically 0.1 %.

Bifluorenyl, bifluorenylidene, 2- and 9-acetoxyfluorenes were synthesized according to known methods.[13-16] Fluorenone (Fluka, Buchs, Switzerland) was used as received. Pentamethylbenzyl acetate, used as standard for GLC analysis, was prepared by the anodic oxidation of hexamethylbenzene.[17]

Electrolyses. Oxidations were carried out in a small (*ca.* 5 cm^3 electrolyte) undivided glass cell



Scheme 3.

with platinum electrodes (1 cm^2) at room temperature. At the end of the electrolysis the electrolyte was slowly pipetted into a separating funnel containing saturated sodium bicarbonate solution and diethyl ether. The aqueous phase was extracted with two more portions of ether and the combined ethereal extracts were then washed with saturated sodium bicarbonate solution and water (twice). Drying (anhydrous sodium sulfate) and concentration gave a solution suitable for analysis by GLC (2 m × 0.3 mm 5 % neopentylglycol succinate on Chromosorb W at 210 °C with pentamethylbenzyl acetate as an internal standard). Isotopic analyses were carried out by GLC/MS. Subsequently the solvent was removed and the residue examined by both ^1H NMR and MS. The yields of fluorene and the acetoxyfluorenes, as determined by ^1H NMR were in agreement with those found by GLC. Note that the GLC/MS method was capable of distinguishing between nuclear and side-chain acetates when the substrate was the dideuterio form, FDD. The identities of all peaks observed on analysis by GLC were confirmed by GLC/MS and comparison of their retention times with authentic samples. The absence (less than 0.1 %) of bifluorenyl and bifluorenylidene was confirmed by GLC (3 % OV1 on Chromosorb W at 270 °C). The efficiency of the extraction procedure was determined by extraction of three samples of fluorene in 0.1 M Bu_4NBF_4 – acetic acid. GLC analysis gave an average "yield" of 102 %. In addition analysis of various electrolysis products before and after extraction gave virtually identical results.

A few experiments were carried out in a divided cell with a platinum foil anode (1 cm^2) and a working compartment containing 15 cm^3 of solution. The work-up and analysis procedure was identical to that described above. Analysis of the catholyte showed that virtually no diffusion of the substrate into the cathode compartment had occurred.

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Short Communications

The Mechanism of the Decomposition of Chlorpromazine Cation Radical in Aqueous Buffers

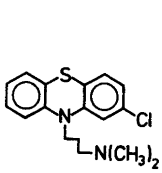
OLE HAMMERICH [a] and VERNON D. PARKER [b]

[a] Department of General and Organic Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark and [b] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

A very complex mechanism with poorly defined intermediates has recently been proposed for the decomposition of chlorpromazine cation radical $1^{\cdot+}$ in aqueous buffer solution.[1] The product of the reaction in acidic buffers was observed to be the oxide *2*. The feature of the postulated mechanism which we find to be unacceptable is reaction (1) which is formally a reaction of a cation radical with

$$1^{\cdot+} + RCO_2^- + H_2O \rightleftarrows 3^{\cdot-} + H^+ \quad (1)$$

a carboxylate ion and hydroxide ion to give an anion radical. The mechanism proposed, in contrast to that from a similar study on the hydroxylation of thianthrene cation radical,[2] is consistent with the observed rate law. We have attempted to formulate



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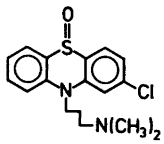

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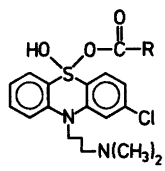

3

reasonable structures of anion radical $3^{\cdot-}$ without success. The purpose of this note is to point out that a reasonably simple mechanism, not requiring any unusual structural features fits the observed kinetics.

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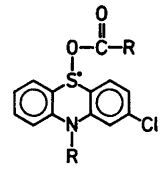
The rate law observed for the decomposition of $1^{\cdot+}$ to *2* is of the form of eqn. (2). The concentration of water was also included in the rate law [1] but

$$\text{Rate} = k_{app}[1^{\cdot+}]^2[RCO_2^-]/[H^+](A[1]+B) \quad (2)$$

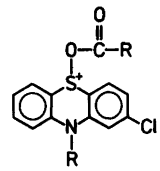
since the experiments were in aqueous solution this is not experimentally justifiable. The constants A and B come from correlation of the observed rate constant with the concentration of *1*. The features of the rate law which differ from the general rate law observed for cation radical reactions with nucleophiles [3-7] are the concentrations of RCO_2^- and H^+ which must be taken into account in formulating a mechanism. The obvious possibility which was not considered is reaction (3) in equilibrium followed by reaction (4) then equilibrium (5) and finally product forming reaction (6).

$$1^{\cdot+} + RCO_2^- \overset{K_3}{\rightleftarrows}$$



4 (3)

$$4 + 1^{\cdot+} \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}}$$



$+1$ (4)

5

$$5 + H_2O \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} 3 + H^+ \quad (5)$$

$$3 \overset{k_6}{\rightarrow} 2 + RCO_2H \quad (6)$$

Rate law (7) is obtained by applying the steady state approximation on intermediate *5* and making the reasonable assumption that $k_4[4][1^{\cdot+}] \gg k_{-5}[3][H^+]$. The latter assumption is surely reasonable

$$\text{Rate} = 2\,k_6K_3K_4K_5[1^{\cdot+}]^2[RCO_2^-]/[H^+]([1] + k_5/k_{-4}) \quad (7)$$

since the concentrations are expected to be of comparable magnitude and k_4 is expected to be very much greater than k_{-5}. Furthermore, it was only necessary to make this assumption in order for the rate law to be *identical* in form to that proposed earlier.[1]

We have recently obtained very strong support for the mechanism that we are proposing for the decomposition of $I^{\cdot+}$ in aqueous buffer solution. The cation radical of 10-methylphenothiazine (MPZ) undergoes reaction with acetate ion in aqueous acetonitrile by mechanism (8)–(10).[8] The acetate moieties in all of the intermediates are considered to be bonded to sulfur. The kinetics are

$$MPZ^{\cdot+} + AcO^- \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} MPZ(OAc)^{\cdot} \quad (8)$$

$$MPZ(OAc)^{\cdot} + MPZ^{\cdot+} \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} MPZ(OAc)^+ + MPZ \quad (9)$$

$$MPZ(OAc)^+ + AcO^- \xrightarrow{k_{10}} MPZ(OAc)_2 \quad (10)$$

somewhat more simple in this case since the nucleophilic attack in reaction (10) is observed kinetically while the comparable reaction with water (5) cannot be detected in aqueous media.

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Identification of 2-Oximino Acids in Yeast by GC – MS

INGA TUNBLAD-JOHANSSON [a] and BENGT Å. ANDERSSON [b,*]

[a] Department of Marine Microbiology, Botanical Institute, University of Göteborg, Carl Skottsbergs Gata 22, S-413 19 Göteborg, Sweden and [b] Department of Structural Chemistry, Faculty of Medicine, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden

In view of its toxic nature[1] and reactivity with carbonyl compounds,[2] hydroxylamine (HA) is most likely to be found in combined form, especially as oximes. Formation of α-keto acid oximes (2-oximino acids) has been suggested as a way for microorganisms to utilize HA as the sole source of nitrogen.[3,4] Oxime formation could be a pure chemical process and/or an enzymatic one. Free HA has mutagenic properties even in extracellular concentrations of 10^{-5} M.[3] However the yeast *Endomycopsis lipolytica* has the ability to grow on HA-concentrations as high as 4×10^{-2} M.[5] This yeast displays a high cellular content of α-keto acids with predominance of 2-oxoglutaric acid (2-oxopentanedioic acid).[6]

In previous investigations only the oxime of pyruvic acid (2-oximinopropanoic acid) was found in microorganisms grown on incompletely reduced nitrogen.[7,8] Pyruvic acid oxime was here converted into acetonitrile with subsequent gas liquid chromatographic (GLC) analysis.[7]

In this study GLC – MS has been used in order to improve the analytical procedure for determining the oximes of glyoxylic-(GOAO) (2-oximinoethanoic acid), pyruvic-(PYAO) (2-oximinopropanoic acid), oxalacetic-(OAAO) (2-oximinobutanedioic acid), and 2-oxoglutaric acid (OGAO) (2-oximinopentanedioic acid) in *E. lipolytica*.

GLC of cell extract of *E. lipolytica* (Fig. 1) revealed the presence of PYAO and OGAO. GOAO could not be detected at all while small amounts [< 2 μg (g^{-1} dry wt)] of OAAO could be traced when a large amount of cell mass was used. However the presence of this oxime could not be confirmed by MS. GLC – MS analyses gave no information about geometric isomers. OAAO, however, is reported to yield two isomers.[9]

Maximum levels of PYAO and OGAO were in the range of 65 – 75 μg (g^{-1} dry wt) for cells in late log phase. At the same stage of growth the levels of pyruvic acid and 2-oxoglutaric acid were 40 and 150 μg (g^{-1} dry wt), respectively.[6] The low content of OGAO in relation to the high amount of 2-oxoglutaric acid is notable. This could imply a fast turnover rate for OGAO or instability of this oxime at conditions prevailing in the cells. The oxime group of OGAO could also be transferred to *e.g.* pyruvic acid by means of transoximases.[10] A non-enzymatic transfer of oxime groups to keto acids was not observed in the present study.

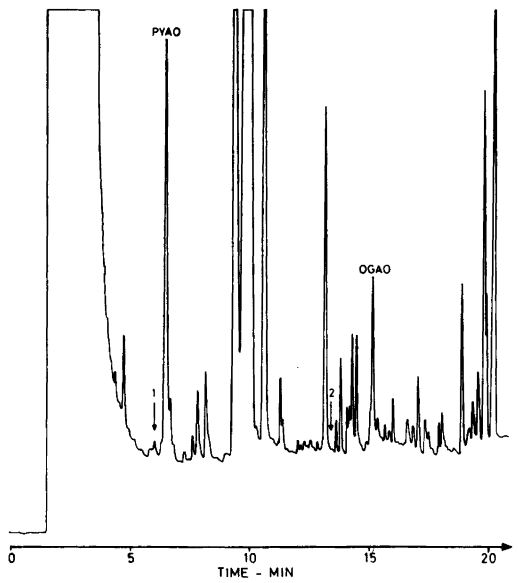

Fig. 1. Gas chromatogram of cell extract from *Endomycopsis lipolytica* grown in hydroxylamine medium. Initial temperature 65 °C for 4 min then 65 – 250 °C at 8 °C/min.

Cryptococcus albidus, a yeast with the ability to grow on nitrate, contained PYAO and OGAO at a lower level than in *E. lipolytica*. These two yeasts together with *Saccharomyces cerevisiae* displayed no oximes when grown on ammonia, indicating the significance of oximes in the reductive assimilation of nitrogen.

Experimental. Extraction. Cells of *E. lipolytica* were grown in a glycerol medium with HA as the sole nitrogen source at a concentration of 8×10^{-3} M.[6] A cell mass corresponding to about 1 g cell dry weight was harvested by centrifugation, washed, suspended in 20 ml pyridine: H_2O (4:1 v/v), and homogenized. The pyridine phase was evaporated and the residue was dissolved in 0.7 ml cold water, saturated with NaCl, and acidified to pH 2. The oximes were extracted four times with 0.6 ml cold ethyl acetate. Diethyl ether and acetone were also

* Present address: EKA Kemi AB, Fack, S-445 01 Surte, Sweden.

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Table 1. Recoveries of α-keto acid oximes from test solution ranging from 25 to 100 μg of each oxime.

Oxime	Average recovery %		
	Ethyl acetate	Diethyl ether	Acetone
GOAO	90	84	75
PYAO	95	95	77
OAAO	60	44	63
OGAO	85	77	68

tested as extraction solvents but, as shown in Table 1, these solvents were found to yield a lower recovery in comparison with ethyl acetate. Moreover, when applied to cell material, acetone extraction resulted in a complicated gas chromatogram with many unresolved peaks.

Trimethylsilylation. The combined ethyl acetate extracts (2.4 ml) were evaporated to dryness and the residue silylated with 100 μl BSTFA containing 1% TMCS. Optimal conditions were obtained when the mixture was warmed at 50 °C for 1 h. Later experiments have shown that silylation for 10 min at room temperature with a mixture of 50 μl pyridine and 50 μl BSTFA:TMCS gave comparable silylation effect.

GLC apparatus. The trimethylsilylated oximes were separated on a Perkin Elmer 3920 instrument and detected by a flame ionization detector. The injections were performed *via* a Grob-type split injector block [11] and the GLC columns used were either OV 101 or SE 30 WCOT glass capillary columns, 25 m long and 0.2 mm i.d. Nitrogen was used as carrier gas with a flow rate of 1.0 ml/min and a split ratio of 1:50. The temperature program was 60 – 250 °C at 8 °C/min.

GLC – MS apparatus. The GLC – MS system used was a combined Carlo Erba Fractovap 2101 gas chromatograph and a Varian MAT 112 mass spectrometer coupled to a Spectro system 100 MS computer. The GLC conditions were the same as above except that helium was used as carrier gas at 2 ml/min. Ionization was performed in EI mode at an IP of 70 eV, emission current of 1500 μA, and an accelerating voltage of 0.8 kV. High resolution MS was performed on reference substances with an AEI, MS 902 instrument operating in EI mode at 70 eV, 200 μA, and 8 kV *via* a direct inlet probe.

Gas chromatograms. In a reference mixture the four α-keto acid oximes used in this study are well separated under the conditions described above. Fig. 1 shows a typical gas chromatogram of a TMS-derivatized extract from *E. lipolytica* where the PYAO and OGAO fractions are seen. GOAO and OAAO are to be expected at fractions 1 and 2, respectively.

Mass spectra. The identity of the four α-keto acid oximes in the cell extracts were determined by high and low resolution mass spectrometry by using reference substances. The diagnostically important ions for these substances were the nitrogen-containing ions *e.g.* M and M – 15. Other ions useful in the identification of the monoacid oximes include M – 43 and a fragment derived from PYAO due to loss of $-COOSi(CH_3)_3$.[12] The latter fragment was dominant in the spectra of the diacid oximes.[13] OGAO also has a prominant ion at *m/z* 170 that originates through loss of $(CH_3)_3-SiOH$ and $-COOSi(CH_3)_3$ from the molecular ion. Other prominent ions (nondiagnostic) typical for silylated compounds were *m/z* 73 $[(CH_3)_3Si]^+$ and *m/z* 147 $[(CH_3)_3Si-O=Si(CH_3)_2]^+$.

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The Oxidation of Hydroquinone by Protonated Quinone

OLE HAMMERICH [a] and
VERNON D. PARKER [b]

[a] Department of General and Organic Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark and [b] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The electrochemical oxidation of hydroquinone has been studied extensively in recent years.[1-9] The overall half-reaction (1) can be considered to be the classical organic redox couple. The mechanism of

$$H_2Q \rightleftarrows Q + 2H^+ + 2e^- \quad (1)$$

the oxidation is complex and involves several coupled electron transfer and proton transfer equilibria. Quinones have been used as electron acceptors during the oxidation of aromatic compounds.[10-13] In some cases [10,11] protonated quinones have been implicated as the active oxidants. We now report data concerned with the reaction of hydroquinone with quinone in the presence of acids to produce two equivalents of hydroquinone cation radical.

We have shown that the reversible oxidation of phenols to the corresponding cation radicals can be achieved by conducting voltammetric measurements at low temperatures in solvents containing strong acids.[14] The function of the acid was to slow down the thermodynamically favorable deprotonation reactions of the phenolic cation radicals. Applying this technique to the voltammetric oxidation of hydroquinone resulted in the voltammograms shown in Fig. 1. In dichloromethane in the absence of acid at −50 °C, an irreversible two electron oxidation was observed (Fig. 1a). Little change in the voltammogram was observed when the oxidation was carried out in the presence of trifluoroacetic acid (TFA) as is evident in Fig. 1b. When methane sulfonic acid (4 %) was added to the same solution, a quasi-reversible two electron oxidation was observed (Fig. 1c). The separation in the peak potentials for the oxidation and reduction processes was found to be dependent upon the acid concentration indicating that reversible protonation was involved. A voltammogram similar to that shown in Fig. 1c could be obtained during the reduction of quinone in dichloromethane containing varying amounts of $HFSO_3$ at −50 °C. In a solution con-

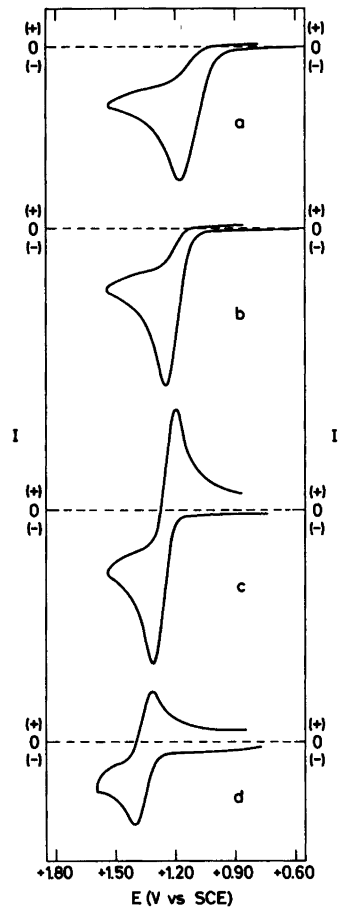

Fig. 1. Cyclic voltammograms for the oxidation of hydroquinone in dichloromethane at −50 °C at 86 mV/s. Supporting electrolyte was Bu_4NBF_4 (0.1 M). (a) No added acid, (b) 2 % TFA, (c) 2 % TFA and 4 % $MeSO_3H$ and (d) the same solution as (c) with 3 % $HFSO_3$ added.

taining TFA (2 %), $MeSO_3H$ (4 %) and $HFSO_3$ (3 %) the oxidation of hydroquinone appeared as a quasi-reversible one electron process (Fig. 1d). The decrease in peak height in going from the voltammogram shown in Fig. 1c to that in Fig. 1d was shown to be a consequence of the number of electrons transferred rather than to destruction of substrate by the independence of the peak height on the amount of $HFSO_3$ added. Thus, under the conditions of the experiments, the hydroquinone cation radical is stable.

When an equimolar solution of hydroquinone and quinone in dichloromethane at −50 °C was treated with $HFSO_3$, the solution immediately as-

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sumed a deep purple color and the visible absorption spectrum showed a maximum at about 550 nm. One electron oxidation of hydroquinone under the same conditions resulted in a solution of the cation radical which showed a visible absorption spectrum identical to that obtained from the oxidation of hydroquinone by quinone.

The oxidation reaction most likely involves protonation of quinone to the dication (2,3). The reduction potential of the dication (eqn. 8, Scheme 1)

$$Q + H^+ \rightleftarrows HQ^+ \quad (2)$$

$$HQ^+ + H^+ \rightleftarrows H_2Q^{2+} \quad (3)$$

is of the order of 500 mV more positive than that for the oxidation of hydroquinone (7). Thus, under conditions where the protonation equilibrium (3) lies far to the right, the equilibrium constant for the electron transfer reaction (4) will be of the order of 10^8 and reaction (4) would be expected to be diffusion controlled. Electron transfer processes involving either quinone (5) or mono-protonated quinone (6) can be ruled out because of very unfavorable equilibrium constants, 10^{-31} and 10^{-18}, calculated from the electrode potentials.

$$H_2Q + H_2Q^{2+} \rightleftarrows 2H_2Q^{\cdot+} \quad (4)$$

A feature of these reactions of further interest is that the equilibria (2)–(4) are very temperature dependent. The deep purple colored solution of hydroquinone cation radical at −50 °C became colorless upon warming quickly to −10 °C. Rapid cooling of the solution once again to −50 °C restored the solution to the original state with very little loss of absorption at 550 nm. Attempts to obtain thermodynamic equilibrium data on reactions (2)–(4) failed due to irreversible side reactions which occur at longer times at temperatures above −50 °C.

Half reaction	E^{rev} *vs.* SCE	
$Q + e^- \rightleftarrows Q^{\cdot-}$	−0.5 V [15]	(5)
$HQ^+ + e^- \rightleftarrows HQ^{\cdot}$	+0.2 V [1]	(6)
$H_2Q^{\cdot+} + e^- \rightleftarrows H_2Q$	+1.3 V	(7)
$H_2Q^{2+} + e^- \rightleftarrows H_2Q^{\cdot+}$	+1.8 V[a]	(8)

Scheme 1. [a]Value estimated from an irreversible oxidation peak.

The results presented here call attention to an important fact not generally recognised. Protonated intermediates in organic reactions can be powerful oxidants. For example, neither the proton nor quinone are oxidizing agents, the reduction potentials being of the order of 0 and −0.5 V (*vs.* SCE), respectively. However, the diprotonated quinone, hydroquinone dication, has a reduction potential of about +2.0 V and is a powerful oxidant. Even in cases where the protonated intermediates are present in very low concentration, oxidation reactions can be very rapid since these high potential oxidants may react at diffusion controlled rates. Thus, electron transfer reactions to protonated intermediates is a favorable reaction pathway.

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Synthesis of *p*-Nitrophenyl 3-*O*-(2-Acetamido-2-deoxy-β-D-gluco-pyranosyl)-α-L-Rhamnopyranoside Corresponding to a Fragment of the *Streptococcus* Group A Cell Wall Polysaccharide

PER J. GAREGG and THOMAS NORBERG

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

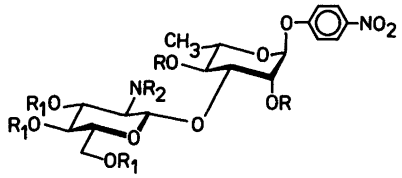
The cell-wall of *Streptococcus* Group A bacteria has been reported[1] to contain the polysaccharide depicted below:

β-D-GlcNAc*p*
1
↓
3
⟨→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→⟩$_n$

The disaccharide β-D-GlcNAc*p*-(1→3)-α-L-Rha*p* has now been synthesized in the form of the *p*-nitrophenyl glycoside *3*, suitable for coupling to proteins. Studies on the antigenic properties of the disaccharide coupled to a protein will be performed.

The free disaccharide has been prepared before[2] and also, recently, the 8-methoxycarbonyloctyl-α-glycoside.[3]

The synthesis of *3* is based on silver triflate-assisted glycosidation[4] of *p*-nitrophenyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[5] with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl bromide.[4] The protected disaccharide *1*, obtained in 64% yield after chromatography, was subsequently deprotected with hydrazine hydrate[4] and acetylated to give the crystalline acetate *2* in an 80% yield. De-*O*-acetylation gave the title disaccharide *3* which, after conversion[6] to the corresponding isothiocyanate derivative, can be used for coupling to proteins.



1: R = Bz, R_1 = Ac, R_2 =

2: R = R_1 = Ac, R_2 = 

3: R = R_1 = H, R_2 = 

Experimental. General methods were the same as those reported before.[7]

p-*Nitrophenyl 3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)-2,4-di-O-benzoyl-α-L-rhamnopyranoside (1).* A mixture of *p*-nitrophenyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[5] (1.20 g, 2.24 mmol), silver triflate (0.82 g, 3.2 mmol) and 2,4,6-trimethylpyridine (0.33 ml, 2.5 mmol) in 1:1 nitromethane – toluene (20 ml) containing powdered 4 Å molecular sieve was stirred and cooled to −20 °C. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl bromide[4] (1.49 g, 3.0 mmol) in 1:1 nitromethane – toluene (10 ml) was added. After 15 min, more bromide (1.0 g) was added, followed by silver triflate (1.0 g) and 2,4,6-trimethylpyridine (0.4 ml). After 4 h at −20 °C, the mixture was diluted with 1:1 diethyl ether – ethyl acetate and filtered. The filtrate was washed successively with aqueous sodium thiosulfate, water, 2 M aqueous sulfuric acid and aqueous sodium hydrogencarbonate. The residue after drying ($MgSO_4$) and concentration was purified by column chromatography on silica gel (250 g). Dichloromethane – ethyl acetate – acetone (40:5:1) eluted pure *1* which upon concentration was obtained as a colorless foam (1.35 g, 64%), $[\alpha]_D$ −14° (*c* 0.5, $CHCl_3$). Further elution gave a fraction containing the dehydrohalogenation product of the bromo sugar[4] (0.82 g).

p-*Nitrophenyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4-di-O-acetyl-α-L-rhamnopyranoside (2).* Disaccharide *1* (1.1 g) was treated with a mixture of 95 % ethanol and 98 % hydrazine hydrate (2:1, 30 ml) at 70 °C for 10 min. After concentration the residue was acetylated (90 °C, 15 min) with pyridine (40 ml) and acetic anhydride (15 ml) and then again concentrated. Purification by column chromatography on silica gel (150 g) using ethyl acetate as eluant gave a solid material which was recrystallized from ethanol. Pure *2* (0.59 g, 80 %) was obtained, m.p. 209 – 213 °C, $[\alpha]_D$ −58° (*c* 0.5, $CHCl_3$). Anal.: $C_{30}H_{38}N_2O_{17}$: C,H,N.

p-*Nitrophenyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside (3).* Compound *2* (0.56 g) was treated with 0.1 M methanolic sodium methoxide (20 ml) at room temperature. After 1 h the mixture was neutralized with Dowex 50 (H^+) and concentrated. The residue, after partitioning between diethyl ether – water and lyophilization of the aqueous phase, was pure *3* (0.36

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g, 92 %) as an amorphous solid, $[\alpha]_D$ −104° (*c* 0.4, H_2O). The 1→3-linkage was demonstrated by methylation analysis – GLC-MS.[8] ^1H NMR (D_2O, 85 °C, external TMS): δ 5.64 (d, $J_{1,2}$ 2.0 Hz, Rha H-1), 4.89 (d, $J_{1,2}$ 7.9 Hz, GlcNAc H-1), 2.03 (acetyl CH_3), 1.20 (d, $J_{5,6}$ 5.4 Hz, Rha CH_3). ^{13}C NMR (D_2O, 25 °C, external TMS), δ 176.0 (acetyl C=O), 161.9, 142.9, 126.9, 117.5 (aromatic C), 104.1 (GlcNAc C-1), 98.5 (Rha C-1), 81.1 – 70.7 (ring carbons), 61.6 (GlcNAc C-6), 56.9 (GlcNAc C-2), 23.4 (acetyl CH_3) 18.0 (Rha CH_3).

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Stereoselectivities in Enzymatic Syntheses of Fluorocitric Acid

SVANTE BRANDÄNGE,[a] OLOF DAHLMAN,[a] ANDERS MÅHLÉN[b] and LARS MÖRCH[a]

[a] Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden and [b] Department of Chemistry, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden

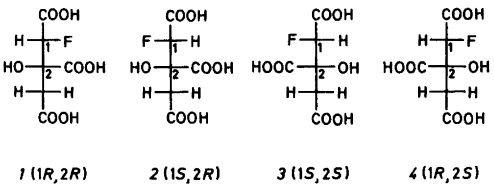

Fig. 1. Absolute configurations of the fluorocitric acids formed in reactions catalyzed by citrate (*si*)-synthase. The reaction with fluoroacetyl-CoA yields *1* together with a diastereomeric minor product (2 −3% of *1*) which is probably *2*. The reaction with fluorooxaloacetate yields *3* and *4* in the ratio 35:65.

Citrate (*si*)-synthase (EC 4.1.3.7) catalyzes the biosynthesis of citric acid from acetyl-CoA and oxaloacetate. The enzyme can also accept fluoroacetyl-CoA as a substrate and a synthesis of toxic fluorocitric acid occurs when fluoroacetic acid, or a compound which can be biologically degraded to it, is administered to living organisms.[1,2] Only one stereoisomer of fluorocitric acid has been detected in *in vitro* experiments and the absolute configuration 1*R*,2*R* * (*1*), proposed by Carrell *et al.*,[3] has recently been proved.[4,5] The use of fluorooxaloacetate, which is also accepted as a substrate by citrate synthase, offers another possibility of obtaining fluorocitric acid by an enzymatic synthesis.[6] Initially only one stereoisomer was found in this reaction, but a recent investigation revealed that two diastereomeric forms of fluorocitric acid are produced in the approximate ratio of 2:1.[7] The initially detected isomer was found to be enantiomeric with the acid formed in the reaction with fluoroacetyl-CoA.[1]

We have reinvestigated these two enzymatic syntheses of fluorocitric acid. The crude reaction mixture obtained in the reaction with fluoroacetyl-CoA was acidified and treated with diazomethane and the mixture of trimethyl esters was analyzed by capillary column GLC. At the retention time of authentic[5] trimethyl (1*R*,2*S*)-fluorocitrate there was a small peak which was not found in a control experiment without enzyme. The trimethyl ester of the previously known product *1* (1*R*,2*R*) was eluted later. In six separate experiments the minor component amounted to 2−3% of the major product *1*. In the mass spectra of diastereomeric trimethyl fluorocitrates the peaks at $m/z = 101$, 160, 161 and 162 show approximately the same relative intensity for the two compounds (37, 0.3, 100 and 7%, respectively).[5] If the minor component is a trimethyl fluorocitrate, the ratio of the intensity for this compound at any of these four m/z values to the intensity at the same m/z value in the mass spectrum of the trimethyl ester of *1* should be constant. After purification of the sample by HPLC, a GLC-MS-SIR (selected ion recording) investigation was undertaken at $m/z = 101$, 160, 161 and 162 and a good constancy in the intensity was obtained: 18, 19, 19 and 18% at the four m/z values, respectively. The GLC, HPLC and MS characteristics of this minor component, taken together with the fact that it is formed enzymatically, strongly indicate that it is a fluorocitric acid belonging to the 1*RS*,2*SR* racemic pair. For mechanistic reasons it is probable that the minor isomer and *1* are epimeric at the fluorine-bearing carbon and that the former thus has the 1*S*,2*R* configuration (*2*).

There are two main ways in which the minor isomer *2* may be formed: directly in the enzymatic synthesis or by non-enzymatic epimerization of *1*, either in the reaction mixture or during work-up or analysis. The reverse epimerization *2*→*1* is excluded since deuterium is retained in *1* formed from (2*R*)-[2-$^2H_1$]fluoroacetyl-CoA[7] but should be lost during epimerization. No increase of *2* was observed after a tenfold prolongation of the reaction time; the weakly alkaline conditions thus caused no epimerization. No formation of diastereomeric acid was seen after a prolonged treatment of (1*S*,2*S*)-fluorocitric acid with stronger hydrochloric acid than that used in the work-up of the enzymatic synthesis. Since authentic trimethyl (1*S*,2*S*)-fluorocitrate on GLC analysis showed the presence of less than 0.2% of diastereomeric ester, no significant epimerization occurs during GLC analysis. We therefore conclude that *2* is formed in the enzymatic synthesis rather than by a non-enzymatic epimerization of *1*.

A reinvestigation of the citrate synthase mediated synthesis of fluorocitric acid from fluorooxaloacetate has also been carried out and, in agreement with previous findings,[7] two diastereomeric acids were detected in the ratio 35:65. These acids were isolated and characterized as

* The numbering is that of 2-hydroxy-1,2,3-alkanetricarboxylic acids.

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molybdate(VI) complexes by CD spectroscopy. Comparison with spectra of reference acids of known configuration[5] gave the absolute configurations of the enzymatically formed fluorocitric acids as 1*S*,2*S* (*3*, minor isomer) and 1*R*,2*S* (*4*, major isomer). The fluorocitric acids *3* and *4* are those expected[7] to be formed in the reaction with fluorooxaloacetate if acetyl-CoA attacks only the *re* face of the fluorooxaloacetate keto carbonyl group (corresponding to the *si* face in oxaloacetate), if both (*R*)- and (*S*)-fluorooxaloacetate can react, and if no inversion of configuration at C-2[8] occurs later in the synthesis. Evidently the reaction with the *R* isomer is the more easy one.

The absolute configuration of *1*,[4,5] combined with the finding[7] that the enzyme selectively abstracts the *pro-S* hydrogen of the fluoroacetyl group during the synthesis of *1*, establishes that an inversion of configuration occurs in the fluoroacetyl group in the synthesis. An inversion also occurs in the reaction with acetyl-CoA.[9,10] The detection of a second isomer of fluorocitric acid (probably *2*) means that the *pro-S* selectivity is only partial (97–98 %), provided that an inversion also occurs in the formation of *2*. A remarkable difference is seen between fluoroacetyl-CoA and propionyl-CoA with regard to their *pro-S*/*pro-R* selectivities. The *pro-S* hydrogen in fluoroacetyl-CoA is the more reactive, but the stereochemically analogous (*pro-R*) hydrogen in propionyl-CoA is the *least* reactive by a factor of about 60.[11,12] The detailed interpretation of this effect requires a knowledge of the topological features of the active site of citrate (*si*)-synthase; studies of the enzyme are being pursued by X-ray methods.[13]

Experimental. GLC of trimethyl fluorocitrates was performed using a Carbowax 20 M fused silica capillary column (50 m × 0.2 mm) mounted in a Hewlett-Packard 5830 A gas chromatograph (electronic integration system) run in the splitless mode (90–190 °C, 20 °C/min). The retention times of the 1*RS*,2*SR* and 1*RS*,2*RS* stereoisomers (17.86 and 18.46 min, respectively) represent a base-line resolution. GLC-EI-MS-SIR (selected ion recording) was performed using the same column mounted in a Finnigan 4000 instrument. HPLC separations of the isomeric trimethyl esters were carried out on a silica gel column (Partisil 10, Reeve Angel, 25 cm × 4.6 mm) with 2,2,4-trimethylpentane–ethyl acetate (5:2) as eluant and using RI-detection. Under the conditions used, the retention times were 6.0 and 7.2 min for the 1*RS*,2*SR* and 1*RS*,2*RS* isomers, respectively, also this representing a base-line resolution. ^{1}H NMR spectra were recorded on a JEOL JNM-FX 100 spectrometer.

Diethyl fluorooxaloacetate was synthesized according to a method given for the difluoro analogue.[14] After distillation (b.p. 78 °C at approximately 100 Pa) it was obtained in a 25 % yield and in a purity exceeding 99 % (GLC, NMR). ^{1}H NMR ($CDCl_3$, TMS): δ 5.94 (d, C*H*F, *J* 47.4 Hz), 4.40 (2H,q), 4.33 (2H,q), 1.40 (3H,t), 1.32 (3H,t).

Fluorooxaloacetic acid. The diethyl ester was hydrolyzed with acetic acid–hydrochloric acid for 25 days at +5 °C.[15] After freeze-drying, the acid was crystallized as in Ref. 16 but working in the temperature interval +20 to −20 °C; m.p. 87 −92 °C (decomp.); lit. m.p. 86–87 °C[15] and 82 −85 °C (decomp.).[17] In the ^{1}H NMR spectrum (D_2O, sodium 2,2-dimethyl-2-silapentane-5-sulfonate as reference) the title compound gave a doublet (J_{HF} 46.9 Hz) at δ 5.26 (*cf.* Ref. 17). Unhydrolyzed ester groups were present to the extent of approximately 9 mol % and only a minute amount (≈ 1 %) of fluoropyruvic acid was present, δ 4.52 (d, J_{HF} 47 Hz) (*cf.* Ref. 18).

Enzymatic synthesis with fluorooxaloacetate. The reaction mixture (11 ml) contained acetyl-CoA (10 mM), fluorooxaloacetate (16 mM), 5,5′-dithiobis(2-nitrobenzoic acid) (17 mM) and pig heart citrate synthase (Boehringer, 50 μg/ml) in 0.05 M Tris-HCl, pH 8.0. The mixture was kept overnight at 23 °C, acidified to pH ≈ 1 with 2 M hydrochloric acid and washed twice with ether to remove 5-nitro-3-thiolobenzoic acid and its corresponding disulfide. Concentration of the aqueous layer to near dryness at 30 °C, addition of excess ethereal diazomethane, drying with $MgSO_4$, filtration and concentration gave a crude product (10 mg) which contained the trimethyl esters of *3* and *4* in the ratio 35:65. After purification by HPLC, the esters weighed 2.7 and 5.8 mg, respectively, corresponding to a total yield of 31 %. The ^{1}H NMR spectra were indistinguishable from those of authentic samples.[5] Hydrolysis of the esters and characterization of the acids as molybdate(VI) complexes by CD were performed as described.[5] CD of minor isomer (*3*) (nm, [Θ] × 10^{-4}): 275, +0.95; 250, −1.2; 234, +1.2; 218, −1.3. CD of major isomer (*4*): 274, +0.70; 250, −0.77; 235, +0.69; 218, −1.4.

Enzymatic synthesis with fluoroacetyl–CoA. Fluoroacetic anhydride (20 μl) was dissolved in tetrahydrofuran (1.5 ml) and part of the solution (200 μl) was added to coenzyme A (14 μmol) in 1 % aqueous $NaHCO_3$ (1 ml). After 40 min at 0 °C, this solution of fluoroacetyl-CoA was transferred in 10 portions during 1 h to a mixture (23 °C) containing oxaloacetic acid (21 μmol), pig heart citrate synthase (Boehringer, 125 μg/ml) and 5,5′-dithiobis(2-nitrobenzoic acid) (21 μmol) in 0.1 M Tris–HCl (pH 7.8, 3.15 ml). The final pH 7.0 was reached after 15 h. Work-up and analysis were performed as above.

When more enzyme was used (360 μg/ml) a higher yield (≈ 20 %) and a purer product was obtained. The relative amount of the minor component was, however, unchanged (2–3 % of *1*).

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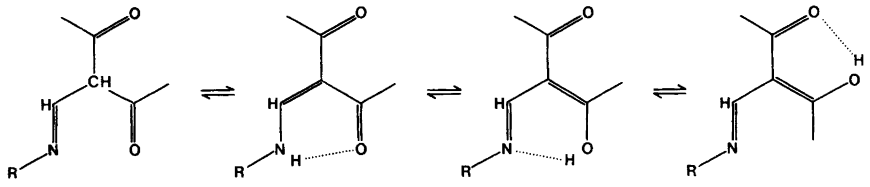
Synthesis and Crystal Structure of the Hydrogen Bonded Molecular Self-complex of *N*-(2,2-Diacetylvinyl)-*o*-phenylenediamine

CHRISTER SVENSSON, INGVAR YMÉN and BARUCH YOM-TOV

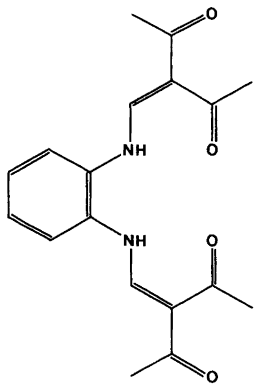
Inorganic Chemistry, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund 7, Sweden

The yellow title compound ($C_{12}H_{14}N_2O_2$) crystallizes with four molecules in a monoclinic unit cell with dimensions $a=10.259(2)$ Å, $b=10.988(3)$ Å, $c=10.896(2)$ Å, $\beta=114.17(1)°$. The space group is $P2_1/c$. The structure was solved by direct methods and refined by the method of least-squares. The final $R=0.036$ for 1738 observed reflections. The tautomer in the crystal is the enamine stabilized by a short intramolecular NH···O hydrogen bond as part of a six-membered ring. The N···O distance is 2.596(3) Å. The azomethino pentane-2,4-dione part is approximately planar, and the molecules form pairs where these parts are overlapped with interplanar distances of 3.45 Å. This self-complex is further strengthened by weak intermolecular hydrogen bonds between the amino groups and carbonyl oxygens.

During the last decade much interest has been focused on the electrocatalytical properties of macrocyclic planar MN_4 chelates of cobalt and iron, *e.q.* phtalocyanins and cobalt dibenzotetraazaannulene.[1] The latter substance has good cata-



1a 1b 1c 1d



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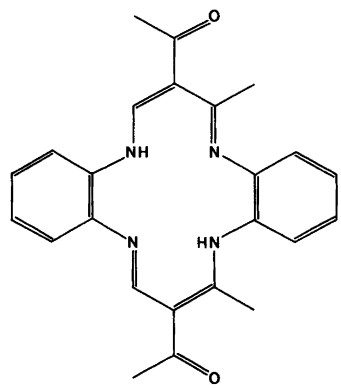

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lytical properties but since it is difficult to synthesize in large quantities the electrocatalytical activity of other chelates of Co and Fe has been investigated.[2]

Attempts to condense 1,2-bis(3-azomethinopentane-2,4-dione)benzene (*2*) with 1,2-diaminobenzene did not lead to the anticipated tetraaza macrocycle (*3*) but to the title compound (*1*). This compound has some interesting properties. It was shown to be versatile both in chelating with cations,[3] as a terdentate ligand, as well as undergoing reactions in aqueous acid solution.[4] The structure determination of (*1*) which is the subject of this paper was performed to throw light on these properties and to study the tautomerism of an azomethino-β-diketone.

The composition of the equilibrium mixture of the keto-enol tautomerism in β-diketones is dependent *i.a.* on the substituents present in the molecules. Thus for example acetylacetone exists to 80 % in the intramolecularly OH···O bonded enol form while the exchange of one methyl for a carbethoxy group results in an equilibrium composition of just 7.5 % enol form.[5] The ketimine–enamine tautomerism was found long ago[6] to be shifted toward the enamine and similarly the amide–iminol tautomeric equilibrium is normally displaced toward the amide form.[7] In these compounds the C–N bonds have a considerable double bond character nevertheless, and for N···O hydrogen bonds, which are operative, there is a general preference for NH···O bonds. In the system reported here several tautomers are feasible. Four of them are shown (*1*a–d) of which *1*b–d have possibilities of forming intramolecular N···O or O···O hydrogen bonds as part of a six-membered ring.

EXPERIMENTAL

Synthesis. A mixture of 1,2-bis(3-azomethinopentane-2,4-dione)-benzene (*2*)[8] (3.3 g, 0.010 mol) and 1,2-diaminobenzene (1.2 g, 0.011 mol) in 60–70 ml toluene was refluxed for 20–25 mins. The yellow hot solution was then filtered. Yellow crystals started crystallizing from the filtrate in a few minutes. After standing for 24 h at room temperature they were filtered off. The title compound (*1*) was obtained practically pure in 90 % (3.9 g, 0.018 mol) yield, m.p. 170–171 °C. Anal. Calc. for $C_{12}H_{14}N_2O_2$: C, 66.03; H, 6.46; N, 12.83; O, 14.66 %. Found: C, 65.9; H, 6.49; N, 12.75; O, 14.89 %. ^{1}H NMR (DMSO-d_6): δ 2.40 [d, 6, (-CH_3)], 5.00 [s, 2, (-NH_2)], 7.33 [m, 4, (benzene)], 8.33 [d, 1, J=6 Hz, (-N=CH-)], 12.47 [d, 1, J=6 Hz, (NH···O)]. MS: *m/e* 218 15 %, 119 100 % (M – acetylacetonyl).

X-Ray study. The lustrous crystals of the title compound are platelets with (100) as the principal face. A crystal with approximate dimensions 0.19 × 0.31 × 0.38 mm^3 was mounted on a glass fibre. Integrated intensity measurements were made on a computer-controlled Enraf-Nonius CAD4 diffractometer using Ni-filtered Cu$K\alpha$ radiation. All reflections within a quarter sphere of reciprocal space with radius $(\sin\theta/\lambda) \leq 0.61$ Å^{-1}, were sampled by the $\omega-2\theta$ scan technique with $\Delta\omega = 0.9° + 0.3° \tan\theta$. The maximum time spent on a reflection was 90 s, resulting in a counting statistics precision about 3 %. Three standard reflections were remeasured every other hour. No significant variation in intensity as a function of exposure time was detected. From the observed data the following systematic absences were derived: *h0l* for *l* odd and *0k0* for *k* odd. Two strong reflections of type *h0l*, 501 and 403, could be shown to originate from the Renninger effect by later remeasurement at varying azimuthal angles. The remaining 2140 independent reflections were corrected for Lorentz, polarization and absorption effects. The transmission factors were in the range 0.80 to 0.88. At the later stages of structure refinement an isotropic extinction correction[9] was applied, resulting in $g = 0.49(8) \times 10^3$. Only one percent of the structure amplitudes were corrected by more than one percent, with a maximum correction of 10 % for *F*(211).

Determination and refinement of the structure. The structure was solved using the MULTAN program.[10] After refinement of all the heavy atom positions, an electron density difference map revealed the hydrogen atoms. In the final refinement cycles, all carbon, nitrogen and oxygen atoms were assigned anisotropic temperature factors and the hydrogen atoms isotropic temperature factors. A total of 202 parameters were refined, with 1738 observed structure amplitudes having $I_{obs} > 3\sigma_c(I_{obs})$. The weights used in the least-squares calculations were derived from $w_i^{-1} = \sigma_c^2(F_{obs}) + (0.01|F_{obs}|)^2 + 0.1$, where $\sigma_c(Fobs)$ is the standard deviation due to counting statistics. The resulting agreement indicators are $R = 0.036$, $R_w = 0.041$ and $S = 1.19$.

The final atomic position coordinates and isotropic temperature factor coefficients are given in Table 1. The final anisotropic temperature factor coefficients for carbon, nitrogen and oxygen and the magnitudes of the 1738 observed and calculated structure factors are available on request.

CRYSTAL DATA

N-(2,2-diacetylvinyl)-*o*-phenylenediamine $C_{12}H_{14}N_2O_2$, M = 218.25 g mol^{-1}; m.p. 444 K, mono-

Table 1. Atomic position coordinates and isotropic thermal parameters. The B_{iso} of atoms O(1) to C(12) were derived from the final anisotropic temperature factor coefficients.

Atom	*x*	*y*	*z*	B_{iso}(Å^2)
O(1)	0.48127(16)	0.20339(12)	0.31323(14)	5.2(1)
O(2)	0.26753(14)	−0.09679(10)	0.36790(12)	4.1(1)
N(1)	0.22874(15)	0.02855(12)	0.55185(14)	3.1(1)
N(2)	0.21690(19)	−0.17700(14)	0.69113(17)	3.9(1)
C(1)	0.30250(17)	0.11254(14)	0.52053(16)	2.8(1)
C(2)	0.35108(17)	0.10369(14)	0.41907(15)	2.7(1)
C(3)	0.43368(17)	0.20500(14)	0.39986(16)	3.0(1)
C(4)	0.46223(24)	0.31662(17)	0.48679(21)	3.8(1)
C(5)	0.32068(17)	−0.00680(14)	0.33762(16)	2.9(1)
C(6)	0.35137(35)	−0.01579(22)	0.21441(24)	5.2(1)
C(7)	0.17624(17)	0.03921(14)	0.65329(16)	2.9(1)
C(8)	0.17466(17)	−0.06685(15)	0.72466(17)	3.1(1)
C(9)	0.12412(20)	−0.05684(18)	0.82520(18)	3.8(1)
C(10)	0.07661(20)	0.05301(19)	0.85263(19)	4.1(1)
C(11)	0.07837(20)	0.15666(18)	0.78159(20)	4.0(1)
C(12)	0.12775(19)	0.14880(16)	0.68094(18)	3.4(1)
H(N1)	0.2169(21)	−0.0413(19)	0.5060(21)	5.2(5)
H(N21)	0.2923(24)	−0.1743(19)	0.6705(21)	5.3(5)
H(N22)	0.2194(22)	−0.2404(21)	0.7458(22)	5.7(5)
H(C1)	0.3253(17)	0.1865(15)	0.5756(16)	3.2(4)
H(C4)	0.3762(25)	0.3662(20)	0.4648(22)	5.7(6)
H(C4)	0.4955(24)	0.2985(21)	0.5815(25)	6.2(6)
H(C4)	0.5366(25)	0.3652(22)	0.4756(24)	6.7(6)
H(C6)	0.3147(30)	−0.0866(27)	0.1717(28)	8.7(8)
H(C6)	0.3096(27)	0.0538(24)	0.1562(26)	7.2(7)
H(C6)	0.4559(33)	0.0000(28)	0.2358(30)	10.0(9)
H(C9)	0.1248(20)	−0.1295(18)	0.8747(19)	4.5(4)
H(C10)	0.0405(21)	0.0584(18)	0.9226(21)	5.5(5)
H(C11)	0.0455(19)	0.2331(18)	0.8004(18)	4.3(4)
H(C12)	0.1243(19)	0.2200(17)	0.6265(18)	3.9(4)

clinic, $P2_1/c$, $a=10.259(1)$ Å, $b=10.988(3)$ Å, $c=10.896(1)$ Å, $\beta=114.17(1)°$, $V=1120.5$ Å^3, $D_{calc}=1.294$ g cm^{-3}, $Z=4$, $F(000)=464$, $\mu(CuK\alpha)=7.39$ cm^{-1}.

DISCUSSION

Molecular dimensions. The bond distances and angles are given in Fig. 1. which also shows the atomic numbering used here. The aromatic ring is planar and it is distorted towards C_{2v} symmetry with the twofold axis along the line C(11)−C(8)−N(2). Assuming that the geometrical effects of the substituents on the bond angles and bond lengths of the benzene ring are additive,[11] one can conclude that the amino group N(2)H$_2$ causes most of the ring distortions. The nitrogen atoms deviate slightly [N(1), 0.011(2) Å; N(2), 0.062(2) Å] from the plane of the benzene ring, *cf.* the torsion angles in Table 2. In 1,2-diaminobenzenes the two nitrogen lone pairs are oriented on opposite sides of the aromatic ring plane to maximize the π-interaction.[12,13] The N(2)H$_2$ group has the same conformation as the amino groups of 1,2-diaminobenzene[13] with one NH bond approximately coplanar with the ring, and the C−N bond is short, 1.384(2) Å. The N(1) configuration is trigonal planar: the lone pair has been donated to the N(1)−C(1)−C(2) bonds and there is little π-donation to the aromatic ring. The C(7)−N(1) bond is long, 1.419(2) Å, and furthermore the plane of the phenyl ring is rotated 36.4(2)° out of the plane of the rest of the molecule.

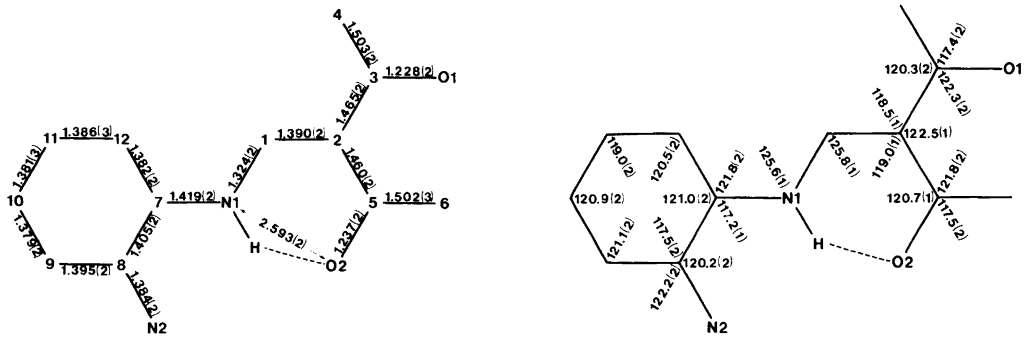

Fig. 1. Bond distances and angles with esd's in parentheses. The atomic numbering used is also shown. The N–H distances are 0.89 to 0.91 Å with esd 0.02 to 0.03 Å. The C–H distances are between 0.96(2) and 1.01(3) Å except one C(6)–H(C6) which is 0.91(2) Å.

Domenicano and Vaciago[14] have pointed out a relation in *e.g.* diphenylaminotriphenylmethane between C(ring)–N torsion angle and distorsions of the endocyclic bond angles: The benzene ring with a C–N torsion of 12.4° is distorted in a manner very similar to that seen in *1* at N(2), but the other ring with a 74.6° torsion angle shows only small bond angle distortions. The observed C(7)–N(1) torsion in the present compound supports the view that the N(2)H_2 group is responsible for the major part of the ring distortions.

Both the bonds N(1)–C(1) and C(1)–C(2) have a high degree of double bond character, the bond lengths being 1.324(2) and 1.390(2) Å, respectively. The non-aromatic part of the molecule approximates to a plane, except for the acetyl group C(6)–C(5)–O(2) which forms an angle of 9.2(2)° to that plane. The non-coplanar orientation of the acetyl group is probably due to the hydrogen bonds accepted by O(2) (*vide infra*).

Table 2. Torsion angles.

Atoms	Angle (°)
N(2)–C(8)–C(7)–N(1)	−2.9(3)
C(8)–C(7)–N(1)–C(1)	−145.0(2)
C(7)–N(1)–C(1)–C(2)	−178.3(2)
N(1)–C(1)–C(2)–C(3)	−178.9(2)
N(1)–C(1)–C(2)–C(5)	−0.2(3)
C(1)–C(2)–C(3)–C(4)	−1.2(3)
C(1)–C(2)–C(3)–O(1)	179.3(2)
C(1)–C(2)–C(5)–C(6)	171.2(2)
C(1)–C(2)–C(5)–O(2)	−8.4(2)

The approximate planarity of the molecule causes crowding shown by the distorsions of the bond angles at the C(2), C(3) and C(5) atoms. Thus for example the C(methyl)–C–O angles are 117.5°

Table 3. Geometry of the hydrogen bonding.

(a) intramolecular	
N(1)···O(2) (Å)	2.593(2)
N(1)–H(N1)	0.90(2)
H(N1)···O(2)	1.88(2)
N(1)–H(N1)···O(2) (°)	135(2)
C(1)–N(1)–H(N1)	114.5(1.3)
C(5)–O(2)···H(N1)	104.5(7)
(b) within a molecular pair	
N(2)···O(1) (Å)	3.130(2)
N(2)–H(N21)	0.89(2)
H(N21)···O(1)	2.28(2)
N(2)–H(N21)···O(1) (°)	160(2)
C(8)–N(2)–H(N21)	115.9(1.4)
C(3)–O(1)···H(N21)	131.0(6)
(c) between pairs	
N(2)···O(2) (Å)	3.056(2)
N(2)–H(N22)	0.91(3)
H(N22)···O(2)	2.16(3)
N(2)–H(N22)···O(2) (°)	167(2)
C(8)–N(2)–H(N22)	115.1(1.4)
C(5)–O(2)···H(N22)	120.8(6)

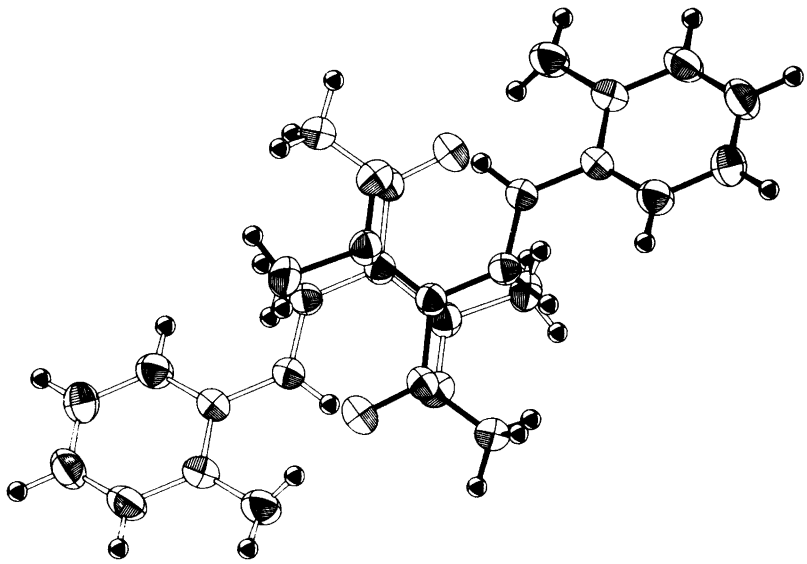

Fig. 2. Overlap diagram of the molecular complex.

while it is normally the angle opposite to the C–O double bond that is less than 120°. The two methyl groups have the same conformation with one of the CH bonds eclipsed with the adjacent carbonyl group.

Intramolecular hydrogen bond. The asymmetric intramolecular hydrogen bond N(1)–H···O has an N···O distance of 2.593(2) Å with the geometry shown in Table 3. The slight rotation of the acetyl oxygen out of the plane of the other atoms in the chelate ring is probably necessary to avoid excessive repulsion. This is a very short hydrogen bond for a neutral molecule, comparable to *e.g.* those in nitromalonamide[15] of 2.580(3) and 2.589(5) Å.

Molecular complex. In the crystal the molecules are arranged in pairs around inversion centres. The approximately planar non-aromatic parts of the molecules overlap as shown in Fig. 2. with an interplanar spacing of 3.45 Å, and the shortest contact, C(3)···O(2), is 3.296(2) Å. Double bonds are arranged alternatingly in the two planes making π-π* electron transfer feasible. This self-complex is further stabilized by two weak hydrogen bonds N–H···O of 3.130(2) Å involving the amino groups and the

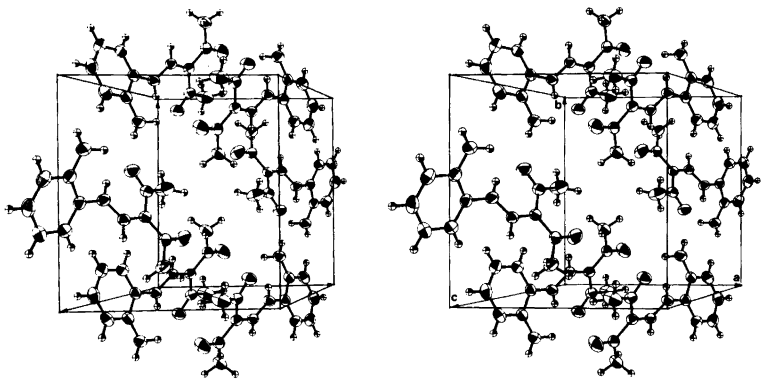

Fig. 3. Stereoscopic view of the molecular packing perpendicular to the *ab*-plane, and with the unit cell outlined.

O(1) atoms. The interplanar spacing is rather long considering the small relative translations in the overlap diagram so the charge transfer interaction is small. The yellow, though intense, colour is another indication of this.

Packing of molecular pairs. The dimeric complexes are arranged in layers, one unit cell thick, parallel to the *bc*-plane. Each pair is connected to the four surrounding units (see Fig. 3) by four hydrogen bonds amino group – carbonyl oxygen O(2) (see Table 3). Thus O(2) accepts one intramolecular and one intermolecular hydrogen bond. Most of the short nonbonded interactions are also within the layer. The weak intermolecular forces in the *a* direction are consistent with the observed crystal morphology.

Acknowledgement. We are indebted to Dr. R. Larsson for valuable discussions.

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Studies of Substituted *N*-Benzoyl-2-pyridinecarboxamides. Reactions with Acyl Chlorides and Other Electrophiles

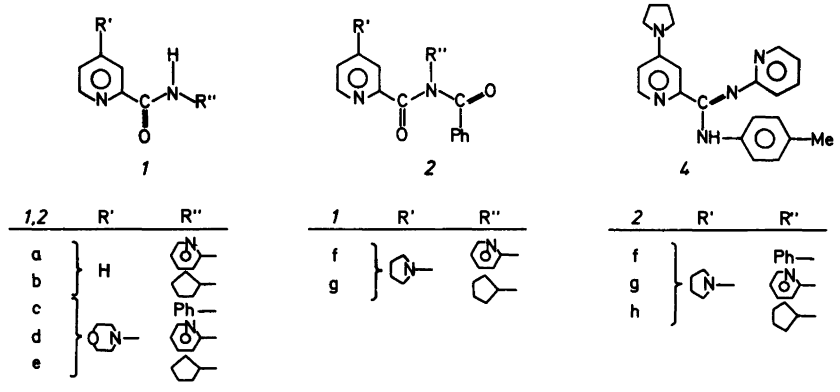
EVA H. MØRKVED

Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

N-Benzoyl-4-(4-morpholinyl)-2-pyridinecarboxamides react as nucleophiles towards benzoyl chloride to give adducts which are stabilized by further addition of one mol of water. The structure of these compounds is elucidated by chemical and spectroscopic methods. The same compounds can be obtained from reactions of 4-(4-morpholinyl)-2-pyridinecarboxamides with two molar equivalents of benzoyl chloride and triethylamine. *N*-Benzoyl-4-(1-pyrrolidinyl)-2-pyridinecarboxamides react with benzoyl chloride to give less stable adducts, whereas *N*-benzoyl-2-pyridinecarboxamides are unreactive towards benzoyl chloride. Methyl iodide, *N*,*N*-diphenylcarbamoyl chloride and acetic anhydride are unreactive towards the 4-*N*,*N*-dialkylamino-2-pyridinecarboxamides and their *N*-benzoyl derivatives. These *N*-benzoylamides give unstable adducts with methyl chloromethanoate, acetyl chloride or *p*-toluenesulfonyl chloride. The adducts decompose to the hydrochlorides of the rearranged *N*-benzoylamides and the acyl imidate hydrochlorides are isolated from these reactions. 2-(Benzoylamino)pyridine reacts with a mixture of thionyl chloride and phosphorus(V) chloride to give the corresponding imidoyl chloride hydrochloride.

Compounds *2*, see Scheme 1, have two important features which are of interest to us. Firstly, the *N*-acylamide function has a spatial arrangement so that an acyl-group transfer from the amide nitrogen to the pyridine nitrogen would go through a five-membered ring transition state. Secondly, the pyridine-4-*N*,*N*-dialkylamino substituent of *2c–h* will enhance the nucleophilicity of the pyridine nitrogen and, therefore, is expected to promote such intramolecular acyl-group transfers.

Recent studies have shown[1] that *N*-acyl-2-pyridinecarboxanilides, which are closely related to *2*, can be prepared by various methods. Presently *2a* is prepared in almost quantitative yield by direct benzoylation of *1a*, *cf.* Scheme 1, whereas 2-



Scheme 1.

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pyridinecarboxanilides were quite resistant to acylation.[1] Therefore, the amide *N*-substituent is of importance for the reactivity of these amides towards acyl halides. The present studies also show the importance of the pyridine-4-*N*,*N*-dialkylamino substituent of both *1* and *2*. For instance, if *2a* or *2d* is treated with benzoyl chloride compound *2a* is recovered unchanged whereas *2d* forms an addition compound with the acid chloride. Moreover, the same addition compound also is obtained from *1d* and two mol equivalents of benzoyl chloride and triethylamine.

RESULTS

Various reaction sequences are expected [1] to give the *N*-benzoylamides *2a–h*. Compounds *2a*, *2d* and *2g* are prepared from the triethylammonium 2-pyridinecarboxylates and *N*-(2-pyridyl)benzimidoyl chloride hydrochloride. A good yield of this imidoyl chloride hydrochloride is obtained from the chlorination of 2-(benzoylamino)pyridine with a mixture of phosphorus(V) chloride and thionyl chloride.[2] Other workers [3a] have reported unsuccessful attempts to prepare this imidoyl chloride by chlorinating 2-(benzoylamino)pyridine with either phosphorus(V) chloride or thionyl chloride. An alternative method of preparing *2a*, *2d* and *2g* would be reaction of benzoic acid and a base with the imidoyl chlorides derived from the amides *1a*, *1d* and *1f*. This method, however, would be of little practical use since the chlorination products of *1a*, *1d* and *1f* are extremely hygroscopic. The chlorination product from *1f* gives a moderate yield of the amidine *4*, *cf.* Scheme 1, from a reaction with 4-methylaniline thus identifying the chlorination product as either the mono- or the dihydrochloride of the imidoyl chloride derived from *1f*.

Compound *2a* also is obtained in excellent yield by direct benzoylation of *1a* with benzoyl chloride in the presence of triethylamine. This reaction may be compared to the rapid dibenzoylation of 2-aminopyridine with benzoyl chloride alone [3b] or in the presence of triethylamine.[3c] Thus, analogous to the postulated mechanism for these reactions [3b] the aminopyridine ring-nitrogen of *1a* might initially be benzoylated and a rapid intramolecular transfer of the benzoyl group to the amide nitrogen would give *2a*. Such a mechanism also would explain the enhanced reactivity of *1a* compared to the reactivity of 2-pyridinecarboxanilides [1] towards benzoyl chloride. Similarly, *1b* is less reactive than *1a* towards benzoyl chloride and a fair yield (40 %) of *2b* is obtained from a reaction of *1b* with benzoyl chloride and triethylamine.

Direct benzoylation of compound *1d* in benzene with two molar equivalents of benzoyl chloride and triethylamine yields a benzene insoluble precipitate which is dissolved in dichloromethane. Triethylammonium chloride is removed by extraction with aqueous sodium hydrogen carbonate and the derivative of *1d* is crystallized from acetone. The same product also is obtained from a reaction of a benzene solution of *2d* with benzoyl chloride. However, the *N*-benzoylamide *2a* is recovered unchanged after treatment with benzoyl chloride. This indicates that the increased nucleophilicity of the 4-(4-morpholinyl)pyridine moiety of *1d* or *2d* is of importance for these reactions. Furthermore, both *2c* and *2e* give similar addition products with benzoyl chloride whereas *2b* is unreactive towards benzoyl chloride. The proposed structures *3a–c*, *cf.* Scheme 2, are indicated by the following observations. Analyses, C, H, Cl and N, for *3a* and *3b* are correct for the addition of 1 mol each of benzoyl chloride and water to *2c* or *2d*. Compound *3a* does not react with triethylamine, and it also is formed in a reaction of *2c* with benzoyl chloride and triethylamine. Therefore, no acidic protons such as pyridinium protons are present in *3a–c*. However, sodium hydride, which is able to react as a base towards hydroxyl hydrogens, does in fact react with *3a* and a 65 % yield of *2c* is obtained. The purple color of this reaction mixture indicates that the fair yield of *2c* may be due to cleavage of the pyridine ring.

A reaction of *3b* with cyclopentylamine in acetonitrile indicates reactions of the amine both as a base and as a nucleophile. Thus, GLC analysis of the reaction mixture shows the presence of equimolar amounts of the three amides *1e*, 2-(benzoylamino)pyridine and *N*-cyclopentylbenzamide. This product mixture may be explained as follows. A nucleophilic attack by cyclopentylamine on the protonated benzoyl group at the pyridine nitrogen of *3b* would give an adduct which by assistance of another molecule of the base would deprotonate. A subsequent dehydration and fragmentation would give equimolar amounts of *N*-cyclopentylbenzamide and *2d*. The latter compound reacts with a third molecule of cyclopentylamine to give *1e* and 2-(benzoylamino)pyridine.

From a similar reaction of cyclopentylamine with

a suspension of *3b* in benzene a 73% yield of cyclopentylammonium chloride was isolated as benzene insoluble material after 21 h. Equimolar amounts of *1e*, 2-(benzoylamino)pyridine and *N*-cyclopentylbenzamide were present in the filtrate which was analyzed by GLC.

IR absorptions also support structures *3a–c*; these compounds show two absorptions of medium strength in the hydroxyl region 3440–3350 cm^{-1}, *3a* and *3b* show two strong absorptions whereas *3c* has one strong absorption in the carbonyl region 1760–1730 cm^{-1}. Finally, the strong absorption at 1655–1660 cm^{-1} shown by these compounds is in the area of an immonium ($C=N^+$) bond.

^1H NMR resonances of nitromethane-d_3 solutions of compounds *3* indicate the presence of two hydroxyl hydrogens, δ 2.3 for *3a*, δ 2.7 for *3b* and δ 7.4 for *3c*. The pyridine protons of *3* resonate at δ 7.1–8.3 and, therefore, structures similar to *3* but with the hydroxyl group bound to the pyridine-6 carbon are ruled out. The latter type of compounds is expected to show a doublet at δ 4–6 due to the proton at the saturated pyridine-6 carbon.[4] Both *N*-acyl-[5a] and *N*-alkoxycarbonyl-[5b,c] 4-*N*,*N*-dimethylpyridines show ^1H NMR resonances at δ 8.6–8.8 for the pyridine H_α and at δ 7–7.2 for the pyridine H_β atoms. These observations therefore are in accordance with the slightly lower δ-value, 8.2–8.3, assigned to the H_α atom at the pyridine-6 carbons of *3a–c*, and the δ-values, 7.1–7.2 assigned to the H_β-atom at the pyridine-5 carbons of *3*.

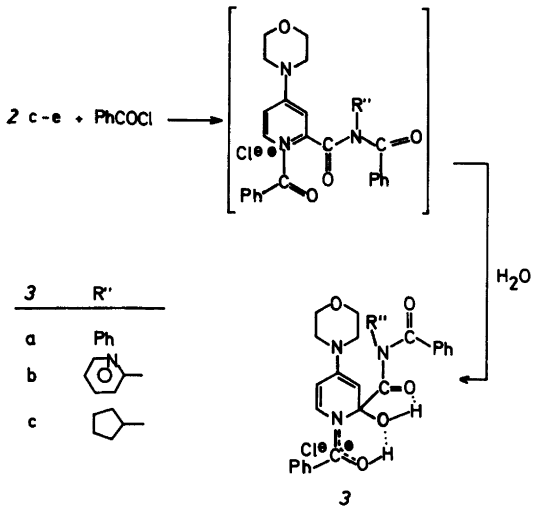
Additional evidence for structure *3* also may be deduced from the ^{13}C NMR resonances which are shown in Table 1. *N*-Protonated pyridines are known to have shielded C_α atoms and a deshielded C_γ compared to pyridine itself.[6] Such changes are observed for the pyridine-carbons 2, 4 and 6 of *2c·HCl* compared to *2c*. However, the δ-values of these atoms in *2c·HCl* and *3a* are about equal and this observation may be explained by two counteracting effects. Since pyridine *N*-acylation[7] has a somewhat less shielding effect than pyridine *N*-protonation,[6] pyridine *N*-benzoylation as in *3a* might deshield the pyridine carbons 2 and 6 as compared to *2c·HCl*. However, loss of aromaticity in the pyridine ring by introduction of the 2-hydroxyl group is expected to have a shielding effect on carbons 2 and 6 and the result might be as observed.

The formation of compounds *3a–c* might deserve some brief comments. Initially an addition of the benzoyl group to the pyridine-nitrogen of *2c–e* certainly occurs. However, Catalin models of 4-(4-morpholinyl)pyridine show a considerable steric hindrance to coplanarity between the pyridine and the morpholine rings. Therefore, even if morpholine will enhance the nucleophilicity of the pyridine nitrogen of *2c–e*, much of the positive charge is expected to remain on the pyridine nitrogen of the initial adducts with benzoyl chloride. These adducts, like most *N*-acylpyridinium salts are expected to be sensitive to moisture.[8] No precautions except using dry solvents were taken to protect these reaction products from moisture, therefore, water easily might add across the pyridine-*N* carbonyl and the pyridine-2 carbon as shown in Scheme 2. This addition may be compared to the formation of Reissert compounds from *N*-acylpyridinium salts. Most acylpyridinium salts are known to react with nucleophiles at the pyridine-4 position giving 1,4-

Table 1. ^{13}C NMR resonances.[a]

Compound	Solvent	Pyridine carbon					Carbonyl	Morpholine
		2	3	4	5	6		
1c	$CDCl_3$	156.2	106.5	150.6	109.7	148.5	162.6	66.2; 46.1
2c	$CDCl_3$	155.6	108.9	152.9	109.9	148.9	173.1; 172.5	66.2; 46.0
2c·HCl	CD_3OD	140.6	104.2	158.4	111.8	136.9	159.8[b]	67.1 [c]
	DMSO-d_6	139.0	102.4	157.0	110.3	135.8	157.6[b]	
3a	$CDCl_3$	139.9	102.1	158.3	114.8	136.0	163.9; 158.3[d]	66,6, 65.9; 49.2, 48.0
	DMSO-d_6	139.2	103.4	157.7	110.7	135.8	163.7; 158.2	65.5; 47.8, 47.0
	DMF-d_7	140.6	104.0	158.9	111.7	136.7	163.7; 161.4	66.5; 48.8

[a] A complete ^{13}C NMR analysis of these and related compounds is in preparation. [b] This resonance probably is due to both C=O and C=N of the acyl imidate structure of this compound, *cf.* Ref. 1. [c] Overlap with CD_3OD resonances. [d] Resolution into two resonances, δ 158.35 and 158.23 at 400 MHz on a Bruker VM-400 instrument.



Scheme 2.

dihydropyridines,[9] but one fairly stable 1,2-dihydropyridine, the first known Reissert compound in the pyridine series, has been isolated.[10] However, initial attack at the pyridine-2 position also has been postulated in some other cases,[11] but unfavorable equilibria might be responsible for the difficulty in isolating such 1,2-dihydropyridines.[10] Thus, we may conclude that nucleophilic attack by water at the pyridine-2 position of the benzoylpyridinium adducts of *2c–e* yields the 1,2-dihydropyridine derivatives *3a–c*. These are stabilized by both charge delocalization and intramolecular hydrogen bonding and, therefore, are isolated without difficulty.

Compounds *3* might be mixtures of two rotational isomers since for instance 1-ethoxycarbonyl-2,4-di-*tert*-butyl-1,2-dihydropyridine was found[4a] to be a mixture of two such isomers. Another explanation for the relatively wide melting range of compounds *3a* and *3c* might be the existence of dimorphic forms which are reported[9b] for some Reissert compounds.

The *N*-acylamides *2f* and *2g* which have pyrrolidine as the pyridine-4 substituent also give addition compounds with benzoyl chloride. However, these compounds seem to be less stable than *3a–c*. Other electrophiles also react with compounds *2*, but pure products are not obtained from reactions of *2c* with either methyl chloromethanoate or with acetyl chloride. The latter product decomposes slowly when dissolved in nitromethane and yields *2c·HCl* after several weeks. Only *2c·HCl* is obtained from a reaction of *2c* with *p*-toluenesulfonyl chloride. No addition compounds are obtained from *1d*, *2d* or *2f* and *N,N*-diphenylcarbamoyl chloride, from *2f* and acetic anhydride or from *2f* and methyl iodide. Even if steric effects might be responsible for the low reactivity *N,N*-diphenylcarbamoyl chloride, electronic effects certainly are important in the other reactions.

Compounds *2c* and *2f* react with hydrogen chloride to give the expected acyl imidate hydrochlorides[1,12] whereas *2d* decomposes when reacted similarly.

The present exploratory studies have clearly demonstrated the effect by the pyridine-4-*N,N*-dialkylamino substituent of compounds *2c–h*. These compounds react as nucleophiles towards acyl chlorides and related electrophiles whereas the unsubstituted compounds *2a–b* are unreactive towards the same electrophiles. However, there are several unanswered questions pertaining to the exact mechanisms of the observed acylations of compounds *1*. There also are unanswered questions about the relative importance of steric and electronic effects for the reactivity of *1* and *2* as nucleophiles or as bases. Further studies of these and similar compounds are in progress.

EXPERIMENTAL

General. IR spectra were recorded on a Perkin Elmer 254 grating spectrometer. Mass spectra were obtained on an AEI MS902 spectrometer with 70 eV bombarding electron energy. ^{1}H NMR and ^{13}C NMR spectra were obtained at 100 MHz on a Jeol JNM-FX100 Fourier Transform NMR spectrometer. GLC analyses were carried out on a Pye Unicam 104 instrument with flame ionization detector and a glass column: 5% OV–17 (150 cm, 2.2 mm i.d.) on Chromosorb W AW–DMCS 80 –100 mesh. All melting points are uncorrected and were obtained on a Büchi "Tottoli" melting point apparatus. Elemental analyses were carried out at Analytische Laboratorium, Elbach, Germany. Silica gel, 63–200 μm, for column chromatography was obtained from Merck, Merck kieselgel 60 F 254 was used for TLC.

Cyclopentylamine, 4-methylaniline, *N,N*-diphenylcarbamoyl chloride, all *purum*, and sodium hydride, 55–60% in oil, practical grade were obtained from Fluka. Methyl chloromethanoate, reagent grade was obtained from BDH, *p*-toluenesulfonyl chloride, reagent grade, from Baker

and methyl iodide, reagent grade, from Merck.

2-Pyridinecarboxamides, 1a – g. The preparation of compound *1c* has been described.[2] The same method of preparation was used for *1a*, *1b*, *1d* – *1g* except that triethylamine was used instead of pyridine as a base.

1a (78%) m.p. 116 – 117 °C (ligroin), lit.[13] no physical constants reported. IR (nujol): 3350 (s), 1695 (s) cm^{-1}.

1b (82%) m.p. 80 – 81 °C (hexane). MS [*m/e* (% rel. int.)]: 190 (28.0, M). Mol. wt., obs. 190.1101, calc. for $C_{11}H_{14}N_2O$ 190.1106. IR (nujol): 3320 (m), 1655 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.46 – 2.17 (8H, m), 4.40 (1H, m), 7.31 – 8.56 (5H, m).

1d (72%) m.p. 140 – 141 °C (benzene). MS [*m/e* (% rel. int.)]: 284 (50.3, M). Mol. wt., obs. 284.1280, calc. for $C_{15}H_{16}N_4O_2$ 284.1273. IR (nujol): 3360 (s), 1700 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 3.43 (4H, m), 3.83 (4H, m), 6.9 – 8.4 (7H, m), 10.49 (1H, broad s).

1e (50 %) m.p. 85 – 87 °C. MS [*m/e* (% rel. int.)]: 275 (23.5, M). Mol. wt., obs. 275.1636, calc. for $C_{15}H_{21}N_3O_2$ 275.1634. IR (nujol): 3250 (s), 1650 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.1 – 2.3 (8H, m), 3.37 (4H, m), 3.77 (4H, m), 4.3 (1H, m). 6.81 (1H, dd, *J* 2.9 Hz), 7.64 (1H, d, *J* 2.9 Hz), 8.0 (1H, s), 8.11 (1H, d, *J* 5.7 Hz).

1f (62%) m.p. 175 – 176 °C (benzene). MS [*m/e* (% rel. int.)]: 268 (44.7, M). Mol. wt., obs. 268.1327, calc. for $C_{15}H_{16}N_4O$ 268.1324. IR (nujol): 3300 (s), 1685 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.1 (4H, m), 3.46 (4H, m), 6.6 – 8.4 (7H, m), 10.5 (1H, broad s).

1g (50 %) m.p. 92 – 94 °C (hexane – diethyl ether). MS [*m/e* (% rel. int.)]: 259 (22.4, M), Mol. wt., obs. 259.1687, calc. for $C_{15}H_{21}N_3O$ 259.1685. IR (nujol): 3290 (m), 1645 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.44 – 2.14 (12H, m), 3.34 (4H, m), 4.34 (1H, m), 6.37 (1H, dd, *J* 2.9 Hz), 7.31 (1H, d, *J* 2.9 Hz), 7.97 (1H, s), 8.06 (1H, d, *J* 5.7 Hz).

N-(2-Pyridyl)benzimidoyl chloride hydrochloride. A solution of 2-(benzoylamino)pyridine[14] (790 mg, 4 mmol) and phosphorus(V) chloride (860 mg, 4.1 mmol) in 6 ml of thionyl chloride was heated at 60 °C for 20 min. Dry benzene (10 ml) was added and the solvents were removed under reduced pressure. Acetone (3 ml) was added to the residue which was filtered and gave 700 mg (69%) of *N*-(2-pyridyl)benzimidoyl chloride hydrochloride m.p. 151 – 155 °C dec. Lit.[3a] m.p. 152 – 157 °C dec. IR (nujol): 1670 (s) cm^{-1}.

N-Cyclopentylbenzimidoyl chloride. A mixture of *N*-cyclopentylbenzamide[15] (1.9 g, 10 mmol) and phosphorus(V) chloride (2.1 g, 10 mmol) in 30 ml of benzene was heated under reflux for 1 h. The solvent was removed under reduced pressure. The residue was boiled with 20 ml of hexane, the hot solution was filtered and 2.0 g (95%) liq. of the title compound was obtained from the filtrate. IR (film): 1665 (s) cm^{-1}.

N-Benzoyl-2-pyridinecarboxamides, 2a – h. Compounds *2c* and *2f* have been described.[16] Compounds *2a*, *2b*, *2d*, *2e*, *2g* and *2h* were prepared by the following procedure. To a mixture of the 2-pyridinecarboxylic acid (2 mmol) and triethylamine (4 mmol) in 30 ml of acetonitrile was added either *N*-(2-pyridyl)benzimidoyl chloride hydrochloride (2 mmol) or *N*-cyclopentylbenzimidoyl chloride (2 mmol). The reaction mixture was heated at 50 – 60 °C for 2 h. The solvent was removed under reduced pressure, and 15 ml of benzene was added to the residue. Triethylammonium chloride was removed by filtration and the filtrate was chromatographed on silica gel. Compounds *2a* and *2b* were eluted with chloroform whereas compounds *2d*, *2e*, *2g* and *2h* were eluted with acetone.

2a (92%) m.p. 130 – 131 °C (diethyl ether and hexane). MS [*m/e* (% rel. int.)]: 303 (39.2, M). Mol. wt., obs. 303.1014, calc. for $C_{18}H_{13}N_3O_2$ 303.1008. IR (nujol): 1710 (s), 1695 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 7.2 – 7.6 (6H, m), 7.8 – 8.0 (5H, m), 8.3 – 8.5 (2H, m).

2b (79%) m.p. 71 – 73 °C. MS [*m/e* (% rel. int.)]: 294 (3.0, M). Mol. wt., obs. 294.1368, calc. for $C_{18}H_{18}N_2O_2$ 294.1368. IR (nujol): 1705 (s), 1650 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.6 – 2.0 (8H, m), 5.0 (1H, m), 7.15 – 7.70 (8H, m), 8.34 (1H, d, *J* 4.9).

2d (45%) m.p. 178 – 181 °C dec. (diethyl ether). MS [*m/e* (% rel. int.)]: 388 (41.7, M). Mol. wt., obs. 388.1540, calc. for $C_{22}H_{20}N_4O_3$ 388.1535. IR (nujol): 1705 (s), 1695 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 3.4 (4H, m), 3.8 (4H, m), 6.7 – 8.4 (12H, m).

2e (44%) m.p. 146 – 147 °C (diethyl ether). MS [*m/e* (% rel. int.)]: 379 (22.7, M). Mol. wt., obs. 379.1900, calc. for $C_{22}H_{25}N_3O_3$ 379.1896. IR (nujol): 1705 (s), 1670 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.5 – 2.2 (8H, m), 3.2 (4H, m), 3.7 (4H, m), 4.9 (1H, m), 6.6 (1H, dd, *J* 2.9 Hz), 7.0 – 7.6 (6H, m), 7.95 (1H, d, *J* 5.7 Hz).

2g (48%) m.p. 170 – 172 °C dec. (diethyl ether). MS [*m/e* (% rel. int.)]: 372 (29.6, M). Mol. wt., obs. 372.1584, calc. for $C_{22}H_{20}N_4O_2$ 372.1586. IR (nujol): 1710 (s), 1690 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.2 (4H, m), 3.6 (4H, m), 6.7 – 8.5 (12H, m).

2h (49%) m.p. 98 – 100 °C (diethyl ether and hexane). MS [*m/e* (% rel. int.)]: 363 (24.3, M). Mol. wt., obs. 363.1945, calc. for $C_{22}H_{25}N_3O_2$ 363.1947. IR (nujol): 1705 (s), 1660 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.5 – 2.15 (12H, m) 3.4 (4H, m), 4.8 (1H, s), 6.6 (1H, dd, *J* 2.9 Hz), 7.25 (1H, d, *J* 2.9 Hz), 7.4 – 7.6 (4H, m), 8.0 – 8.15 (2H, m).

Reactions of *1* with Acyl Chlorides

Benzoylation of 1a. A solution of *1a* (200 mg, 1 mmol), benzoyl chloride (140 mg, 1 mmol) and triethylamine (152 mg, 1.5 mmol), in 8 ml of benzene

was heated at 80 °C for 17 h. Triethylammonium chloride (140 mg, 1 mmol) was removed by filtration. The benzene filtrate was concentrated, chromatographed on silica gel with chloroform as the eluent and 270 mg (90%) of *2a* m.p. 129–130 °C was obtained. The IR absorptions, the TLC Rf (chloroform) and the molecular weight of this compound were identical to those of *2a* which has been obtained from triethylammonium 2-pyridinecarboxylate and *N*-(2-pyridyl)benzimidoyl chloride hydrochloride.

Benzoylation of 1b. Benzoyl chloride (112 mg, 0.8 mmol) was added to a solution of *1b* (150 mg, 0.79 mmol) and triethylamine (80 mg, 0.8 mmol) in 10 ml of benzene. The reaction mixture was heated under reflux for 44 h, triethylammonium chloride (75 mg, 0.55 mmol) was removed by filtration and the filtrate was chromatographed on silica gel. The chloroform eluate yielded 80 mg of a liquid which was shown by TLC to be a mixture of *1b* and *2b*, and 90 mg (39%) of *2b* m.p. 70–72 °C.

Benzoylation of 1d. To a 10 ml benzene solution of benzoyl chloride (155 mg, 1.1 mmol) and triethylamine (120 mg, 1.2 mmol) was added *1d* (150 mg, 0.5 mmol). The yellow solution was heated at 60–80 °C for 1.5 h. The solvent was removed under reduced pressure and the solid residue was extracted with dichloromethane and aqueous sodium hydrogencarbonate. The dichloromethane extract gave, after drying and removal of the solvent, a solid residue. Addition of acetone to this residue yielded 160 mg (55%) of *3b* m.p. 159–160 °C dec.

Anal. $C_{29}H_{27}ClN_4O_5$: C, H, Cl, N. IR (nujol): 3430 (m), 3360 (m), 1760 (s), 1730 (s) and 1660 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.74 (2H, broad s), 3.89 (4H, s), 4.0 (4H, s) 7.14–8.3 (17H, m).

Benzoylation of 1f. Benzoyl chloride (141 mg, 1 mmol) was added to a solution of *1f* (134 mg, 0.5 mmol) and triethylamine (101 mg, 1 mmol) in 10 ml of benzene. The reaction mixture was heated at 50 °C for 2 h, and then left at ambient temperature for 60 h. The white precipitate was removed by filtration and yielded 320 mg m.p. 155–250 °C dec. IR (nujol): 3450 (m), 3350 (m), 2600 (s), 2490 (s), 1770 (s), 1730 (s), 1665 (s), 1575 (s). This product mixture was extracted with 40 ml of chloroform and 15 ml of water. The chloroform extract was dried over magnesium sulfate and yielded an oily residue upon removal of the solvent. Diethyl ether (10 ml) was added to the residue which crystallized and was filtered after 2h, dried at 0.1 mm Hg and gave 180 mg m.p. 127–137 °C dec. IR (nujol): 3400–3300 (m), 1750–1730 (s), 1665 (s), 1575 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.0–2.6 (6H, broad s), 3.65–4.0 (4H, m), 7.1 (1H, dd, *J* 2.9 Hz), 7.2–8.3 (16H, m).

Benzoylation of 1g. Benzoyl chloride (141 mg, 1 mmol) was added to a solution of *1g* (130 mg, 0.5 mmol) and triethylamine (101 mg, 1 mmol) in 10 ml of benzene. White needles were removed by filtration after 1 h at 55 °C and yielded 120 mg (0.87 mmol) of triethylammonium chloride, m.p. 257–260 °C subl. The filtrate was left at ambient temperature for 2h, some white crystalline material was removed by filtration and gave 150 mg m.p. 95–117 °C gas ev. IR (nujol): 3440–3340 (s), 2600 (w), 2500 (w), 1740 (s), 1660 (s), 1575 (s) cm^{-1}. This product was extracted with 15 ml of chloroform and 3 ml of water. The dried chloroform extract yielded, upon removal of the solvent, and oily residue. The residue was triturated with diethyl ether, the solid was filtered, dried at 0.1 mm Hg and gave 95 mg m.p. 92–110 °C gas ev.; 165–175 °C dec. IR (nujol): 3440–3340 (s), 1740 (s), 1665 (s), 1575 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.5–2.0 (4H, m), 2.2 (4H, m), 2.52 (6H, s), 3.6–3.8 (3H, m), 6.8–8.2 (8H, m).

Reaction of 1d *with* N,N-*diphenylcarbamoyl chloride.* A solution of *N,N*-diphenylcarbamoyl chloride (163 mg, 0.7 mmol) in 5 ml of benzene was added to a solution of *1d* (200 mg, 0.7 mmol) and triethylamine (100 mg, 1 mmol) in 10 ml of benzene. The clear solution was heated at 60 °C for 1 h and then left at ambient temperature for 60 h. The solvent was removed under reduced pressure, 4 ml of diethyl ether was added to the residue and 170 mg (85%) of *1d*, m.p. 137–139 °C was removed by filtration. The filtrate was concentrated and yielded 130 mg (80%) m.p. 79–83 °C of *N,N*-diphenylcarbamoyl chloride.

Reactions of *2* with Acyl Chlorides

Reactions with benzoyl chloride. Benzoyl chloride was added to a benzene solution of an equimolar amount of *2*. After a given reaction time at a specified temperature the product was removed by filtration, washed with benzene and was dried at ambient temperature at 0.1 mmHg.

Benzoyl chloride and 2a gave no benzene-insoluble material after 21 h at ambient temperature; *2a* (85%) m.p. 128–130 °C was recovered from the benzene solution.

Benzoyl chloride and 2b gave no benzene insoluble material after 3 h at 50 °C. The benzene solution was chromatographed on silica gel and *2b* (85%) m.p. 71–72 °C was obtained from the chloroform eluate.

Benzoylation of 2c yielded after 1 h at 30–40 °C a product m.p. 148–158 °C dec. This product was stirred with 3 ml of acetone for 1 h and yielded after filtration and drying *3a* (64%) m.p. 158–168 °C dec. Anal. $C_{30}H_{28}ClN_3O_5$: C, H, Cl, N. IR (nujol): 3420 (m), 3350 (m), 1755 (s), 1735 (s), 1655 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.3 (2H, s), 4.0 (8H, m), 7.1–8.2 (18H, m). Compound *3a* (71%) m.p. 156–164 °C dec. also was obtained from the same reactants except that acetone instead of benzene was used as the solvent.

In another experiment equimolar amounts of benzoyl chloride and triethylamine were dissolved in benzene. A benzene solution of one molar equivalent of *2c* was added. The white precipitate was filtered after 30 min at 35 °C, then triturated with water and yielded after drying *3a* (85%) m.p. 160–170 °C dec. This product showed identical IR absorptions and ^{1}H NMR resonances to *3a* which had been obtained from *2c* and benzoyl chloride.

Benzoylation of 2d yielded after 2 h at 40 °C *3b* (67%) m.p. 161–163 °C dec. IR absorptions and ^{1}H NMR resonances of this product were identical to those of the product which was obtained by benzoylation of *1d*.

Benzoylation of 2e yielded after 1 h at ambient temperature *3c* (93%) m.p. 137–145 °C gas ev. IR (nujol): 3440 (m), 3360 (m), 1745 (s), 1660 (s), cm^{-1}. ^{1}H NMR (CD_3NO_2): δ 1.4–2.3 (8H, m), 4.33 (8H, m), 7.15–8.25 (16H, m). Compound *3c* was recovered unchanged from a nitromethane solution. MS [*m/e* (% rel. int.)]: 379 (8.1, M–PhCOCl $-H_2O$).

Benzoylation of 2f. To a solution of *2f* (75 mg, 0.2 mmol) in 8 ml of benzene was added benzoyl chloride (30 mg, 0.2 mmol). The reaction mixture was stirred at 45 °C for 30 min. The white precipitate was filtered, washed with benzene and yielded after drying 85 mg m.p. 124–126 °C dec. Anal. Found: C, 67.77; H, 5.25; Cl, 6.14; N, 7.34. Calc. for $C_{30}H_{28}ClN_3O_4$ (*2f*+PhCOCl+H_2O): C, 67.98; H, 5.32; Cl, 6.69; N, 7.92. IR (nujol): 3350 (m, broad), 1740 (s), 1665 (s), 1580 (s) cm^{-1}. ^{1}H NMR (CD_3NO_2): δ 2.2 (4H, m), 3.7–4.0 (5H, m), 7.0–8.2 (18H, m).

Benzoylation of 2g. To a solution of *2g* (56 mg, 0.15 mmol) in 5 ml of benzene was added benzoyl chloride (20 mg, 0.15 mmol). The reaction mixture was stirred at 35 °C for 15 min, the white precipitate was filtered, washed with benzene and yielded 75 mg m.p. 115–120 °C gas ev. IR (nujol): 3400–3300 (m), 1750–1730 (s), 1665 (s), 1575 (s) cm^{-1}.

This product had decomposed after 3–4 weeks at ambient temperature.

Reactions of *2* with other electrophiles

The electrophile was added to a benzene solution of an equimolar amount of *2*. After a given reaction time at a specified temperature the product was removed by filtration, washed with benzene and was dried at 0.1 mmHg.

2c *and methyl chloromethanoate* were reacted for 30 min. at 30 °C and gave white crystals m.p. 110–120 °C gas ev. Anal. Found: C, 64.39; H, 5.14; N, 9.36. Calc. for $C_{25}H_{24}ClN_3O_5$ (*2c*+$ClCOOCH_3$): C, 62.30; H, 5.01; N, 8.71. Calc. for $C_{23}H_{22}ClN_3O_2$ (*2c*+HCl): C, 67.72; H, 5.44; N, 10.30. IR (nujol): 3400–3320 (w) 1770 (s, broad), 1665 (s) cm^{-1}. ^{1}H NMR (CD_3NO_2): δ 3.4–3.57 (4H, m), 3.76–4.1 (7H, m), 6.8–8.11 (15H, m).

2c *and acetyl chloride* were reacted for 30 min at 30–35 °C and gave white crystals m.p. 130–145 °C gas ev. IR (nujol): 3420–3320 (w), 1770 (sh), 1750 (s), 1660 (s) cm^{-1}. ^{1}H NMR (CD_3NO_2): δ 2.36 (3H, s), 3.5–4.0 (10H, m), 7.1–8.2 (13H, m). This product had decomposed to *2c·HCl* after four weeks in a nitromethane solution.

2c *and* p-*toluenesulfonyl chloride* gave no immediate precipitate at ambient temperature. White crystals formed slowly and were filtered after 120 h to yield 91% of *2c·HCl* m.p.149–152 °C dec. The product was identified by IR and ^{13}C NMR.

2c *and hydrogen chloride*. Hydrogen chloride was led over a benzene solution of *2c* for about 1 min. The oily precipitate crystallized slowly, the crystals were filtered off after 1 h and gave *2c·HCl* (99%) as a white powder, m.p. 149–152 °C dec. IR (nujol): 1740 (s), 1660 (s) cm^{-1}. ^{1}H NMR ($(CD_3)_2SO$): δ 3.79 (8H, s), 7.3–7.8 (12H, m), 8.35 (1H, d, *J* 8 Hz), 10.67 (1H, s). ^{1}H NMR ($CD(O)N(CD_3)_2$): δ 3.9 (4H, s, broad), 4.3 (4H, s, broad), 7.3–7.7 (11H, m), 7.8 (1H, d, *J* 2.9 Hz), 8.45 (1H, d, *J* 7.4 Hz), 11.7 (1H, s, broad).

2d *and* N,N-*diphenylcarbamoyl chloride*. Compound *2d* (80%) was recovered from the clear solution after 18 h at 50 °C.

2d *and hydrogen chloride*. The oily precipitate which formed was triturated with diethyl ether for 15 min and gave crystals m.p. 90 °C gas ev.; 150 °C dec. The product was somewhat hygroscopic and a good nujol mull could not be prepared. IR (nujol): 1780 (sh), 1710–1700 (s) cm^{-1}.

2f *and* N,N-*diphenylcarbamoyl chloride*. Compound *2f* (82%) was recovered after 29 h at 30–40 °C.

2f *and acetic anhydride*. Compound *2f* (80%) was recovered after 2 h at 70 °C.

2f *and methyl iodide*. Compound *2f* (95%) was recovered after 1 h at 30–40 °C.

2f *and hydrogen chloride*. The oily precipitate which formed crystallized in about 5 min and yielded *2f·HCl* (97%) m.p. 137–139 °C dec. MS [*m/e* (% rel. int.)]: 371 (9.3, M–HCl). IR (nujol): 1740 (s), 1670 (s) cm^{-1}. ^{1}H NMR (CD_3NO_2): δ 2.2 (4H, m), 3.7 (5H, m), 6.9 (1H, dd, *J* 2.9 Hz), 7.3–7.6 (11H, m), 7.95 (1H, d, *J* 7.1 Hz).

Reactions of *3*

3a *and triethylamine*. To a suspension of *3a* (55 mg, 0.1 mmol) in 6 ml of benzene was added triethylamine (50 mg, 0.5 mmol). The reaction mixture was stirred at 40–50 °C for 2 h and was filtered. The white solid was washed with benzene and yielded 50 mg (91%) of *3a* m.p. 156–163 °C dec.

3a *and sodium hydride.* To a solution of *3a* (65 mg, 0.12 mmol) in 8 ml of dichloromethane was added sodium hydride (12 mg, 0.24 mmol). The reaction mixture was filtered after 15 min at ambient temperature, and the filtrate yielded 30 mg (65 %) of *2c* m.p. 160 – 165 °C. The identity of *2c* was verified by TLC and IR. The liquid components of the filtrate were not identified, but the purple color indicates some decomposition.

3b *and cyclopentylamine.* A solution of *3b* (18 mg, 0.03 mmol) and cyclopentylamine (40 mg, 0.5 mmol) in 5 ml of acetonitrile was analyzed by GLC at 290 °C after 1 h at ambient temperature. Equimolar amounts of *1e*, 2-(benzoylamino)pyridine and *N*-cyclopentylbenzamide were the only products present in this reaction mixture. In another experiment, *3b* (140 mg, 0.26 mmol) was suspended in 7 ml of benzene, cyclopentylamine (140 mg, 1.6 mmol) was added and the reaction mixture was stirred at ambient temperature for 21 h. Undissolved material was removed by filtration and yielded 23 mg (73 %) of cyclopentylammonium chloride m.p. 199 – 201 °C subl. GLC analysis at 300 °C of the filtrate showed the presence of equimolar amounts of *1e*, 2-(benzoylamino)pyridine and *N*-cyclopentylbenzamide.

Preparation of 4. A solution of *1f* (430 mg, 1.6 mmol) and phosphorus(V) chloride (350 mg, 1.7 mmol) in 10 ml of dichloromethane was stirred at 20 °C for 15 min. The solvent was removed under reduced pressure and a solution of 4-methylaniline (170 mg, 1.6 mmol) in 5 ml of dichloromethane was added to the yellow hygroscopic residue. Triethylamine (200 mg, 2 mmol) was added and the reaction mixture was stirred at 20 °C for 2 h. The solvent was removed, the residue was dissolved in 15 ml of aqueous sodium carbonate and was extracted with 2 × 15 ml of benzene. The benzene extracts gave a liquid residue upon removal of the solvent and the residue was extracted with a mixture of tetrachloromethane (10 ml) and hexane (5 ml). Some undissolved material, 230 mg, m.p. 115 – 150 °C was removed by filtration and was found to be *1f* in admixture with a small amount of *4*. The tetrachloromethane – hexane extract yielded 130 mg (23 %) of *4*, m.p. 98 – 101 °C. MS [*m/e* (% rel. int.)]: 357 (100, M). Mol. wt., obs. 357.1950, calc. for $C_{22}H_{23}N_5$ 357.1953. IR (nujol): 3300 (m), 1640 (m), 1610 (s) cm^{-1}.

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Metabolites of the Pyridylallylamine Zimelidine Syntheses *via* α,β-Unsaturated Aldehydes with Conservation of Stereochemistry

THOMAS HÖGBERG and JAN LUNDSTRÖM

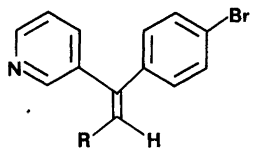
Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

Some tentative metabolites and metabolic intermediates of (*Z*)-3-(4-bromophenyl)-*N*,*N*-dimethyl-3-(3-pyridyl)allylamine (*1*, zimelidine) were synthesized. (*Z*)-3-(4-Bromophenyl)-3-(3-pyridyl)-2-propenal (*8*) and its *N*-oxide (*15*) were used in syntheses of *N*-methyl-hydroxylamines (*17*, *18*), the pyridyl *N*-oxide of zimelidine (*16*) and a nitrone (*19*). The nitrone was also formed by oxidation of the allylic carbon of the corresponding *N*-methylhydroxylamine *17* with silver oxide. The compounds were diastereomerically pure and shown to possess the *Z*-configuration by means of UV and ^{1}H NMR.

Recently, we described the identification and syntheses of the principal metabolites *2*–*10* of the antidepressant zimelidine (*1*) (see Table 1).[1] Some minor metabolites have not yet been identified and several possible intermediates may be postulated in the different metabolic pathways found.[1] Therefore, it was of interest to provide reference material for further metabolic investigations. This paper concerns the syntheses of such tentative metabolites and intermediates.

Both nitrogens in *1* form *N*-oxides *in vivo* and two metabolites (*4* and *5*) have been identified. Thus, it was of interest to synthesize the third possible *N*-oxide *16*, having only the pyridine nitrogen oxidized. Hydroxylamines and nitrones may be formed as metabolic intermediates in the dealkylation and deamination of alkylamines in vivo.[2,3] The compounds *17*, *18* and *19* are examples of such compounds, which may be formed in the biodegradation of *1* (Table 1), or more specifically the secondary amine *2*.

Table 1.



	R	Pyridine *N*-oxide
1	CH_2NMe_2	16
2[a]	CH_2NHMe	
3[a]	CH_2NH_2	
4[a]	$CH_2N(O)Me_2$	5[a]
6[a]	CH_2NHAc	7[a]
8[a]	CHO	15
9[a]	COOH	10[a]
11	$\overline{CHOCH_2CH_2O}$	12
13	CH_2OH	14
17	$CH_2N(OH)Me$	18
19	$CH=N(O)Me$	

[a] Zimelidine metabolites.

Zimelidine (*1*) has the *Z*-configuration[4,5] and the isolated metabolites *2*–*10* possessed this geometry.[1] However, both diastereomers of the acrylic acids *9* and *10*, which can isomerize under the aqueous conditions,[1] were isolated. We expected difficulties in separating the relatively hydrophilic *Z*- and *E*-isomers of *16*–*19* and therefore stereospecific syntheses of the *Z*-forms were required.

A well-known synthetic method to amines and hydroxylamines is reductive amination of the corresponding carbonyl compound with sodium cyano-

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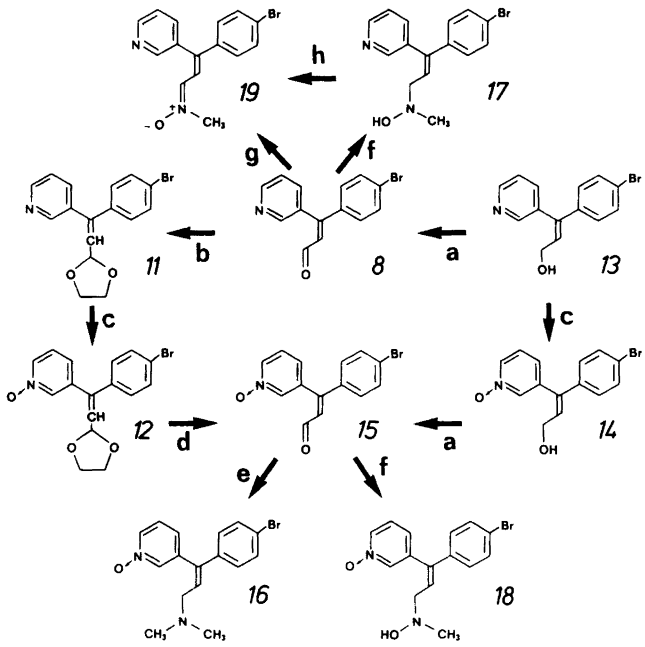

Scheme 1. a. MnO_2, $CHCl_3$; b. $HOCH_2CH_2OH$, H_2SO_4, C_6H_6, Δ; c. MCPBA, $CHCl_3$; d. aq HCl, EtOH; e. $HNMe_2 \cdot HCl$, $NaBH_3CN$, MeOH; f. HONHMe, $NaBH_3CN$, MeOH, H_2O, pH 5; g. HONHMe, Et_2O, CH_2Cl_2; h. Ag_2O, Et_2O.

borohydride in the presence of an amine.[6,7] Accordingly, it was desirable to obtain the aldehydes *8* and *15* as the pure *Z*-forms, for subsequent transformations with conservation of the stereochemistry. Moreover, the *N*-oxide *15* is also a conceivable metabolite, since it may be formed in the metabolic route to *10*.

Direct oxidation of *8*[1] with *meta*-chloroperbenzoic acid (MCPBA) gave a mixture from which *15* was difficult to obtain in pure form. In another attempted synthesis of *15* from *8*, the aldehyde function was protected as an acetal prior to oxidation. However, the isolated ethylene acetal *11* consisted of an isomeric mixture, which was converted to a *Z/E* mixture of *15* (Scheme 1). We have previously shown that the allylic alcohol *13* is easily oxidized with manganese dioxide to *8* with retained stereochemistry.[1] Thus the corresponding conversion of *14* to *15* is likely to take place. Direct oxidation of *13* with MCPBA gave *14* in *Z*-form after flash chromatography and recrystallization in 35 % yield (*cf.* the direct *N*-oxidation of morphine[8]). Subsequent treatment of *14* with manganese dioxide gave isomerically pure *15* (Scheme 1).

Previous experiments on reductive amination of *8* with sodium cyanoborohydride in anhydrous methanol showed that a low pH was essential for conversion to *1*, while minimizing isomerization.[9] Thus, the corresponding reductive amination of *15* was carried out with dimethylamine hydrochloride in methanol without addition of base (*cf.* Ref. 10) and the *N*-oxide *16* was obtained with a purity of >97 % *Z*-form (Scheme 1).

The reductive *N*-methylhydroxylaminations of the aldehydes *8* and *15*, respectively, were performed with sodium cyanoborohydride in aqueous methanol at pH ≈5 in analogy with a procedure described by Morgan and Beckett.[7] The reaction was rapid and produced *17* and *18*, respectively, in high yields. The work-up of *17* was straightforward, but the more hydrophilic *N*-oxide *18* was isolated from the aqueous reaction mixture by absorption on an XAD-2 column.

The stereochemical assignments of the new compounds above were based on UV and NMR spectra as described previously.[1] It was found that the pyridine *N*-oxides of the allylic derivatives have a λ_{max} (UV in 0.1 M HCl) at 5 – 6 nm longer wavelength

than the corresponding nonoxidized derivatives (*cf.* *1*–*16*, *4*–*5*, *13*–*14* and *17*–*18*). Furthermore, the 2,6-pyridyl protons are shifted upfield in the NMR spectra of the *N*-oxides *12*, *14*, *15*, *16* and *18*.

The oxidation of the hydroxylamine *17* to a nitrone can either involve the allylic position or the methyl group. The allylic carbon is likely to be more vulnerable to oxidation since we have shown that *1* is oxidized with manganese dioxide to the aldehyde *8*.[1] Oxidation of *17* was accomplished with silver oxide[11] and as expected the α,β-unsaturated nitrone *19* was obtained. Alternatively, *19* was prepared by condensation of *8* with *N*-methylhydroxylamine in the presence of molecular sieves, a reaction which is identical to the first step in the reductive *N*-methylhydroxylamination of *8* (Scheme 1). The conjugated nitrone *19* was unusually stable, since it was not hydrolyzed directly in aqueous hydrochloric acid (*cf.* UV spectra of *8* and *19*). The shifts of the 2,6-pyridyl protons in the NMR spectrum of *19* indicated the *Z*-configuration of the olefinic bond (*cf.* Ref. 1). Besides, the nitrone could be in *syn* or *anti* form, but no conclusive evidence of this could be obtained by ^{1}H NMR (200 MHz). The mass spectrum of *19* has the abundant fragments *m/z* 161 (M − C_6H_4Br) and *m/z* 240/238 (M − pyridyl), which may be explained by formation of stabilized 1,2-oxazoles by elimination of the aryl radicals during ring closure (Fig. 1).

EXPERIMENTAL

General procedures. Melting points were determined on a Mettler FP61 apparatus in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Varian T-60 or a Jeol FX-200 spectrometer. UV spectra were obtained on a Zeiss DMR 21 spectrophotometer. Mass spectra (70 eV) were recorded on an LKB 9000 instrument. GLC were run on an OV-17 column. TLC were run on precoated plates (Merck, Silica Gel F_{254}). Elemental analyses, performed by Analytische Laboratorien, Elbach, W. Germany, were within ±0.4 % of the theoretical calues unless otherwise noted. Amberlite XAD-2 was purchased from Rohm and Haas, Philadelphia. Molecular sieves were of 3 Å.

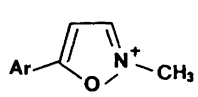

Fig. 1. Major fragments in the mass spectrum of the nitrone *19* (Ar = C_6H_4Br or C_5H_4N).

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)-2-propenal (8). Prepared according to Ref. 1. UV [0.1 M HCl (ε)]: λ_{max} 312 (16400), λ_{min} 273 (9500), λ_{max} 236 (13000), λ_{min} 218 (11200) nm; UV [EtOH (ε)]: λ_{max} 302 (22000), λ_{min} 253 (11500), λ_{max} 230 (18100), λ_{min} 221 (17700)nm; TLC (EtOAc/MeOH/H_2O 8:3:2) R_F = 0.75.

1-(4-Bromophenyl)-3-ethylenedioxy-1-(3-pyridyl) propene (11). Compound *8* (0.58 g, 2 mmol) was heated under reflux with 5 g ethylene glycol and 4 drops of conc. H_2SO_4 in 25 ml benzene for 2 days in a Dean-Stark apparatus. After cooling the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$ and NaCl solutions. Drying ($MgSO_4$) and evaporation gave 0.7 g of an oil, which on GLC was shown to be an isomeric mixture (2:3) of the acetals *11*. MS [*m/z* (rel. int.)]: 333/331 (26/27, M), 261/259 (50/50), 260/258 (20/14), 180 (100), 179 (33), 152(24), 73 (36).

1-(4-Bromophenyl)-3-ethylenedioxy-1-(3-pyridyl) propene N-*oxide (12).* To the crude acetal mixture, *11* (2 mmol) dissolved in 12 ml chloroform 3-chloroperbenzoic acid (0.41 g, 2.1 mmol, 90 %) was added with cooling in an ice-bath. The temperature was allowed to rise to ambient and the mixture was stirred for 4 h. Filtration through 40 g alumina, subsequent elution with chloroform and evaporation of the solvent gave 0.35 g (50 %) of an oil. ^{1}H NMR ($CDCl_3$): δ 3.8–4.3 (m, 4, ethylene), 5.23 (d [overlapping], 1, OCHO), 6.20 6.23 (two d, 1, vinyl), 7.0–7.8 (m, 6, aromatic), 8.1–8.4 (m, 2, 2,6-pyridyl); MS [*m/z* (rel. int.)]: 349/347 (21/22, M), 333/331 (35/35, [M − O]), 261/259 (53/54, [M − O − CO_2 − CH_2CH_2]), 260/258 (30/23), 180 (100, [261/259 − Br]), 179 (46), 73 (48).

Hydrolysis in aqueous ethanol with HCl gave 0.26 g (43 % overall yield from *8*) of a *Z/E* (2:1) mixture of *15*. ^{1}H NMR ($CDCl_3$): δ6.65, 6.59 (two d, 1, vinyl), 7.1–7.8 (m, 6, aromatic), 8.1–8.4 (m, 2, 2,6-pyridyl), 9.54, 9.58 (two d, 1, CHO).

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)-2-propen-1-ol (13). Prepared according to Ref. 1. UV [0.1 M HCl (ε)]: λ_{max} 248 (19700), λ_{min} 224 (11600)nm.

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)-2-propen-1-ol N-*oxide (14).* 3-Chloroperbenzoic acid (2.3 g, 12 mmol, 90 %) was added to a solution of *13* (2.90 g, 10 mmol) in 15 ml chloroform with cooling in an ice-bath. After stirring for 0.5 h at 0 °C and 2 h at room temperature the solution was filtered through 30 g aluminia, which was washed with chloroform–methanol 3:1.

Evaporation of the solvent and recrystallization from chloroform–diisopropyl ether gave 1.7 g of crystals, mp 173–176 °C. Flash chromatography of the product on a column of silica gel (40 g, 0.040–0.063 mm) with methanol–ethyl acetate 1:5 (250 ml) and 1:1 (200 ml) and subsequent recrystallization from chloroform–diisopropyl ether afforded 1.07

g (35 %) pure title compound, mp 182–184 °C.

^{1}H NMR ($CDCl_3/CD_3OD$): δ 4.13 (d, 2, allyl), 4.55 (s, 1, OH), 6.41 (t, 1, vinyl), 7.1–7.7 (m, 6, aromatic), 8.16 (m, 1, 2-pyridyl), 8.27 (dt, 1, 6-pyridyl); MS [*m/z* (rel. int.)]: 307/305 (24/27, M) 291/289 (19/37, [M–O]), 290/288 (42/51), 289/287 (37/18, [M–H_2O]), 278/276 (26/27), 262/260 (15/19), 248/246 (35/38), 210 (100, [M–O–Br]); UV [0.1 M HCl (ε)]: λ_{max} 253 (16400), λ_{min} 228 (10700) nm; Anal. $C_{14}H_{12}BrNO_2$: C, H, Br, N, O.

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)-2-propenal N-oxide (15). Compound *14* (0.15 g, 0.5 mmol) was dissolved in 6 ml chloroform and stirred with 1 g MnO_2 (Merck, gefällt) under a nitrogen atmosphere at ambient temperature overnight. TLC (EtOAc–MeOH–H_2O 8:3:2) showed that a complete conversion had taken place leading to a compound with R_F = 0.54.

To the reaction mixture 10 ml chloroform and 10 ml methanol were added prior to filtration through Celite. The filter cake was washed with hot chloroform–methanol 1:1. Evaporation of the filtrate *in vacuo* gave 0.15 g (98 %) of an oil. ^{1}H NMR ($CDCl_3$): δ 6.65 (d, 1, vinyl) 7.1–7.8 (m, 6, aromatic), 8.18 (m, 1, 2-pyridyl), 8.31 (dt, 1, 6-pyridyl), 9.57 (d, 1, CHO); MS [*m/z* (rel. int.)] 305/303 (85/87, M), 289/287 (37/39, [M–O]), 288/286 (38/34, [M–OH]), 260/258 (19/17, [M–O–CHO]), 208 (100, [M–O–Br]), 180 (64), 179 (66), 165 (44), 155 (63), 153 (17), 152 (48); UV [0.1 M HCl (ε)]: λ_{max} 309 (14400), λ_{min} 280 (9300), λ_{max} 253 (14400), λ_{max} 241 (14800), λ_{min} 225 (13300)nm; UV [EtOH (ε)]: λ_{max} 299 (sh, 12000), λ_{max} 269 (16800), λ_{min} 246 (14400), λ_{max} 234 (sh, 15000)nm.

(Z)-3-(4-Bromophenyl)-N,N-*dimethyl-3-(3-pyridyl* N′-*oxide)allylamine (16).* Dimethylamine hydrochloride (0.41 g, 5 mmol) and *15* (0.15 g, 0.49 mmol) were dissolved in 5 ml methanol. After addition of $NaBH_3CN$ (0.035 g, 0.5 mmol, 90 %) and 3 g molecular sieves the reaction mixture was stirred under nitrogen for 24 h. To the mixture was added 10 ml 6 M HCl and the methanol was removed under reduced pressure. The residue was made alkaline with 45 % NaOH and extracted twice with ether and once with dichloromethane. After drying ($MgSO_4$) the extracts were combined and evaporated to give 0.12 g (76 %) of colourless oil. ^{1}H NMR ($CDCl_3$, base): δ 2.31 (s, 6, CH_3), 3.07 (d, 2, allyl), 6.36 (t, 1, vinyl), 7.0–7.7 (m, 6, aromatic), 8.10 (m, 1, 2-pyridyl), 8.21 (dt, J = 6.2 and 1.5 Hz, 1, 6-pyridyl); MS [*m/z* (rel. int.)]: 334/332 (0.22/0.24, M), 333/331 (0.74/0.74), 318/316 (10/11, [M–O]), 317/315 (22/21), 303/301 (2.4/2.6), 275/273 (6.8/10), 274/272 (26/27), 240/238 (4.8/5.8), 193 (100, [M–O–NMe_2–Br]), 192 (16), 161 (4.8), 70 (13), 58 (41), 42 (23); UV [0.1 M HCl (ε)]: λ_{max} 256 (22900), λ_{min} 230 (15300).

(Z)-3-(4-Bromophenyl)-N-*hydroxy*-N-*methyl-3-(3-pyridyl)allylamine (17).* *N*-Methylhydroxylamine hydrochloride (0.67 g, 8 mmol) and *8* (2.0 g, 6.9 mmol) were dissolved in 3 ml water and 12 ml methanol with addition of a few drops of conc. HCl. Immediately after the dissolution the pH was adjusted to 5 with 45 % NaOH. $NaBH_3CN$ (0.50 g, 7 mmol, 90 %) was added in one portion and during the reaction at room temperature the pH was kept at 4.7–5.3 by incremental addition of 2 M HCl. After 0.5 h no further increase in pH occurred and after 1.5 h TLC (EtOAc–MeOH–H_2O 8:3:2, title compound *17* R_F = 0.61) showed no trace of the aldehyde *8*. After 4 h the excess of hydride was destroyed with conc. HCl and the solution was diluted with saturated aqueous NaCl, made alkaline and extracted twice with ether. The combined ether extracts were dried ($MgSO_4$) and evaporated to give 1.9 g (86 %) of crude product. The amine was converted to its oxalate salt and recrystallized twice from ethanol to give 1.85 g (56 %) of the pure oxalate of 17, mp 149–150 °C (dec.). ^{1}H NMR ($CDCl_3$, base): δ 2.59 (s, 3, CH_3), 3.35 (d, 2, allyl), 6.37 (t, 1, vinyl), 7.0–7.7 (m, 6, aromatic), 8.47 (m, 1, 2-pyridyl), 8.59 (dd, 1, 6-pyridyl); MS [*m/z* (rel. int.)]: 320/318 (3.4/3.6, M), 304(11), 303 (7.0), 302 (21), 301/299 (50/46), 275/273 (36/41, [M–ONMe]), 274/272 (24/21, [M–HONMe]), 193 (100, [M–HONMe–Br]), 192 (22), 46(25, [HONMe]), 45 (43), 44(38); TMS-derivative: 392/390 (5.2/5.0, M), 274/272(32/33, [M–TMSONMe]), 193 (100, [M–TMSONMe–Br]); UV [0.1 M HCl (ε)]: λ_{max} 250 (16300), λ_{min} 226 (11100). Anal. $C_{15}H_{15}BrN_2O \cdot 1.75\ C_2H_2O_4$:C (calc. 46.60, found 47.07), H, N, O, Br.

(Z)-3-(4-Bromophenyl)-N-*hydroxy*-N-*methyl-3-(3-pyridyl)allylamine* N-*oxide (18).* *N*-Methylhydroxylamine hydrochloride (0.13 g, 1.5 mmol) and *15* (0.26 g, 0.85 mmol) were dissolved in 0.6 ml water and 2.5 ml methanol. The pH was adjusted to 5 with 45 % NaOH and $NaBH_3CN$ (0.09 g, 1.3 mmol, 90 %) was added. The pH was maintained at about 5 by addition of 2 M HCl at room temperature. TLC after 3 h showed no aldehyde *15*. The solution was allowed to stand overnight before work-up. The reaction mixture was acidified and after the evolution of gas bubbles had ceased the mixture was made alkaline. The aqueous solution was allowed to pass through an XAD-2 column (20 g, prewashed with methanol and water). The eluent was passed through the column once more and then the column was washed with distilled water. The adsorbed product was eluted with methanol and TLC (EtOAc–MeOH–H_2O 8:3:2) revealed only traces of impurities in the product with R_F = 0.44. Chloroform was added to the methanolic solution and the solvent was evaporated *in vacuo* to leave 0.27 g (95 %) of an oil. An oxalate was prepared and recrystallized from ethanol–diisopropyl ether

giving a hygroscopic product, which was deliquescent but resolidified on standing, mp 99–102 °C (dec.). ^{1}H NMR (CD_3OD, oxalate): δ 2.98 (s, 3, CH_3), 3.53 (d, 2, allyl), 6.49 (t, 1, vinyl), 7.1–7.8 (m, 6, aromatic), 8.27 (m, 1, 2-pyridyl), 8.36 (dt (partly concealed), 1, 6-pyridyl); MS [*m/z* (rel. int.)]: 336/334 (1.0/1.0, M), 320/318 (1.5/2.2, [M–O]), 304 (2.1), 303 (1.9), 302 (5.1), 301 299 (13/12), 275/273 (9.0/11, [M–O–ONMe]), 274/272 (9.6/9.3, [M–O–HONMe]), 193 (37, [M–O–HONMe–Br]), 192 (9.2), 46 (58, [HONMe]) 45 (100), 44 (50); UV [0.1 M HCl (ε)]: λ_{max} 256 (22500), λ_{min} 229 (15900); Anal. $C_{15}H_{15}BrN_2O_2 \cdot C_2H_2O_4$:C, H, Br, N, O.

N-[*(2Z)-3-(4-Bromophenyl)-3-(3-pyridyl)-2-propenylidene*]*methylamine* N-*oxide* (*19*). A. *Oxidation of* 17. The base of *17* (extracted from 0.5 mmol oxalate) was dissolved in 30 ml anhydrous ether under a nitrogen atmosphere at room temperature. Molecular sieves (4 g) and Ag_2O (0.70 g, 3 mmol, Fluka AG) were added and the mixture was stirred for 16 h. TLC showed an incomplete conversion and additional Ag_2O (0.46 g, 2 mmol) was added and the mixture was stirred overnight (total 40 h). TLC (EtOAc–MeOH–H_2O 8:3:2) showed a single compound having R_F=0.41 and no starting material *17*. Ether (20 ml) and acetone (20 ml) were added to the reaction mixture. After stirring and filtration through Celite the solvent was evaporated *in vacuo*. Dichloromethane was added to the residue and evaporation gave 81 mg (51 %) of a crystalline product *19*, mp 173–176 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.69 (s, CH_3), 7.01 and 7.49 (two d, J=10.3 Hz, HC=CH), 7.10 and 7.46 (AA'BB', J=8.8 Hz, phenyl), 7.40 (ddd, J=7.8, 4.9 and 0.9 Hz, 5-pyridyl), 7.53 (dt, J=7.8 and 1.9 Hz, 4-pyridyl), 8.51 (narrow m, 2-pyridyl), 8.69 (dd, J= 4.9 and 1.9 Hz, 6-pyridyl); MS [*m/z* (rel. int.)]: 319 (5.3), 318/316 (32/33, M), 301/299 (77/78, [M–OH]), 274 (13), 273/271 (39/39), 272/270 (37/24), 240/238 (86/86, [M–C_5H_4N]), 192 (32), 191 (36), 161 (100, [M–C_6H_4Br]), 131 (36), 42 (57); UV [0.1 M HCl (ε)]: λ_{max} 337 (21000), λ_{min} 273 (9200), λ_{max} 250 (15400), λ_{min} 222 (10500); UV [EtOH(ε)]: λ_{max} 341 (16000), λ_{min} 276 (6300), λ_{max} 249 (11400), λ_{min} 220 (6600).

B. *Condensation of* 8 *with* N-*methylhydroxylamine*. *N*-Methylhydroxylamine hydrochloride (42 mg, 0.5 mmol), NaOH (20 mg, 0.5 mmol) and *8* (144 mg, 0.5 mmol) were dissolved in 5 ml ether and 3 ml dichloromethane at room temperature. Molecular sieves (3 g) were added and the mixture was stirred under nitrogen overnight. TLC showed that most of *8* had been converted to *19*. Another 0.1 mmol (8.4 mg) of *N*-methylhydroxylamine hydrochloride was added and the mixture was stirred for a further 6 h. TLC showed only traces of *8* and 10 ml dichloromethane and 10 ml ether were added and the mixture was stirred. Filtration through Celite and evaporation of the solvent gave 50 mg (32 %) of the title compound *19* as a crystalline product, mp 174–176 °C. The product was chromatographically and spectroscopically identical with *19* obtained by oxidation of *17*.

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Synthesis of the Antidepressant Zimelidine and Related 3-(4-Bromophenyl)-3-(3-pyridyl)allylamines. Correlation of their Configurations

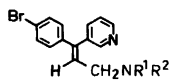
BERNT CARNMALM, TOMAS DE PAULIS, THOMAS HÖGBERG, LARS JOHANSSON, MAJ-LIZ PERSSON, SETH-OLOF THORBERG and BENGT ULFF

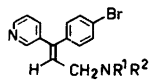
Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

Various methods for the synthesis of the antidepressant (*Z*)-3-(4-bromophenyl)-*N*,*N*-dimethyl-3-(3-pyridyl)allylamine, zimelidine, are described. In addition, syntheses of the analogous secondary and primary amines, as well as the corresponding tertiary, secondary and primary *E*-isomers are shown. The steric interrelations of these amines are discussed on the basis of chemical and spectral evidence (NMR, UV).

(*Z*)-3-(4-Bromophenyl)-*N*,*N*-dimethyl-3-(3-pyridyl)-allylamine (*1*), zimelidine, is a new antidepressant with secured clinical effect.[1,2] In connection with the evaluation of this drug, the investigation was extended to the corresponding secondary *3* and primary *5* amines as well as to the *E*-isomers *2*, *4* and *6*, see Fig. 1. This paper describes various synthetic routes to these compounds.

Originally *1* was prepared from the acetophenone *7* *via* the Mannich base *8* and the amino-alcohol *9* (Scheme 1).[3,4] Dehydration of *9* yielded a mixture of the diastereomers *1* and *2* in a ratio of 3:1. The separation of *1* and *2* was effectively achieved by utilizing the differing solubilities of their hydrochlorides. Precipitation in acetone gave the hydrochloride of *1* in high purity and good yield, and from the mother liquor *2* was isolated as the oxalate.





Z-Isomers			*E*-Isomers		
No.	R^1	R^2	No.	R^1	R^2
1	CH_3	CH_3	*2*	CH_3	CH_3
3	CH_3	H	*4*	CH_3	H
5	H	H	*6*	H	H

Fig. 1. Stereoisomers of the 3-(4-bromophenyl)-3-(3-pyridyl)allylamines discussed in this paper.

It was of interest to develop other more general routes to zimelidine, which could also be applied for synthesis of the secondary and primary amines *3*–*6*. A key intermediate, the ketone *10*, was prepared essentially as previously described.[5]

As shown in Scheme 1, a Reformatsky reaction of *10* gave the hydroxy-ester *11*, which was subsequently reduced to the diol *12*. A mixture of isomeric allylic bromides *13* was obtained either by treating *12* with phosphorus tribromide or by bromination of the isomer mixture *14*. The latter was formed in a Wittig reaction, which gave a *Z*–*E*-ratio of 1:1, while the bromination of *12* gave an isomer ratio of 4:1. The bromides were treated *in situ* with dimethylamine or with methylamine giving mixtures of *1* and *2* or of *3* and *4*, respectively, (Schemes 1 and 2). A mixture of *1* and *2* was also formed directly from the ketone *10*, using the ylide from 2-(dimethylamino)ethyltriphenylphosphonium bromide.[6] This reaction, carried out in tetrahydrofuran, gave the isomers *1* and *2* in a ratio of 3:2.

Furthermore, the hydroxy-ester *11* was used in a reaction sequence, leading *via* the amide *17* and the amino-alcohol *18* to the secondary amines *3* and *4* (Scheme 2). These were formed as a mixture in approximately the same *Z*–*E*-ratio as were *1* and *2* in the dehydration of *9*. The separation of *3* and *4* was achieved by precipitation of their oxalates

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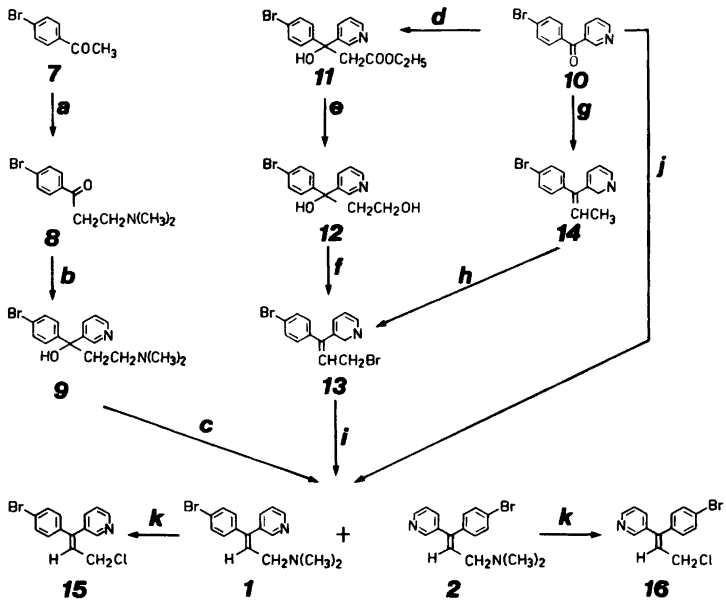
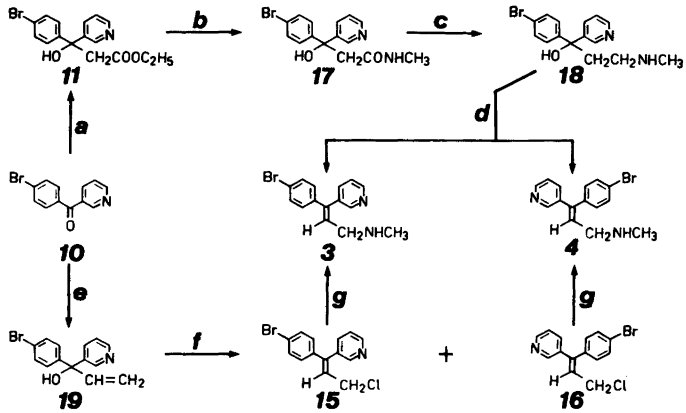


Scheme 1. Syntheses of the tertiary amines *1* and *2*. [a] HCHO, $HNMe_2 \cdot HCl$, EtOH. [b] 3-Lithiopyridine, Et_2O. [c] H_2SO_4, Ac_2O. [d] Zn, $BrCH_2COOEt$, C_6H_6. [e] $LiAlH_4$, Et_2O. [f] PBr_3, CH_2Cl_2. [g] $Ph_3P{=}CHCH_3$, DMSO, THF. [h] NBS, AIBN, CCl_4. [i] $HNMe_2$, CH_2Cl_2. [j] $Ph_3P{=}CHCH_2NMe_2$, THF. [k] $ClCOOCH_2CCl_3$, C_6H_6.

from different solvents.

As the isomerically pure tertiary amines *1* and *2* had become available from large scale preparations, they were attractive as starting materials for the synthesis of *3* and *4*. Thus, dealkylation experiments with various chloroformates were performed. No secondary amines were isolated, but when using trichloroethyl chloroformate, *1* and *2* gave the allylic chlorides *15* and *16*, respectively, in good yields (Scheme 1). No *cis*–*trans*-isomerization was observed. These findings are in agreement with independent results of others, published after the completion of this part of our work.[7] The allylic chlorides were isolated as crystalline and stable



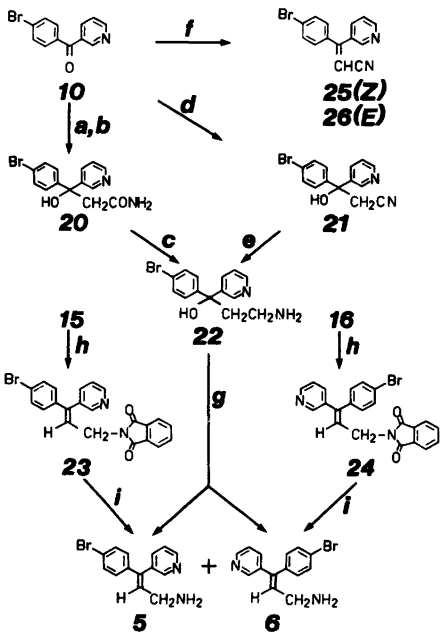
Scheme 2. Syntheses of the secondary amines *3* and *4*. [a] Zn, $BrCH_2COOEt$, C_6H_6. [b] H_2NMe (30% aq.). [c] $NaBH_4$, $BF_3 \cdot Et_2O$. [d] H_2SO_4 (75% aq.). [e] $CH_2{=}CHMgBr$, THF. [f] PCl_5, CH_2Cl_2. [g] H_2NMe, EtOH.

hydrochlorides. Each chloride (*15*, *16*) gave the corresponding secondary amine (*3*, *4*) upon treatment with methylamine (Scheme 2).

Another way of obtaining the allylic halides has been found in the acidic rearrangement of the tertiary allylic alcohol *19*. Treatment of *19* with phosphorus pentachloride afforded predominantly the isomer *15*.[8] This constitutes a convenient route to the *Z*-isomers of amines such as *1* and *3*.[8,9]

The tertiary and secondary amines *1*–*4* could all be obtained by dehydration of the appropriate alcohols. Thus, in order to synthesize the primary amines *5* and *6*, the amino-alcohol *22* was prepared as shown in Scheme *3*. Dehydration of *22* gave a mixture of *5* and *6*, but only *5*, formed in slight excess, could be isolated. The isomer *6* was obtained from the allylic chloride *16* using the Gabriel reaction. By this method amine *5* was also obtained, starting with *15*.

Another route to the primary amines was investigated *via* the nitriles *25* and *26*, which could easily be prepared and separated (Scheme *3*). However, reduction to the primary amines *5* and *6* could not be achieved using a mixture of lithium aluminium hydride and aluminium chloride or lithium aluminium hydride alone.



Scheme 3. Syntheses of the primary amines *5* and *6*. [a] Zn, $BrCH_2COOEt$, C_6H_6. [b] NH_3 (conc.), EtOH. [c] $NaBH_4$, $BF_3.Et_2O$. [d] $LiCH_2CN$, THF, −50 °C. [e] $LiAlH_4$, THF, −30 °C. [f] $(EtO)_2P(O)=CHCN$, DMSO, THF. [g] H_2SO_4 (70 % aq.). [h] Potassium phtalimide, DMF. [i] $H_2NNH_2.H_2O$, MeOH.

STRUCTURAL RELATIONSHIPS

The steric correlations between the amines depicted in Fig. 1 reside on chemical as well as spectral evidence. Chemically, the three amines *1*, *3* and *5* are interrelated *via* the allylic chloride *15*, and the amines *2*, *4* and *6* *via* the chloride *16* (*cf.* Schemes 1–3). From each of these reactions only one isomer was detected indicating that no isomerization occurred. Furthermore, *3* was converted to *1* on *N*-methylation, giving a closed reaction sequence (*1*–*15*–*3*–*1*).

In the ^1H NMR spectra there are easily recognizable features which permit the isomeric amines to be divided into two series. Firstly, the triplet of the olefinic proton appears at a field slightly lower for *1*, *3* and *5* than for *2*, *4* and *6*, respectively. The amine mixtures show two well-resolved triplets, useful in determination of the ratio of the isomers. Secondly, in the low field part of the aromatic region, the signals of the 2- and 6-pyridyl protons are separated in the first series, while overlapping in the second. These shift differences give rise to characteristic patterns as shown in Fig. 2. Thirdly, the width of the AA'BB' spin system of the *p*-bromophenyl protons is *ca.* 0.15 ppm smaller in the former series than in the latter (*cf.* Ref. 4).

The same distinction could be made on the basis of UV-spectra. In hydrochloric acid solution *1*,

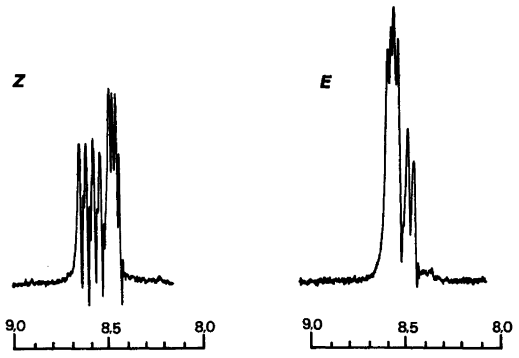


Fig. 2. The NMR-pattern of the 2- and 6-pyridyl protons of the *Z*- and *E*-isomers, exemplified by compounds *3* and *4*.

Table 1. Europium-induced shift in NMR spectra of compounds *25* and *26*. Values in ppm from TMS, measured in $CDCl_3$ at 37 °C.

	25		*26*	
	vinyl	2-py	vinyl	2-py
Plain compound[a]	5.87	8.65	5.77	8.57
With $Eu(fod)_3$[b]	6.42	12.75	6.85	13.68
LIS	0.55	4.10	1.08	5.11
LIS-ratio	0.13		0.21	
Standard compound	*1*		*2*	
LIS-ratio[c]	0.15		0.23	

[a] Concentration *ca.* 0.4 M. [b] [$Eu(fod)_3$]/[nitrile] *ca.* 0.2. [c] Calculated from figures in Ref. 11.

3 and *5* have a λ_{max} at about 250 nm and *2*, *4* and *6* at about 220 nm.

The configuration of zimelidine (*1*) has been established to be *Z* by an X-ray single-crystal analysis.[10] Furthermore, the configuration of both *1* and *2* have been determined by the use of lanthanide shift reagents, the LIS-technique.[11] These results together with the correlations above give the configurations shown in Fig. 1 for the amines *1*–*6*.

The allylic chlorides *15* and *16* give spectral data in parallel with those of the amines (NMR and especially UV). Isomer *15* belongs to the *Z*-, and *16* to the *E*-series, thus confirming the chemical evidence.

The nitriles *25* and *26* have conjugated systems differing from those of the amines and, as a consequence, the UV and simple NMR spectra do not give reliable steric information. A similar ambiguous situation has been described for the corresponding carboxylic acids and aldehydes.[4] Nevertheless, a correlation with the amines *1* and *2* has been possible using the LIS-technique and observing the vinyl and the 2-pyridyl protons. $Eu(fod)_3$ was used as the shift reagent and it was assumed that a one to one complex with the pyridine nitrogen atom is determining the LIS. It was recently shown that this is true for the complexes of *1* and *2*.[11] Good additional evidence for the validity of this assumption is that the LIS-ratios found for *25* and *26* are very close to those for *1* and *2*. According to these results *25* has the *Z*- and *26* has the *E*-configuration.

The steric division of the amines *1*–*6* into two groups is also reflected in their biological effects. One well recognized screening method for potential antidepressants measures the inhibition of the neuronal uptake of biogenic amines. *In vitro*, each of the *Z*-isomers, *1*, *3* and *5*, inhibits the uptake of 5-hydroxytryptamine more strongly than the corresponding *E*-isomer, *2*, *4* or *6*. For noradrenaline the selectivity is reversed.*

EXPERIMENTAL

If not otherwise stated the following applies. Melting points are uncorrected. Elemental analyses gave figures within ±0.4 % from the calculated values. Spectra were recorded on the following instruments and are reported in the following way (significant peaks only). Mass: LKB 9000 (purified salt, direct inlet, 70 eV), MS: *m/e* (rel. int. %). Ultraviolet: Zeiss DMR 21 (in 0.1 M HCl), UV: λ nm(ε). Proton magnetic resonance: Varian T 60 (free amine recovered from the purified salt, solvent $CDCl_3$, internal TMS), NMR: δ (multiplicity).

(Z)-3-(4-Bromophenyl)-N,N-*dimethyl-3-(3-pyridyl)allylamine (1).* (a) *Dehydration of* 9. Concentrated H_2SO_4 (6 ml) was added dropwise to *9* (14.5 g, 43 mmol) dissolved in 125 ml acetic anhydride. The solution was heated under reflux for 30 min, cooled and poured into 250 ml ice-water. The solution was made alkaline with 500 ml 5 M NaOH and extracted with 3 × 250 ml ether. The ether layer was treated with charcoal, dried (Na_2SO_4) and the solvent evaporated to give 13.8 g crude amine as an oil containing *1* and *2* in a ratio of 3:1. The amine mixture was dissolved in acetone and concentrated HCl (75 mmol) was added, giving a solid precipitate.

Recrystallization from aqueous ethanol gave 9.6 g (55 %) of the dihydrochloride monohydrate of *1*. M.p. 195–198 °C. Anal. $C_{16}H_{17}BrN_2.2HCl.H_2O$: C, H, Br, Cl, N, O. MS: 318/316 (29/29, M), 58 (100). UV: 250 (19700, max), 225 (14000, min). NMR: 8.60 (dd, 1, 6-pyridyl), 8.46 (m, 1, 2-pyridyl), 7.42, 7.08, 7.6–7.3 (AA'BB'+m, 6, C_6H_4+4,5-pyridyl), 6.30 (t, 1, CH), 2.98 (d, 2, CH_2), 2.23 (s, 6, CH_3).

(b) *Bromination of* 14 *to* 13 *and amination of crude* 13. The olefin mixture *14* (1.0 g, 3.5 mmol) and *N*-bromosuccinimide (0.62 g, 3.5 mmol) in 180 ml carbon tetrachloride were heated with stirring and azoisobutyronitrile (0.1 g) was added at 70 °C. After heating under reflux for about 2 h the mixture was cooled and the succinimide formed was filtered off. The resulting solution of crude *13* was stirred at room temperature with aqueous dimethylamine

*Concentration of amines *1*–*6* (μM) giving 50% inhibition of the neuronal uptake in mouse brain slices *in vitro*: 5-Hydroxytryptamine: *1* (1.7), *2* (6.1), *3* (0.10), *4* (2.5), *5* (2,4), *6* (6.0). Noradrenaline: *1* (>24), *2* (6.1), *3* (1.52), *4*(0.8), *5* (>24), *6* (3.0).[12]

(43 %, 10 ml, 100 mmol) for 4 h and then extracted with 3 × 75 ml 0.5 M HCl. The aqueous layers were made alkaline with 30 % NaOH and extracted with 2 × 100 ml dichloromethane. Drying ($MgSO_4$) and evaporation gave 0.65 g (59 %) of an oil consisting of *1* and *2* in a ratio of about 1:1 according to NMR.

(c) *From the propanediol* 12. The diol *12* (0.92 g, 3.0 mmol) and PBr_3 (1.62 g, 6.0 mmol) in 60 ml dichloromethane were heated under reflux overnight. After cooling in an ice-bath a solution of dimethylamine (4.5 g, 100 mmol in 5 ml CH_2Cl_2) was added and the mixture was then stirred at room temperature for 1 h. Alkalization with 50 ml 2 M NaOH, extraction with dichloromethane, washing with water, drying ($MgSO_4$), and evaporation gave a mixture of *1* and *2* as an oil, 0.81 g (85 %), in the ratio 82:18 (GLC, OV-17 on Chromosorb, 2 m column, 200 °C). The dihydrochloride of *1* was precipitated from acetone and crystallized from aqueous 2-propanol, giving 0.50 g (41 %) dihydrochloride monohydrate. M.p. 191–195 °C, ≤0.5 % of *2* (GLC).

(d) *Wittig reaction of* 10. Butyllithium (10 mmol) in hexane was added to 2-(dimethylamino)ethyltriphenylphosphonium bromide (4.14 g, 10 mmol) in 30 ml dry tetrahydrofuran at ambient temperature during 1 min. After stirring for 30 min a suspension of *10* (2.62 g, 10 mmol in 15 ml THF) was added to the solution of the dark red ylide. The mixture was heated at 60 °C for 5 h and after cooling 25 ml 2 M HCl were added. The solvent was evaporated *in vacuo* after addition of toluene to the mixture. Additional 2 M HCl was added and the aqueous phase was washed twice with toluene, filtered, made alkaline and extracted twice with ether. Drying ($MgSO_4$) and evaporation gave 2.2 g (69 %) of amines as a yellow oil. Integration of an Eu $(fod)_3$-shifted NMR showed *1* and *2* in a ratio of 62:38. Concentrated HCl (1 ml, 11 mmol) was added to a solution of 1.2 g of the amine mixture in acetone. After heating to reflux the mixture was cooled and the acetone was decanted from the smeary precipitate. Crystallization from aqueous 2-propanol gave 1.20 g (30 %) of the dihydrochloride monohydrate of *1*. M.p. 192–194 °C.

(e) *Eschweiler-Clarke methylation of* 3. A mixture of *3* (0.6 g, 1.8 mmol), formic acid (0.6 g, 13 mmol) and formalin (0.4 ml 36 % aqueous solution, 4.8 mmol) was heated on a steam bath for 3 h. GLC (as in procedure (c)) revealed a complete conversion of *3* to *1*. Precipitation of the dihydrochloride from acetone and recrystallization twice from aqueous 2-propanol gave 0.33 g (45 %) dihydrochloride monohydrate. M.p. 192–200 °C. No melting point depression was found on admixture with a sample of *1* from procedure (a).

(E)-3-(4-Bromophenyl)-N,N-*dimethyl-3-(3-pyridyl)allylamine (2)*. The mother liquor from the precipitation of the hydrochloride of *1*, method (a), was concentrated *in vacuo*. The oily residue was dissolved in water, made alkaline, and extracted with ether, Washing with water, drying (Na_2SO_4), and evaporation gave a colourless oil. To an ice-cooled solution of this oil in dry acetone an equivalent amount of oxalic acid in dry acetone was added dropwise. The formed crystalline precipitate was washed with cold acetone, dried *in vacuo*, and recrystallized three times from methanol. M.p. 174–176 °C. The analyses showed the compound to be the "sesquioxalate" of *2*. Anal. $C_{16}H_{17}BrN_2.1.5\ C_2H_2O_4$: C, H, Br, N, O. MS: identical with *1*. UV: 237 (18100, shoulder), 219 (21900, max). NMR: 8.53 (m, 1, 2-pyridyl), 8.50 (dd, partly concealed, 1, 6-pyridyl), 7.55, 7.05, 7.6–7.0 (AA'BB'+m, 6, C_6H_4+4,5-pyridyl), 6.27 (t, 1, CH), 3.01 (d, 2, CH_2), 2.23 (s, 6, CH_3).

(Z)-3-(4-Bromophenyl)-N-*methyl-3-(3-pyridyl)-allylamine (3)*. (a) *Dehydration of* 18. The aminopropanol *18* (1.1 g, 3.4 mmol) was dissolved in H_2SO_4 (75 %, 12 ml) and the solution heated with stirring on a steam bath for 25 min. After cooling, ice and NaOH (2 M, *ca.* 130 ml) were added to pH 10. The mixture was extracted with 3 × 75 ml ether. The combined ether layers were extracted with 3 × 100 ml 1 M HCl. After alkalization (30 % NaOH) extraction with methylene chloride, drying ($MgSO_4$) and evaporation, a mixture of *3* and *4* in a ratio of 3:1 was obtained. A hot solution of the amine mixture (0.58 g, 1.9 mmol) and oxalic acid (0.20 g, 2.2 mmol) in ethanol gave the oxalate of *3* on cooling. The salt was recrystallized from aqueous ethanol (93 %) giving 0.58 g (44 %). M.p. 206–208 °C. The dihydrochloride monohydrate of *3* was prepared from a solution of the free amine (12 g) in 100 ml ethanol by adding 6.5 ml concentrated HCl giving 8.3 g. M.p. 155–158 °C. Anal. $C_{15}H_{15}BrN_2.2\ HCl.H_2O$: C, H, Br, Cl, N, O. MS: 304/302 (94/100, M), 44 (84). UV: 248 (19200, max), 224 (12500, min). NMR: 8.61 (dd, 1, 6-pyridyl), 8.47 (m, 1, 2-pyridyl), 7.43, 7.08, 7.5–7.3 (AA'BB'+m, 6, C_6H_4+4,5-pyridyl), 6.28 (t, 1, CH), 3.26, (d, 2, CH_2), 2.39 (s, 3, CH_3), 1.38 (s, 1, NH).

(b) *Amination of* 15. The hydrochloride of *15* (13.8 g, 40.0 mmol) was stirred with methylamine (12 ml, 240 mmol) in 1200 ml ethanol at room temperature for 24 h. After concentration *in vacuo* 50 ml 2 M HCl was added. The mixture was washed with ether, made alkaline with 2 M NaOH and extracted with dichloromethane. Drying and evaporation gave 12 g of *3* as an oil, ≥95 % purity by NMR.

(E)-3-(4-Bromophenyl)-N-*methyl-3-(3-pyridyl)-allylamine (4)*. (a) *Dehydration of* 18. The residue from precipitation of the oxalate of *3* (procedure (a)) contains *3* and *4* in the ratio 2:3. A solution of this amine mixture (2.7 g, 8.8 mmol) and oxalic acid

(0.81 g, 8.9 mmol) in 90 ml warm aqueous acetonitrile (85 %) gave on cooling 1.9 g oxalate of *4*. Recrystallization (aq. CH_3CN) gave 1.1 g. M.p. 198 – 201 °C. Anal $C_{15}H_{15}BrN_2.C_2H_2O_4$: C, H, Br, N, O. MS: 304/302 (90/100, M), 147 (57), 44 (46). UV: 236 (18800, shoulder), 220 (20800, max). NMR: 8.54 (m, 1, 2-pyridyl), 8.48 (dd, partly concealed, 1, 6-pyridyl), 7.55, 7.05, 7.6 – 7.0 (AA'BB' + m, 6, C_6H_4 + 4,5-pyridyl), 6.23 (t, 1, CH), 3.28 (d, 2, CH_2), 2.40 (s, 3, CH_3), 1.31 (s, 1, NH).

(b) *Amination of* 16. Compound *4* was prepared from the hydrochloride of *16* (7.0 g, 23 mmol), as described for compound *3* method (b). The oxalate of *4* was prepared (6.0 g, 86 %). M.p. 195 – 197 °C. Anal. $C_{15}H_{15}BrN_2.C_2H_2O_4$: C, H, Br, N, O.

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)allylamine (5). (a) *Dehydration of* 22. The amino-propanol *22* (4.7 g, 16 mmol) was mixed with 12 ml 70 % H_2SO_4 and the solution was heated at 80 °C for 30 min. Ice-water was added and then 35 ml of 30 % NaOH. The reaction mixture was extracted with ether. Drying (Na_2SO_4) and evaporation gave 4.5 g of an oil. This was dissolved in 50 ml methanol and oxalic acid (2.0 g, 16 mmol) in 25 ml 2-propanol and 5 ml water was added giving 2.5 g of crystals. Recrystallization (55 ml MeOH – 2-PrOH – H_2O, 5:5:1) gave 1.8 g (30 %) of the oxalate of *5* containing one mol equiv. of methanol. M.p. 160 – 162 °C. Anal. $C_{14}H_{13}BrN_2.C_2H_2O_4.CH_4O$: C, H, Br, N, O. UV: 247 (19600, max), 224 (12700, min). NMR: 8.55 (dd, 1, 6-pyridyl), 8.42 (m, 1, 2-pyridyl), 7.35, 7.01, 7.5 – 7.2 (AA'BB' + m, 6, C_6H_4 + 4,5-pyridyl), 6.19 (t, 1, CH), 3.31 (d, 2, CH_2).

(b) *Hydrazinolysis of* 23. The pthalimide *23* (2.8 g, 6.7 mmol) was dissolved in 95 ml methanol and a solution of hydrazine hydrate (0.8 ml, 16 mmol) in 5 ml methanol was added dropwise with stirring at 40 °C. The solution was heated at 60 °C for 5 h, and then concentrated *in vacuo*. The residue, combined with 100 ml 2M NaOH was extracted with ether and this extract with diluted HCl. The aqueous layer was made alkaline with 30 % NaOH, and extracted with dichloromethane. Drying and evaporation gave 1.25 g of an oil. A warm solution of this oil and and oxalic acid dihydrate (0.63 g, 5.0 mmol) in 50 ml acetonitrile gave on cooling 1.61 g (63 %) of the oxalate of *5*. M.p. 162 – 164 °C, undepressed on admixture with a sample prepared by method (a).

(E)-3-(4-Bromophenyl)-3-(3-pyridyl)allylamine (6). The pthalimide *24* (1.6 g, 3.8 mmol), treated as described in method (b) for preparation of 5, gave 1.3 g (90 %) of the oxalate of *6*. Analytical sample, m.p. 169 – 171 °C (MeOH – CH_3CN, 8:1). Anal. $C_{14}H_{13}BrN_2.\frac{1}{2}C_2H_2O_4$: C, H, Br, N, O. UV: 237 (15400, shoulder), 221 (17500, max), 214 (17000, min). NMR: 8.45 (m, 1, 2-pyridyl), 8.41 (dd, partly concealed, 1, 6-pyridyl), 7.44, 6.96, 7.6 – 6.9 (AA'BB' + m, 6, C_6H_4 + 4,5-pyridyl), 6.13 (t, 1, CH), 3.30 (d, 2, CH_2).

1-(4-Bromophenyl)-3-dimethylamino-1-propanone (8). From 4-bromoacetophenone (*7*) compound *8* was obtained according to Ref. 13. Yield 73 % of the hydrochloride. M.p. 191 – 193 °C (lit.[13] 196 °C). The free amine *8* was prepared and recrystallized from hexane – ethanol. M.p. 66 – 68 °C.

1-(4-Bromophenyl)-3-(dimethylamino)-1-(3-pyridyl)propan-1-ol (9). 3-Bromopyridine (118 g, 0.78 mol) in 100 ml dry ether was added dropwise under nitrogen at −60 °C during 45 min to a solution of butyllithium (1.00 mol) in 425 ml hexane mixed with 200 ml ether. After stirring for another 15 min, a solution of *8* (102 g, 0.40 mol, free amine) in 600 ml ether was added dropwise at −40 to −30 °C during 50 min. The mixture was stirred for 2.5 h while the temperature was allowed to rise to ambient and then poured into 1500 g ice and 150 ml conc. HCl. The water layer was separated, the pH was adjusted to 4, washed with dichloromethane, made alkaline and extracted with ether three times. The ether layer was treated with charcoal, dried (Na_2SO_4) and concentrated. The residue was triturated with hexane and then recrystallized from 2-propanol to give 68.3 g (51 %) of *9*. M.p. 123 – 125 °C. Anal. $C_{16}H_{19}BrN_2O$: C, H, Br, N, O. NMR: 2.42 (s, 4, CH_2CH_2), 2.25 (s, 6, CH_3).

4-Bromophenyl 3-pyridyl ketone (10). This compound was prepared according to Ref. 5.

Ethyl 3-(4-bromophenyl)-3-hydroxy-3-(3-pyridyl)-propionate (11). A mixture of *10* (100 g, 0.38 mol) and zinc (50 g, 0.76 mol) in 200 ml dry benzene was refluxed under nitrogen, while ethyl bromoacetate (112 g, 0.70 mol) in 100 ml benzene was slowly added during 30 min. The mixture was further refluxed for 4 h, cooled and diluted with 600 ml benzene. The solution was washed three times with 10 % aqueous acetic acid. Ether (400 ml) was added and the hydrochloride of *11* was formed as an oil after addition of 10 % HCl. The salt was made alkaline and the amine extracted with benzene. The hydrochloride of *11* was precipitated by addition of HCl in ether to give 135 g (92 %). M.p. 177 – 182 °C (2-propanol). Anal. $C_{16}H_{16}BrNO_3.HCl$: C, H, Br, Cl, N, O. NMR: 4.03 (q, 2, CH_2O), 3.21 (s, 2, CH_2CO).

1-(4-Bromophenyl)-1-(3-pyridyl)-1,3-propanediol (12). A solution of *11* (9.5 g, 27 mmol, free amine) in 50 ml ether was added dropwise to an ice-cold mixture of $LiAlH_4$ (1.0 g, 27 mmol) and 150 ml ether. The reaction mixture was heated under reflux for 5 h, cooled and a saturated Na_2SO_4 solution was added. Filtration and evaporation gave a residue which was crystallized from chloroform to give 6.3 g (76 %) of *12*. M.p. 130 – 132 °C. Anal. $C_{14}H_{14}BrNO_2$: C, H, Br, O. NMR: 3.80 (t, 2, CH_2O), 2.50 (t, 2, CH_2).

3-(4-Bromophenyl)-3-(3-pyridyl)allyl bromide

(13). Compound 13 was not isolated. See preparation of *1* method (b) and (c).

1-(4-Bromophenyl)-1-(3-pyridyl)propene (14). Sodium hydride (3.0 g, 50 % in oil, 63 mmol) was heated in 100 ml anhydrous dimethyl sulfoxide at 85 °C for 30 min under nitrogen. After cooling to room temperature a solution of ethyltriphenylphosphonium iodide (26 g, 62 mmol, in 100 ml DMSO) was added with stirring. After 30 min a deep red colour had developed, and a solution of *10* (11.2 g, 42 mmol) in 100 ml tetrahydrofuran was added at room temperature. After another hour the mixture was poured into 1.5 l of ice-water. Extraction with 3 × 250 ml ether, washing with water, drying (Na_2SO_4) and evaporation gave 27.2 g of a semicrystalline residue. On trituration with ether–isopropylether (200 ml 1:1) at −5 °C for 1 h a solid was formed, which was filtered off. The filtrate was concentrated and distilled to give 10.8 g (90 %) of *14*. B.p. 110–120 °C/1–2 Pa, n_D^{25} 1.6272, *E*–*Z* ratio 1:1 (from NMR).

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)allyl chloride (15). A solution of 2,2,2-trichloroethyl chloroformate (24.8 g, 117 mmol) in 200 ml dry benzene was added dropwise to a stirred solution of *1* (20.0 g, 63 mmol, free amine) in 900 ml dry benzene. The reaction mixture was refluxed for 2 h, cooled, filtered and the solvent evaporated. Recrystallization from acetone gave 13.6 g (70 %) of the hydrochloride of *15*. M.p. 190–192 °C. Anal. $C_{14}H_{11}BrClN.HCl$: C, H, Br, Cl, N. UV: 251 (19900, max), 226 (12400, min). NMR (hydrochloride in $CDCl_3$): 9.06 (dq, 1, 6-pyridyl), 8.68 (m, 1, 2-pyridyl), 8.4–8.0 (m, 2, 4,5-pyridyl), 7.47, 7.01 (AA′BB′, 4, C_6H_4), 6.49 (t, 1, CH), 4.05 (d, 2, CH_2).

(E)-3-(4-Bromophenyl)-3-(3-pyridyl)allyl chloride (16). The hydrochloride of *16* was prepared from *2* in analogy with *15*. Yield 57 %. M.p. 208–210 °C (acetone). Anal. $C_{14}H_{11}BrClN.HCl$: C, H, Br, Cl, N. UV: 240 (18700, shoulder), 224 (20500, max). NMR (hydrochloride in $CDCl_3$): 8.84 (dm, 1, 6-pyridyl), 8.65 (m, 1, 2-pyridyl), 8.4–7.8 (m, 2, 4,5-pyridyl), 7.61, 7.09 (AA′BB′, 4, C_6H_4), 6.56 (t, 1, CH), 4.09 (d, 2, CH_2).

3-(4-Bromophenyl)-3-hydroxy-N-methyl-3-(3-pyridyl)propionamide (17). The hydrochloride of *11* (19.4 g, 50 mmol) was dissolved in a mixture of aqueous methylamine (40 %, 200 ml) and 30 ml ethanol. After stirring for 24 h at room temperature a precipitate had formed. It was collected and recrystallized from 150 ml 2-propanol, giving 13.2 g (79 %) of *17*. M.p. 188–191 °C. Analytical sample, m.p. 190–191 °C (2-PrOH). Anal. $C_{15}H_{15}BrN_2O_2$: C, H, Br, N, O. NMR: 3.42 (s, 1, OH), 3.20 (s, 2, CH_2), 2.57 (d, 3, CH_3).

1-(4-Bromophenyl)-3-(methylamino)-1-(3-pyridyl)propan-1-ol (18). To a mixture of *17* (5.0 g, 15 mmol) and $NaBH_4$ (4.0 g, 106 mmol) in 300 ml dry tetrahydrofuran, boron trifluoride etherate (23 ml, 183 mmol, in 100 ml THF) was added slowly at 0 °C. After 7 h at 0 °C another 4.0 g of $NaBH_4$ and boron trifluoride etherate (23 ml) were added as above and the mixture stirred for 50 h at room temperature. Water was then added slowly followed by 2 M NaOH to pH 11 and the mixture extracted with ether. Washing with water, drying (Na_2SO_4) and evaporation gave 15 g of a solid, which was treated with 50 ml 50 % H_2SO_4 at 110 °C for 10 min. After neutralization with saturated Na_2CO_3, extraction with ether gave 5.1 g of an oil. A warm solution of this oil and oxalic acid (1.4 g, 16 mmol) in 200 ml acetone gave on cooling the oxalate of *18*. Recrystallization from 250 ml ethanol gave 2.3 g (38 %). M.p. 179–182 °C. Anal. $C_{15}H_{17}BrN_2O.C_2H_2O_4$: C, H, Br, N, O. NMR: 4.67 (broad, 2, OH, NH), 2.62 (m, 2, CH_2), 2.32, 2.25 (m + s, 5, $CH_2 + CH_3$).

1-(4-Bromophenyl)-1-(3-pyridyl)-2-propen-1-ol (19). This compound was prepared according to Ref. 8.

3-(4-Bromophenyl)-3-hydroxy-3-(3-pyridyl)propionamide (20). The hydroxy-ester *11* (0.8 g, 2.5 mmol) was dissolved in 10 ml ethanol, concentrated NH_3 (50 ml) was added and the mixture was stirred at room temperature for 24 h. The precipitate formed was collected and recrystallized from 2-propanol. Yield 0.45 g (56 %) M.p. 213–214 °C. Anal. $C_{14}H_{13}BrN_2O_2$: H, Br, N, O. Found: C 51.9 Calc.: C 52.4. NMR: 3.16 (s, 2, CH_2).

3-(4-Bromophenyl)-3-hydroxy-3-(3-pyridyl)propionitrile (21). Acetonitrile (6.5 g, 0.16 mol) in 50 ml dry tetrahydrofuran was added dropwise under nitrogen at −50 °C to a mixture of butyllithium in hexane (100 ml, 1.5 M) and 50 ml tetrahydrofuran and allowed to react for 35 min. A solution of *10* (36.5 g, 0.14 mol, in 250 ml THF) was added at −50 °C and the mixture was set aside to reach room temperature and then poured into 500 g ice-water and 500 ml dichloromethane. The aqueous layer was further extracted with 2 × 200 ml dichloromethane. Washing with water, drying and evaporation gave 39.7 g of an oil. It was dissolved in 500 ml warm 2-propanol and 35 ml 4 M HCl-ether (0.14 mol) in 100 ml 2-propanol was added. Cooling gave 34.6 g (74 %) of the hydrochloride of *21*. M.p. 163–165 °C. Anal. $C_{14}H_{13}BrN_2O.HCl$: C, H, Br, N. Found: O 5.35. Calc.: O 4.71. NMR: 3.18 (s, 2, CH_2).

3-Amino-1-(4-bromophenyl)-1-(3-pyridyl)propan-1-ol (22). (a) *From hydroxy-nitrile* 21. A solution of *21* (17.2 g, 56 mmol) in 175 ml tetrahydrofuran was diluted with 200 ml ether. After cooling to −35 °C $LiAlH_4$ (4.0 g, 112 mmol) was added in portions under nitrogen. The mixture was kept at 0 °C for 2 h and then at 15 °C for 2 h. The reaction was quenched by slow addition of 20 ml saturated

Na_2SO_4. After stirring for 1/2 h, the inorganic salts were filtered off and extracted with 2 × 100 ml ether. Washing, drying and evaporation gave 14.7 g of an oil. This was dissolved in 500 ml warm 2-propanol and a solution of oxalic acid (4.3 g, 48 mmol) in 300 ml 2-propanol was added. After cooling overnight 11.8 g (53 %) crystals were collected. M.p. 98–105 °C. The free amine *22* was isolated and recrystallized from 2-propanol. M.p. 118–120 °C. MS: 308/306 (28/28, M), 106 (68), 30 (100). NMR: 2.90 (m, 2, CH_2), 2.33 (m, 2, CH_2).

(b) *From hydroxy-amide* 20. To a mixture of *20* (5.7 g, 17 mmol) and $NaBH_4$ (3.8 g, 100 mmol) in 200 ml tetrahydrofuran, boron trifluoride etherate (18.4 g, 130 mmol, in 50 ml THF) was added slowly under nitrogen at 0 °C. After stirring for 48 h at room temperature the mixture was made alkaline with 2M NaOH and extracted with chloroform. Evaporation gave 4 g of an oil, which crystallized on treatment with ether–hexane, yielding 2.3 g (63 %) of *22*. M.p. 95–115 °C. NMR: As for (a).

(Z)-1-(4-Bromophenyl)-3-pthalimido-1-(3-pyridyl)propene (23). Potassium pthalimide (4.7 g, 25 mmol) was added to a solution of the hydrochloride of *15* (5.2 g, 17 mmol) in 60 ml dimethylformamide and the mixture was heated with stirring at 80 °C for 3 h. Addition of ice-water, extraction with ether, drying and evaporation gave 5.8 g of a crystalline residue. Recrystallization from 140 ml methanol gave 2.9 g (41 %) of *23*. M.p. 136–140 °C. Anal. $C_{22}H_{15}BrN_2O_2$: C, H, Br, N, O.

(E)-1-(4-Bromophenyl)-3-pthalimido-1-(3-pyridyl)propene (24). Using the procedure described for compound *23*, the hydrochloride of *16* (5.2 g) gave 1.9 g (35 %) of *24*. M.p. 136–137 °C. Anal. $C_{22}H_{15}BrN_2O_2$: C, H, Br, N, O. NMR: 8.47 (m, 2, 2,6-pyridyl), 7.72, 7.55, 7.19, 7.9–7.0 (d + AA'BB' + m, 10, phthalimido + C_6H_4 + 4,5-pyridyl), 6.11 (t, 1, CH), 4.38 (d, 2, CH_2).

(Z)-3-(4-Bromophenyl)-3-(3-pyridyl)acrylonitrile (25). A mixture of NaH (20 g, 50 % in oil, 0.40 mol) and 200 ml dry dimethyl sulfoxide was heated at 80 °C for 40 min with stirring under nitrogen. After cooling a solution of diethyl cyanomethylphosphonate (53 g, 0.30 mol, in 150 ml DMSO) was slowly added and the mixture was stirred for 1.5 h at room temperature. A solution of *10* (60 g, 0.23 mol) in 350 ml tetrahydrofuran was slowly added at 23 °C. Stirring was continued for 1 h. The reaction mixture was poured into 1000 ml of ice-water. Extraction with 5 × 150 ml ether, washing of each portion with 100 ml water, drying of the combined extracts and evaporation of the solvent gave 73 g of an oil. This was dissolved in 200 ml benzene and separated on 800 g silica (0.063–0.200 mm) in benzene. When 2 l eluate had been collected, the solvent was changed to methanol–isopropylether–benzene (1:10:10). Subsequent fractions gave 19.6 g of compound *25*, 5.7 g of a mixture of *25* and *26*, and 1.9 g of *26*. TLC using methanol–isopropylether (1:25) showed R_F 0.36 for *25* and R_F 0.18 for *26*. The 19.6 g *25* was recrystallized from 60 ml ethanol giving 11.3 g. M.p. 122–123 °C. Anal. $C_{14}H_9BrN_2$: C, H, Br, N. MS: 286/284 (58/62), 205 (100). NMR: 5.87 (s, 1, CH).

(E)-3-(4-Bromophenyl)-3-(3-pyridyl)acrylonitrile (26). Fractions from the preparation of *25*, which contained *26*, were combined and recrystallized from isopropylether giving pure *26*. M.p. 93–94 °C. Anal. $C_{14}H_9BrN_2$: C, H, Br, N. MS: 286/284 (66/68), 205 (100). NMR: 5.77 (s, 1, CH). The hydrochloride was prepared and crystallized from acetone. M.p. 116–120 °C. Anal. $C_{14}H_9BrN_2$.HCl: C, H, N.

Stereostructure of the nitriles 25 and 26. Dry solutions of *25* and *26*, respectively, in deuteriochloroform (Merck Uvasol) were prepared. Solid $Eu(fod)_3$, tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium, (Ciba-Geigy, used as purchased) was added in portions until each solution showed an easily observable induced shift difference (LIS). The figures found are shown in Table *1*.

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Synthetic Inhibitors of Alcohol Dehydrogenase. Pyrazoles Containing an Unsaturated Hydrocarbon Residue in the 4-Position

BO-RAGNAR TOLF,[a] RICHARD DAHLBOM,[a] HUGO THEORELL[b] and ÅKE ÅKESON[b]

[a] Department of Organic Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden and [b] Laboratory for Enzyme Research, Nobel Institute of Biochemistry, Karolinska Institute, Solnavägen 1, Box 60 400, S-104 01 Stockholm 60, Sweden

A series of pyrazoles containing an unsaturated hydrocarbon residue in the 4-position has been synthesized and tested for ability to inhibit the activity of the enzyme liver alcohol dehydrogenase. These compounds were found to be less active than the corresponding saturated analogues.

Previous work in our laboratories has shown that pyrazoles substituted with an alkyl group in the 4-position are very potent inhibitors of the enzym horse liver alcohol dehydrogenase (LADH) *in vitro*.[1,2] The activity seems to be correlated to the lipophilicity of the substituent as the inhibitory power is increased for each carbon atom that is added to an unbranched chain and decreased by branching or cyclization of the chain. A number of these inhibitors have been tested in rats. It was found that pyrazole derivatives with long alkyl substituents strongly inhibit the ethanol metabolism *in vivo* too. However, the duration turned out to be shorter than that of the lowest homologue in the series, 4-methylpyrazole.[3] This may be due to different rates of metabolism and/or different distribution. These findings induced us to make a number of modifications of the substituent in the 4-position in the hope of finding compounds with longer duration. The present paper describes the synthesis of some pyrazoles containing an unsaturated hydrocarbon residue in the 4-position and results from *in vitro* tests for their ability to inhibit the activity of LADH.

CHEMISTRY

Pyrazoles having a substituent in the 4-position containing a triple bond have been synthesized from copper(I) acetylides and 4-halogenopyrazoles,[4,5] or by dehydrohalogenation of 4-halogenoacylpyrazoles [6] or 2-bromo-3-(4-pyrazolyl)acrylic acid derivatives.[7] However, these methods require forcing reaction conditions and do not offer convenient procedures for the preparation of the desired compounds.

During the last years, coupling of acetylenes and organometallic compounds with aryl or alkenyl halides using transition metal catalysts has received considerable attention.[8-14] It has been reported[9] that coupling of acetylenes with iodoarenes, bromopyridines and bromoalkenes proceeds smoothly at room temperature in diethylamine using cuprous iodide and bis(triphenylphosphine)palladium dichloride as catalysts. We found that this reaction could be used for the synthesis of 4-alkynylpyrazoles in good yields. However, 4-iodopyrazole could not be used as starting material apparently due to the acidity of the NH proton. The introduction of a protecting group was therefore necessary and the 2,4,6-trimethylbenzoyl group was found to be excellent. Unlike the benzoyl and *o*-toluyl groups, which were also tried it is stable during the reaction yet is easily removed. Thus, reaction of 4-iodo-1-(2,4,6-trimethylbenzoyl)pyrazole (*1*) with the appropriate acetylene compound in diethylamine in the presence of catalytic amounts of bis(triphenylphosphine) palladium dichloride and cuprous iodide afforded the pyrazoles *2a* – *2c* (Scheme 1) in excellent yields (83 – 97 %).

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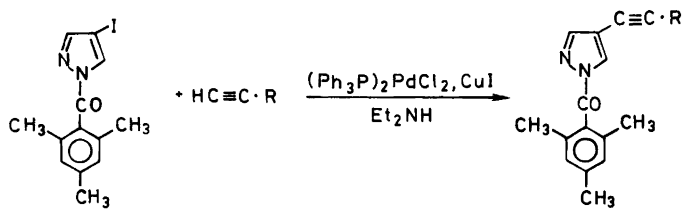


1

2a R = CH_2OH

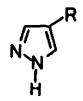
b R = C_4H_9

c R = C_6H_5

Scheme 1.

Compounds *2b* and *2c* were then treated with sodium hydroxide in aqueous methanol under mild conditions giving *5* and *6*.

4-Ethynylpyrazole (*4*) could not be prepared directly by this method since the use of acetylene would give a disubstituted acetylene.[9] However, Atkinson and co-workers[15] have shown that α,β-acetylenic aldehydes easily undergo deformylation, and the synthesis of *4* was accomplished by coupling *1* with 2-propyn-1-ol and subsequent oxidation of the resulting alcohol *2a* to the aldehyde *3* with active manganese dioxide in benzene at room temperature. Treatment of *3* with sodium hydroxide in aqueous methanol at 50 °C resulted in simultaneous deformylation and removal of the protecting group, thus affording *4*. Hydrogenation of *4* and *5* over a Lindlar catalyst gave the olefins *7* and *8a* (*Z* isomer), respectively. The *E* isomer *8b* was obtained in a rather sluggish reaction by treatment of *5* with lithium aluminium hydride in diglyme[16]



	R		R
4	C≡CH	*11*	$CH(OH)\cdot C_6H_5$
5	$C\equiv C\cdot C_4H_9$	*12*	$CH_2\cdot C_6H_5$
6	$C\equiv C\cdot C_6H_5$	*13*	$CH_2\cdot CH_2\cdot C_6H_5$
7	$CH=CH_2$	*14*	$(CH_2)_3\cdot Br$
8a	$CH=CH\cdot C_4H_9$ *(Z)*	*15*	$(CH_2)_3\cdot C\equiv CH$
8b	$CH=CH\cdot C_4H_9$ *(E)*	*16*	$(CH_2)_3\cdot CH=CH_2$
9a	$CH=CH\cdot C_6H_5$ *(Z)*	*17*	$(CH_2)_4\cdot CO\cdot C_6H_5$
9b	$CH=CH\cdot C_6H_5$ *(E)*	*18*	$(CH_2)_5\cdot C_6H_5$

at 155 °C. The geometrical isomers of 4-(2-phenylethenyl)pyrazole (*9a,b*) were obtained similarly from *6*.

Some compounds with the unsaturated residue separated from the pyrazole ring by one or more methylene groups were also prepared.

4-Benzylpyrazole (*12*) was obtained in the following manner. Reaction of 4-bromo-1-(2-tetrahydropyranyl)pyrazole (*10*) with butyllithium at −78 °C gave a 4-lithiopyrazole intermediate, which on reaction with benzaldehyde and subsequent hydrolysis of the protecting group afforded 4-(α-hydroxybenzyl)pyrazole (*11*). Reduction of *11* with hydrogen over a palladium catalyst gave the desired 4-benzylpyrazole (*12*). 4-(2-Phenylethyl)-pyrazole (*13*) was obtained by catalytic hydrogenation of *6*. 4-(3-Bromopropyl)pyrazole (*14*), obtained from the corresponding alcohol by treatment with thionyl bromide, reacted with sodium acetylide to give 4-(4-pentynyl)pyrazole *15* which was subsequently hydrogenated to the olefine *16*. Treatment of *14* with the sodium salt of ethyl benzoylacetate followed by hydrolysis of the ester and decarboxylation afforded 4-(5-oxo-5-phenylpentyl)pyrazole (*17*), which was submitted to Wolff-Kishner reduction to yield 4-(5-phenylpentyl)pyrazole (*18*).

EXPERIMENTAL

General. Melting points were determined in an electrically heated metal block using open capillary tubes and calibrated Anschütz thermometers. ^1H NMR spectra were recorded in $CDCl_3$ unless otherwise stated with TMS as internal standard, using a Perkin-Elmer R 12 B spectrometer. IR spectra were recorded on a Perkin-Elmer 157 G spectrophotometer, using KBr discs. GLC analyses

were run on a Varian 1700 chromatograph. Columns: 3 m long glass column packed with 3 % XE 60 or 5 % OV 25 on Chromosorb W (80–100 mesh). Individual compounds were isolated on a 250 × 0.9 cm glass column packed with 10 % XE 60 on Chromosorb W (60–80 mesh). For column chromatography silica gel 60, Merck, was used. Microanalyses were carried out at the Microanalytical Laboratory, Royal Agricultural College, Uppsala. All reactions with bis(triphenylphosphine)palladium dichloride were performed under nitrogen.

Starting materials. 4-Bromopyrazole,[17] 4-iodopyrazole[17] and 4-(3-hydroxypropyl)pyrazole[18] were prepared according to the literature. 2,4,6-Trimethylbenzoyl chloride was prepared from the commercially available 2,4,6-trimethylbenzoic acid. Bis(triphenylphosphine)palladium dichloride, 2-propyn-1-ol, 1-hexyne and phenylacetylene were commercially available.

4-Iodo-1-(2,4,6-trimethylbenzoyl)pyrazole (1). 2,4,6-Trimethylbenzoyl chloride (18.3 g, 0.1 mol) was added dropwise at room temperature to a solution of 4-iodopyrazole (17.6 g, 0.091 mol) and triethylamine (10.1 g, 0.1 mol) in dry benzene–ether (120 ml, 5:1). The reaction mixture was refluxed for 8 h and filtered. The filtrate was washed with water and after drying ($MgSO_4$) and evaporation of the solvent *in vacuo* the solid residue was recrystallized from hexane affording 21.8 g (71 %) of product, m.p. 84–85 °C. Anal. $C_{13}H_{13}IN_2O$: C, H, N. IR: 1725 cm^{-1}. ^1H NMR: δ 2.14 (6H, s), 2.32 (3H, s), 6.93 (2H, s), 7.71 (1H, s), 8.44 (1H, s).

4-(3-Hydroxy-1-propynyl)-1-(2,4,6-trimethylbenzoyl)pyrazole (2a). To a stirred solution of *1* (10.0 g, 0.029 mol) and 2-propyn-1-ol (1.7 g, 0.03 mol) in diethylamine (100 ml) a catalytic amount of bis(triphenylphosphine)palladium dichloride and cuprous iodide was added. The mixture was stirred at room temperature and the reaction was followed by TLC (silica gel; ether–light petroleum 1:2 until completion (24 h). The solvent was then evaporated *in vacuo* and ether was added to precipitate the salts. After filtration and evaporation, the crude product was purified on a silica gel column (ether–light petroleum) affording 7.7 g (97 %) of a slowly crystallizing product. A small sample was recrystallized from ether–hexane, m.p. 72.5–73.5 °C. Anal. $C_{16}H_{16}N_2O_2$: C, H, N. IR: 2240, 1720 cm^{-1}. ^1H NMR: δ 2.13 (6H, s), 2.32 (3H, s), 6.42 (2H, s), 6.95 (2H, s), 7.77 (1H, s), 8.41 (1H, s).

4-(1-Hexynyl)-1-(2,4,6-trimethylbenzoyl)pyrazole (2b) was prepared from *1* (2.5 g) and 1-hexyne (0.7 g) according to the procedure described above. After purification on a silica gel column (ether–light petroleum) the title compound was obtained as an oil (2.0 g, 90 %). Anal: $C_{19}H_{22}N_2O$: C, H, N. IR (film): 2240, 1720 cm^{-1}. ^1H NMR: δ 0.93 (3H, t), 1.2–1.8 (4H, m), 2.30 (6H, s), 2.31 (3H, s), 2.39 (2H, t), 6.92 (2H, s), 7.71 (1H, s), 8.34 (1H, s).

4-Phenylethynyl-1-(2,4,6-trimethylbenzoyl)pyrazole (2c) was prepared similarly from *1* (2.5 g) and phenylacetylene (0.8 g). After column chromatography (silica gel, ether) and recrystallization (methanol), 1.9 g (83 %) of *2c* was obtained, m.p. 122–123 °C. Anal. $C_{21}H_{18}N_2O$: C, H, N. IR: 1720 cm^{-1}. ^1H NMR: δ 2.16 (6H, s), 2.31 (3H, s), 6.93 (2H, s), 7.20–7.60 (5H, m), 7.82 (1H, s), 8.47 (1H, s).

4-Formylethynyl-1-(2,4,6-trimethylbenzoyl)pyrazole (3). To a solution of *2a* (7.0 g, 0.026 mol) in dry benzene (150 ml) was added active manganese dioxide (34 g, 0.4 mol). The reaction mixture was shaken vigorously for 10 h until the starting material was consumed (TLC). After filtration through celite, the solvent was evaporated *in vacuo* affording 6.0 g (87 %) of a yellow oil, which was used without additional purification in the next step. IR (film): 2740, 2200, 1720, 1660 cm^{-1}. ^1H NMR: δ 2.14 (6H, s), 2.32 (3H, s), 6.94 (2H, s), 7.88 (1H, s), 8.51 (1H, s), 9.39 (1H, s).

4-Ethynylpyrazole (4). To a solution of *3* (6.0 g, 0.023 mol) in methanol (120 ml), 5 M NaOH (40 ml) was added at 50 °C. The solution was stirred at 50 °C for 0.5 h and was then concentrated during 0.5 h to *ca.* 50 ml under reduced pressure. Water (40 ml) was added and the solution was extracted with ether in a Soxhlet apparatus. The ethereal solution was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified on a silica gel column using ether–hexane as eluent, affording 1.4 g (70 %) of *4*, m.p. 101–103 °C (solidified oil). Anal. $C_5H_4N_2$: C, H, N. IR: 3280, 2110 cm^{-1}. ^1H NMR: δ 3.03 (1H, s), 7.80 (2H, s). *Hydrochloride:* m.p. 118–119 °C (dec.) from ethanol–ether). Anal. $C_5H_4N_2 \cdot HCl$: C, H, N.

4-(1-Hexynyl)pyrazole (5) was prepared from *2b* (2.0 g) using the procedure described for *4*. Purification on a silica gel column (ether–light petroleum) afforded 0.89 g (89 %) of the title compound, m.p. 37–38 °C (solidified oil). Anal. $C_9H_{12}N_2$: C, H, N. IR: 2220 cm^{-1}. ^1H NMR: δ 0.93 (3H, t), 1.1–1.8 (4H, m), 2.38 (2H, t), 7.67 (2H, s). *Hydrochloride:* m.p. 93.5–94.5 °C (dec.) (from ethanol–ether). Anal. $C_9H_{12}N_2 \cdot HCl$: C, H, N.

4-Phenylethynylpyrazole (6) was prepared similarly by hydrolysis of *2c* (1.8 g) during 1 h. Extraction with ether, drying ($MgSO_4$) and evaporation of the solvent *in vacuo* afforded a crystalline residue which was recrystallized from ether–hexane to give 0.85 g (88 %) of the title compound, m.p. 134–134.5 °C. Anal. $C_{11}H_8N_2$: C, H, N. IR: 2220 cm^{-1}. ^1H NMR: δ 7.2–7.7 (5H, m), 7.80 (2H, s). *Hydrochloride:* m.p. 125.5–126.5 °C (dec.) (from ethanol–ether). Anal. $C_{11}H_8N_2 \cdot HCl$: C, H, N.

4-Ethenylpyrazole (7). A solution of *4* (1.0 g,

0.011 mol) in ethanol (25 ml) was hydrogenated at room temperature over a Lindlar catalyst at atmospheric pressure until the starting material had been consumed (48 h), the reaction being followed by GLC. After filtration through celite and evaporation of the solvent, the residue was recrystallized (light petroleum) affording 0.4 g (40 %) of *7*, m.p. 88–89 °C. Anal. $C_5H_6N_2$: C, H, N. ^{1}H NMR: δ 5.0–5.7 (2H, m), 6.4–6.9 (1H, m) 7.71 (2H, s). *Hydrochloride:* m.p. 124–126 °C (dec.) (from ethanol–ether). Anal. $C_5H_6N_2 \cdot HCl$: C, H, N.

*(Z)-4-(1-Hexenyl)pyrazole (*8a*).* A solution of *5* (0.8 g) in ethanol (25 ml) was hydrogenated similarly over a Lindlar catalyst for 24 h. After filtration through celite and evaporation of the solvent *in vacuo*, the product was isolated by preparative GLC affording 0.23 g (29 %) of *8a*, m.p. 39.5–40.5 °C (solidified oil). ^{1}H NMR: δ 0.91 (3H, t), 1.1–1.7 (4H, m), 2.0–2.6 (2H, m), 5.25–6.15 (2H, m), 7.59 (2H, s). *Hydrochloride:* m.p. 111.5–112.5 °C (dec.) (from ethanol–ether). Anal. $C_9H_{14}N_2 \cdot HCl$: C, H, N.

*(E)-4-(1-Hexenyl)pyrazole (*8b*).* A solution of *5* (1.5 g) in diglyme (30 ml) was treated with lithium aluminium hydride (0.77 g) at 155 °C for 6 h. After hydrolysis with water and filtration, ether was added. The ethereal solution was washed with water and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by preparative GLC affording 0.33 g (22 %) of *8b*, m.p. 85.5–86.5 °C. ^{1}H NMR: δ 0.92 (3H, t), 1.1–1.6 (4H, m), 1.9–2.4 (2H, m) 5.6–6.45 (2H, m), 7.53 (2H, s). *Hydrochloride:* m.p. 137–138 °C (dec.) (from ethanol–ether). Anal. $C_9H_{14}N_2 \cdot HCl$: C, H, N.

*(Z)-4-(2-Phenylethenyl)pyrazole (*9a*).* A solution of *6* (1.0 g, 5.9 mmol) in ethanol (30 ml) was hydrogenated over a Lindlar catalyst as described above for compound *7*. Purification on a silica gel column with ether as eluent afforded 0.75 g (75 %) of the title compound, m.p. 51–52.5 °C (solidified oil). ^{1}H NMR: δ 6.38 (2H, s), 7.1–7.5 (7H, m). *Hydrochloride:* m.p. 131–132 °C (from ethanol–ether). Anal. $C_{11}H_{10}N_2 \cdot HCl$: C, H, N.

*(E)-4-(2-Phenylethenyl)pyrazole (*9b*).* A solution of *6* (1.0 g, 5.9 mmol) in diglyme (40 ml) was treated with $LiAlH_4$ (0.5 g) at 120 °C for 5 h as described for *8b*. Yield 0.5 g (50 %), m.p. 216–217 °C (from acetone). Anal. $C_{11}H_{10}N_2$: C, H, N. ^{1}H NMR ($CDCl_3$, CD_3COCD_3): δ 6.88 (1H, d, J=17 Hz), 7.04 (1H, d, J=17 Hz), 7.2–7.5 (5H, m), 7.75 (2H, s). *Hydrochloride:* m.p. 185.5–187 °C (dec.) (from ethanol–ether). Anal. $C_{11}H_{10}N_2 \cdot HCl$: C, H. N.

*4-Bromo-1-(2-tetrahydropyranyl)pyrazole (*10*).* A mixture of 4-bromopyrazole (2.0 g, 13.6 mmol) and catalytic amounts of *p*-toluenesulfonic acid in dihydropyran (15 ml) was refluxed for 7 h. Ether (100 ml) was added and the mixture was washed with saturated Na_2CO_3 solution and water. After drying (Na_2CO_3) and evaporation of the solvent *in vacuo*, the residue was purified by column chromatography [silica gel, ether–light petroleum (1:2)] affording 2.15 g (69 %) of the title compound as a liquid. ^{1}H NMR: δ 1.2–2.3 (6H, m), 3.35–4.2 (2H, m), 5.31 (1H, t), 7.47 (1H, s), 7.62 (1H, s). The compound was used in the next step without being analyzed.

*4-(α-Hydroxybenzyl)pyrazole (*11*).* To a solution of *10* (2.0 g, 8.7 mmol) in dry THF (30 ml) was added dropwise a solution of butyllithium in hexane (7.4 ml, 1.28 *M*) at −78 °C. After 10 min at this temperature benzaldehyde (1.1 g, 10.4 mmol) in THF (5 ml) was added dropwise. After 2 h at −78 °C the reaction mixture was stirred at room temperature overnight. The reaction mixture was treated with saturated NH_4Cl solution and ether (100 ml) was added. The organic phase was washed with water, dried (Na_2CO_3) and evaporated *in vacuo*. The residue was hydrolyzed at room temperature in a mixture (200 ml) of 1 *M* HCl and ethanol (1:1) overnight. The mixture was evaporated to a volume of about 50 ml and extracted with ether, made alkaline with saturated Na_2CO_3 and extracted with $CHCl_3$. After drying ($MgSO_4$) and evaporation *in vacuo* the residue was recrystallized from acetone–hexane affording 0.4 g (26 %) of the title compound, m.p. 92–94 °C. Anal. $C_{10}H_{10}N_2O$: C, H, N. ^{1}H NMR: δ 5.58 (1H, s), 6.9–7.4 (7H, m).

*4-Benzylpyrazole (*12*).* A solution of *11* (0.35 g, 2 mmol) in HOAc (25 ml) was hydrogenated in the presence of a small amount of 10 % Pd/C in a Parr low pressure hydrogenation apparatus. The catalyst was filtered off, the solvent evaporated *in vacuo* and the residue purified on preparative TLC (silica gel, ether) affording 0.05 g of an oil, which was converted to its *oxalate*, m.p. 150–151 °C (dec.) (from ethanol–ether). Anal. $C_{10}H_{10}N_2 \cdot (CO_2H)_2$: C, H, N. ^{1}H NMR (base): δ 3.76 (2H, s), 7.1–7.5 (7H, m).

*4-(2-Phenylethyl)pyrazole (*13*).* A solution of *6* (0.5 g, 3 mmol) in ethanol (20 ml) was hydrogenated at room temperature over a palladium catalyst (5 % on Al_2O_3) at atmospheric pressure for 3 h, when the calculated amount of hydrogen had been consumed. Filtration and evaporation of the solvent left a solid residue (0.5 g, 100 %). A small amount was recrystallized from hexane, m.p. 94–95 °C. Anal. $C_{11}H_{12}N_2$: C, H, N. ^{1}H NMR: δ 2.82 (4H, s), 7.20 (5H, s), 7.31 (2H, s). *Oxalate:* m.p. 157–158 °C (from ethanol–hexane). Anal. $C_{11}H_{12}N_2 \cdot (CO_2H)_2$: C, H, N.

*4-(3-Bromopropyl)pyrazole (*14*).* To a stirred suspension of 4-(3-hydroxypropyl)pyrazole (5.0 g, 0.04 mol) in dry benzene (25 ml) was added a solution of thionyl bromide (8.2 g, 0.04 mol) in dry benzene benzene (25 ml). The mixture was refluxed for 1 h and cooled. After addition of ether, the product

was filtered off and recrystallized from chloroform–ether to give the *hydrobromide* of the title compound (9.3 g, 87 %), m.p. 147–148 °C. Anal. $C_6H_9BrN_2 \cdot HBr$: C, H, N. The free base had m.p. 58–59 °C. ^1H NMR: δ 2.10 (2H, m), 2.70 (2H, t), 3.41 (2H, t), 7.48 (2H, s).

4-(4-Pentynyl)pyrazole (15). To a solution of sodium acetylide, prepared from sodium (1.2 g, 0.052 mol) in liquid ammonia (100 ml) and acetylene, a solution of *14* (4.0 g, 0.021 mol) in ether (25 ml) was added dropwise at −45 °C. The reaction mixture was stirred at this temperature for 3.5 h when the ammonia was allowed to evaporate. Ice-water was added and the mixture was extracted with ether (3 × 60 ml). The ethereal solution was washed with NH_4Cl solution and dried ($MgSO_4$). Evaporation of the ether *in vacuo* and distillation afforded *15* (0.9 g, 32 %), b.p. 114–115 °C/1 mmHg, m.p. 39–40 °C (solidified oil). The yield was improved considerably (75 %) if the product was purified by column chromatography (silica gel, ether) instead of distillation. Anal. $C_8H_{10}N_2$: C, H, N. IR: 3290, 2120 cm^{-1}. ^1H NMR: δ 1.6–2.5 (5H, m), 2.66 (2H, t), 7.47 (2H, s). *Semioxalate:* m.p. 152–153 °C. Anal. $C_8H_{10}N_2 \cdot 0.5\,(CO_2H)_2$: C, H, N.

4-(4-Pentenyl)pyrazole (16). A solution of *15* (1.5 g, 0.011 mol) and an equivalent amount of oxalic acid in ethanol (40 ml) was hydrogenated at room temperature over a small amount of Lindlar catalyst at atmospheric pressure until the calculated amount of hydrogen had been consumed and no trace of starting material remained (GLC). The catalyst was filtered off and the solvent evaporated *in vacuo*. The residue was treated with aqueous Na_2CO_3 and extracted with ether. After drying ($MgSO_4$) the ethereal solution was fractionated under reduced pressure affording 1.1 g (73 %) of product, b.p. 90–91 °C/0.3 mmHg. Anal. $C_8H_{12}N_2$: C, H, N. ^1H NMR: δ 1.2–2.3 (4H, m), 2.47 (2H, t), 4.80–5.05 (2H, m), 5.47–6.12 (1H, m), 7.33 (2H, s). *Oxalate:* m.p. 113–114 °C (from ethanol–ether). Anal. $C_8H_{12}N_2 \cdot (CO_2H)_2$: C, H, N.

4-(5-Oxo-5-phenylpentyl)pyrazole (17). To a solution of sodium ethoxide, prepared from sodium (1.2 g, 0.052 mol) and absolute ethanol (60 ml), ethyl benzoylacetate (10.3 g, 0.054 mol) was added and the mixture heated to reflux. A solution of the bromide *14* (6.7 g, 0.035 mol) in absolute ethanol (30 ml) was added dropwise and the reaction mixture was refluxed for 16 h. After filtration, evaporation *in vacuo* and addition of water (20 ml), conc. H_2SO_4 (20 ml) was added dropwise. The mixture was refluxed for 1 h, extracted with ether (4 × 50 ml) and made alkaline with NaOH (50 %). Extraction with ether (6 × 50 ml), drying ($MgSO_4$) and evaporation of the solvent *in vacuo* afforded the title compound, (5.3 g, 65 %), m.p. 83–84 °C (from ethanol–water). IR: 1680 cm^{-1}. ^1H NMR: 1.4–2.1 (4H, m), 2.55 (2H, t), 2.97 (2H, t), 7.25–8.1 (7H, m). *Oxalate:* m.p. 170–171 °C (from ethanol–light petroleum). Anal. $C_{14}H_{16}N_2O \cdot (CO_2H)_2$: C, H, N.

4-(5-Phenylpentyl)pyrazole (18). A solution of the oxo compound *17* (1.5 g), hydrazine hydrate (1 ml, 85 %) and KOH (1.1 g) in diethylene glycol was heated at 140 °C for 1.5 h and then at 200 °C for 1.5 h. After cooling, the reaction mixture was poured into water (50 ml) and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$), evaporated *in vacuo* and the residue distilled affording *13* (1.0 g, 71 %), b.p. 186 °C/0.6 mmHg. ^1H NMR: δ 1.2–2.0 (6H, m), 2.50 (2H, t), 2.60 (2H, t), 7.23 (5H, s), 7.40 (2H, s). *Oxalate:* m.p. 126–127.5 °C (from ethanol–light petroleum). Anal. $C_{14}H_{18}N_2 \cdot (CO_2H)_2$: C, H, N.

RESULTS AND DISCUSSION

The inhibitory power of the pyrazole derivatives on LADH activity was tested fluorometrically by observing the change of fluorescence of the coenzyme on its reduction with ethanol as substrate, and the inhibition constant, K_I, was determined as previously described.[1,2] The inhibition constants are shown in Table 1 which also includes some corresponding saturated compounds for comparison.

Table 1 shows that the introduction of a double or a triple bond in the substituent at the 4-position affords compounds which are less active than the corresponding saturated analogues. Compounds *4*, *5* and *7* are considerably less active (17–70 times) than the corresponding saturated derivatives 4-ethyl- and 4-hexylpyrazole, whereas *8a*, *8b*, *15* and *16* are only 4–9 times weaker than the saturated analogues. Apparently, the influence of a multiple bond in the latter compounds is more strongly correlated to the decrease in lipophilicity [20] of the substituents than is the case with compounds *4*, *5* and *7*. We have earlier found a positive correlation between the inhibitory power of 4-substituted alkylpyrazoles and the lipophilicity of the substituent [2] but the present data indicate that also electronic factors have an influence on the inhibitory activity. Some previously published results [1,2] suggest the same trend. Thus, 4-trifluoromethylpyrazole was found to be about 6 times less active than the methyl analogue in spite of its higher lipophilicity, and the difference in inhibitory power between 4-phenyl- and 4-cyclohexylpyrazole is greater than can be expected only from the

Table 1. Inhibitory power of pyrazole derivatives on LADH.

Compound	K_I, μM
4-Ethynylpyrazole (*4*)	0.50
4-(1-Hexynyl)pyrazole (*5*)	0.019
4-Phenylethynylpyrazole (*6*)	0.005
4-Ethenylpyrazole (*7*)	0.12
(*Z*)-4-(1-Hexenyl)pyrazole (*8a*)	0.0045
(*E*)-4-(1-Hexenyl)pyrazole (*8b*)	0.0025
(*Z*)-4-(2-Phenylethenyl)pyrazole (*9a*)	0.03
(*E*)-4-(2-Phenylethenyl)pyrazole (*9b*)	0.0016 [f]
4-Benzylpyrazole (*12*)	0.011
4-(2-Phenylethyl)pyrazole (*13*)	0.0011 [f]
4-(4-Pentynyl)pyrazole (*15*)	0.0073
4-(4-Pentenyl)pyrazole (*16*)	0.0034
4-(5-Phenylpentyl)pyrazole (*18*)	0.0005 [f]
Pyrazole [a]	0.22
4-Methylpyrazole [a]	0.013
4-Ethylpyrazole [a]	0.007
4-Pentylpyrazole [a]	0.0008
4-Hexylpyrazole [b]	0.0005
4-Phenylpyrazole [a]	0.1
4-Cyclohexylpyrazole [b]	0.0078
4-Cyclohexylmethylpyrazole [b]	0.0021
1*H*-Indazole (benzopyrazole) [c]	14
4,5,6,7-Tetrahydro-1*H*-indazole [d]	0.75
1-Methyl-4-propylpyrazole [e]	100

[a] From Ref. 1. [b] From Ref. 2. [c] From Ref. 19. [d] The inhibition constant for this compound was erroneously reported to be 0.075 in Ref. 2. [e] The inhibition constant for this compound was erroneously reported to be 0.10 in Ref. 2. [f] Corrected for the amount of the inhibitor bound by the enzyme.

difference in lipophilicity of the substituents. The high inhibitory activity of 4-phenylethynylpyrazole (*6*) cannot be anticipated when the activities of compound *4* and 4-phenylpyrazole are considered. This is also the case with the *E* isomer of 4-(2-phenylethenyl)pyrazole (*9b*). Evidently, conjugation between the pyrazole and benzene nuclei in compounds *6* and *9b* strongly reinforces the inhibitory activities. In compounds *12* and *13*, where the conjugation is broken, the phenyl group contributes to a lesser extent to the increase in activity when compared to the activities of the parent compounds 4-methyl- and 4-ethylpyrazole.

The pronounced difference in activity between the geometric isomers *9a* and *9b*, the *E (trans)* isomer being about 20 times more potent than the *Z (cis)* isomer, is noteworthy. However, inspection of molecular models shows the likely explanation.

It is reasonable to assume that the two rings are coplanar, and in such a case the 3(5)-position of the pyrazole ring in the *Z* isomer will be effectively blocked by the benzene ring thus making the compound less active, probably owing to steric hindrance. On the other hand, there is little difference in activity between the isomers of 4-(1-hexenyl)-pyrazole (*8a*, *8b*), the *E* isomer being 1.8 times more potent than the *Z* isomer. This may reflect the great conformational flexibility of the 1-hexenyl chain.

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But There Is Oxidized Glutathione in *Streptococcus faecalis*

REIJO LAHTI, PAULI VUORINEN and MIKKO SUONPÄÄ

Department of Biochemistry, University of Turku, SF-20500 Turku 50, Finland

Redox state of glutathione in *Streptococcus faecalis* was examined. Cells taken from the exponential phase of growth contained reduced and oxidized glutathione at concentrations of 2.5 and 1.1 mM, respectively, whereas at the stationary phase the corresponding values were 0.35 and 0.3 mM, Our results indicate that, contrary to common opinion, as much as 23 – 31 % of total glutathione is in its oxidized form.

It has been generally thought that there is no oxidized glutathione (GSSG) in bacterial cells.[1,2] However, we think that this view is due in part to a lack of reliable methods for the determination of GSSG.[3] The most commonly used method, presented by Tietze,[4] involves complexing of reduced glutathione (GSH) with *N*-ethylmaleimide (NEM), extraction of the unreacted NEM with ether, and measurement of the yellow colour formed as a result of incubation of GSSG in the presence of glutathione reductase (EC 1.6.4.2), NADH, and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). However, we[3] and others[5,6] have shown that complete extraction of NEM, a potent inhibitor of glutathione reductase,[7] is very difficult. So it is obvious that the most commonly used method for the determination of GSSG decribed by Tietze[4] should not be used since it may lead to a severe underestimation of intracellular GSSG levels.

We have recently described[3] a simple modification of Tietze's[4] method in which CuCl instead of NEM is used in the precipitation of GSH. Making use of this method we determined intracellular GSSG concentrations in *Streptococcus faecalis*, and found that there is oxidized glutathione in bacteria. The details of these studies are described in this paper.

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MATERIALS AND METHODS

Streptococcus faecalis ATCC 8043 was grown in a rich medium at 37 °C in a rotary shaker and growth was followed by measuring the turbidity of the culture with a Klett-Summerson colorimeter as described by Lahti & Heinonen.[8] Samples (2.5 ml) taken from the culture were rapidly chilled by 1:1 dilution with ice-cold 0.15 M NaCl containing 10 mM sodium azide. Cells were harvested by centrifugation (5000 *g*, 10 min, 4 °C), and washed once with ice-cold 0.15 M NaCl containing 10 mM sodium azide. The cells were stored at −70 °C.

Glutathione was extracted from the cells by shaking (20 min 37 °C) in buffer-toluene mixture (0.1 ml toluene in 5 ml of 0.1 M potassium phosphate buffer pH 7.5 containing 5 mM Na-EDTA). The extracts were centrifuged (5000 *g*, 10 min, 4 °C) and glutathione was measured in the supernatant.

Total glutathione was determined as described by Tietze.[4] In this method GSSG is reduced to GSH by glutathione reductase and NADH, and a yellow GSH-5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) complex is formed which can be measured with a Klett-Summerson colorimeter using filter 42 (390 – 440 nm). The reaction mixture was as follows: 4 ml of diluted cell extracts (dilution with 0.1 M potassium phosphate buffer pH 7.5 containing 5 mM Na-EDTA) containing up to 0.3 and 0.2 nmol glutathione per reaction mixture for total and oxidized glutathione, respectively; 1 ml of 1.1 mM Na-NADH, 0.025 ml (about 3.5 units) of glutathione reductase, and 0.1 ml of 10 mM DTNB.

GSSG was determined in the same way after precipitation of GSH with CuCl.[3] CuCl as powder (20 mg) was added to 5 ml of diluted extracts containing up to 0.25 nmol glutathione. After 30 s of vigorous mixing with a Vortex mixer at room temperature (about 20 °C) the suspension was

centrifuged (5000 *g*, 10 min, 4 °C) and GSSG was determined in the supernatant. As 95 % of GSH was precipitated by CuCl, this had to be taken into account in the calculations. Furthermore CuCl was slightly oxidized under our experimental conditions (the solution became blue), resulting in a partial inhibition of glutathione reductase.[3] To overcome this effect a GSSG standard was determined in the presence of CuCl. For the estimation of intracellular glutathione the volume of the bacterial cells in each sample was calculated as described by Moses and Sharp.[9]

RESULTS AND DISCUSSION

We[3] have recently introduced a simple modification of Tietze's method[4] for the determination of GSSG in which CuCl is used instead of NEM in the precipitation of GSH. In this system care must be taken not to exceed the capacity of CuCl to precipitate glutathione. Therefore when oxidized glutathione was determined the samples taken from the culture were diluted (with potassium phosphate buffer pH 7.5 containing 5 mM Na-EDTA) so that they contained at most 0.20 nmol glutathione per reaction mixture in order to ensure efficient precipitation of GSH (Fig. 1).

Table 1 shows the concentrations of oxidized and reduced glutathione found in the exponential and stationary phase of growth in *Streptococcus faecalis*.

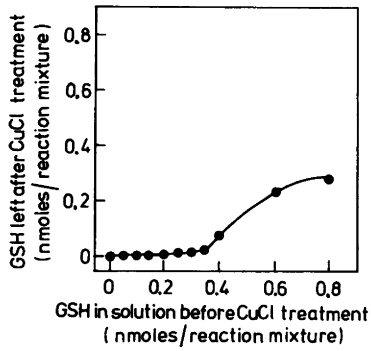

Fig. 1. Precipitation of GSH with CuCl. Different dilutions of GSH standard were made in 0.1 M potassium phosphate buffer pH 7.5 containing 5 mM Na-EDTA. Then CuCl treatment and GSH measurement were performed as described in Materials and Methods. ●, indicates the concentration of GSH observed in the solution after CuCl treatment.

Table 1. Glutathione content in *Streptococcus faecalis*. E and S indicate samples taken from the exponential and stationary phase of growth, respectively. KU_{62} denotes to the turbidity of the culture as Klett-Summerson colorimetric units. For further detailes see Materials and Methods.

Phase of growth	GSH (mM)	GSSG (mM)	GSSG (% of total)[a]
E_1 (KU_{62} = 43)	2.44	1.07	23.4
E_2 (KU_{62} = 62)	2.54	1.10	23.2
S_1 (KU_{62} = 195)	0.37	0.34	32.3
S_2 (KU_{62} = 195)	0.33	0.25	30.1

[a] Total glutathione = GSH + 2 × GSSG.

The concentrations of total glutathione calculated from Table 1 are close to those observed with other bacteria.[1,10,11] However, it is most remarkable that, contrary to common opinion,[1,2] there is a significant amount of oxidized glutathione in *S. faecalis;* 23 and 31 % of total glutathione in the exponential and stationary phases, respectively (Table 1). Complete recoveries for GSH (data not shown) and GSSG (Table 2) indicate that GSH is not oxidized to GSSG during the manipulation of samples. This is further supported by the fact that similar amounts of GSSG were observed by extracting glutathione from the cells by ethanol immediately after filtration of the samples taken from the culture. However, for some unknown reason, the ethanol extraction was not precise enough to be used routinely, and so it was omitted.

We think it quite natural that there is GSSG in the cells because in other redox-systems (NAD^+ −NADH, $NADP^+$ −NADPH) the oxidized form exists as a majority.[12-16]

Rather low K_m-values for GSSG (0.01 − 0.1 mM) have generally been observed with glutathione reductase, and this has been thought to support the view that the intracellular concentration of GSSG is very low.[17-19] However, it must be recognized that a K_m-value in two-substrate systems is a rather complicated concept; the magnitude of K_m-value may depend, for instance, on the concentrations of both substrates.[20] No matter how tightly the enzyme binds GSSG, complete reduction will not occur unless the concentration of NADPH available for glutathione reductase at least equals the GSSG concentration. In addition to glutathione reductase, several enzymes catalyzing biosynthetic reactions require NADPH as a reducing agent. Hence it is

Table 2. Recovery of oxidized glutathione. Oxidized glutathione (GSSG) was added to the samples taken from the exponential ($KU_{62}=20$) and stationary ($KU_{62}=180$) phases of growth. Preparation of the cell extracts by toluene, precipitation of GSH by CuCl, and GSSG measurement were carried out as described in Materials and Methods.

Phase of growth	GSSG observed[a] in the sample	GSSG added[a] to the sample	GSSG observed[a] from the mixture	Recovery (%)
Exponential phase of growth	0.054	0.05	0.106	102
Stationary phase of growth	0.049	0.05	0.095	96

[a] nmol GSSG/reaction mixture.

difficult to anticipate the concentration of NADPH available for glutathione reductase in the cells. It can be mentioned, for interest, that with glutathione reductase of *Chromatium vinosum* Chun and Hurlbert[21] observed a rather high K_m-value for GSSG (7 mM). They thought that the real substrate for this enzyme could not be GSSG but some other disulfide existing in cells in much higher concentrations. The internal concentrations of GSSG found in *S. faecalis* would make it possible for the glutathione reductase of *C. vinosum* to use GSSG as a substrate.

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2-Oxo-1,3,2-dioxathianes. I. Preparation of the Alkyl-substituted Derivatives

TERTTU VIRTANEN and HANNU NIKANDER*

Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku 50, Finland

2-Oxo-1,3,2-dioxathiane, all methyl- and several other alkyl-substituted 2-oxo-1,3,2-dioxathianes have been synthesized by condensing 1,3-alkanediols and thionyl chloride. The amount of the S=O-axial and S=O-equatorial isomers can be controlled by adding pyridine to the reaction mixture.

2-Oxo-1,3,2-dioxathianes are cyclic sulfurous acid esters, cyclic sulfites. The parent compound, 2-oxo-1,3,2-dioxathiane has been shown to exist in a chair conformation with the S=O group axially oriented.[1,2] An axial S=O group has been found to be about 15 kJ mol^{-1} more stable than an equatorial S=O group.[2] Substituted derivatives exist preferentially in a chair conformation with an axial or equatorial S=O group, or in a mixture of two interconverting chair forms.[1,2]

2-Oxo-1,3,2-dioxathianes can be synthesized by condensing an appropriate 1,3-alkanediol and thionyl chloride (Fig. 1). They are easily hydrolyzed by base to the corresponding diols. Since diastereoisomeric 2-oxo-1,3,2-dioxathianes are relatively easy to separate from each other they offer a useful method for the preparation of the diol isomers.

In order to carry out a thorough and definite structural analysis of this ring system 2-oxo-1,3,2-dioxathiane, all methyl- and several other alkyl-substituted derivatives have been synthesized. In the present paper two variations of the preparation method are briefly discussed.

2-Oxo-1,3,2-dioxathianes can be prepared by refluxing the ethereal solution of diol and thionyl chloride or by adding thionyl chloride dissolved in benzene to a diol–pyridine solution. In the first

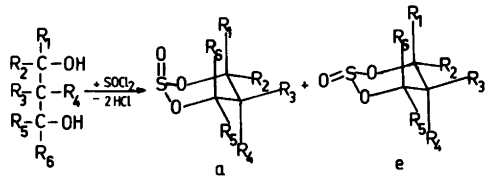

Fig. 1. Preparation of 2-oxo-1,3,2-dioxathianes.

method the liberated hydrogen chloride equilibrates isomers *a* and *e* (Fig. 1) and the final product consists mainly of the more stable isomer *a*. In the other method pyridine reacts with hydrogen chloride forming pyridine hydrochloride and the proportion of isomer *e* depends on the pyridine–diol ratio and the reaction time (Fig. 2). When the pyridine–diol ratio is close to two, the mol fraction of isomer *e* decreases with the increasing reaction time. When this ratio is equal to or greater than eight the mol fraction of isomer *e* is time-independent since the concentration of the hydrogen chloride is

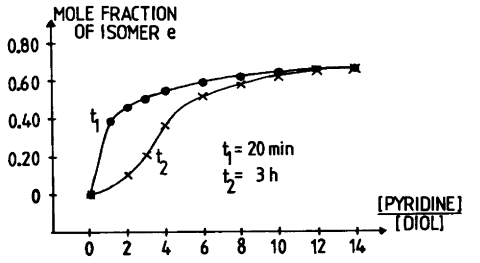

Fig. 2. Dependence of the mol fraction of S=O-equatorial isomer *e* on the pyridine–diol ratio and reaction time (reactants 0.03 mol butanediol and 0.03 mol thionyl chloride in benzene, 273 K, determinations were made by gas chromatography).

* To whom correspondence should be addressed.

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Table 1. 2-Oxo-1,3,2-dioxathianes prepared in this study.

	Substitution in 2-oxo-1,3,2-dioxathianes	B.p./K kPa^{-1} or m.p./K	n_D^{293}
1	–	343/2.3	1.4529
2	*r*-2-*t*-4-Me	343 – 344/1.3	1.4563
3	*r*-2-*c*-4-Me	351 – 353/1.3	1.4550
4	*r*-2-*c*-5-Me	331 – 333/1.2	1.4482
5	*r*-2-*t*-5-Me	338 – 342/1.1	1.4520
6	4,4-Me_2	343/1.1	1.4500
7	5,5-Me_2	331/1.2	1.4451
8[a,b]	*r*-2-*t*-4,*c*-5Me_2	359 – 361/1.6	1.4509
9	*r*-2-*t*-4,*t*-5-Me_2		
10[a,b]	*r*-2-*c*-4,*c*-5-Me_2	376 – 378/1.8	1.4668
11	*r*-2-*c*-4,*t*-5-Me_2		
12	*r*-2-*t*-4,*t*-6-Me_2	343 – 347/1.6	1.4410
13	*r*-2-*c*-4,*c*-6-Me_2	320	
14	*r*-2-*c*-4,*t*-6-Me_2	353 – 355/1.6	1.4495
15[a,b]	*r*-2-4,4,*c*-5-Me_3	363/1.6	1.4583
16	*r*-2-4,4*t*-5-Me_3		
17	*r*-2-4,4,*t*-6-Me_3	353/1.3	1.4450
18	*r*-2-4,4,*c*-6-Me_3	315	
19[a,b]	*r*-2-*t*-4,5,5-Me_3	373/2.3	1.4523
20	*r*-2-*c*-4,5,5-Me_3		
21[a,b]	*r*-2-*t*-4,*c*-5,*t*-6-Me_3		
22	*r*-2-*t*-4,*t*-5,*t*-6-Me_3	363 – 383/1.5	1.4525
23	*r*-2-*c*-4,*c*-5,*t*-6-Me_3		
24	*r*-2-*c*-4,*t*-5,*t*-6-Me_3		
25[c]	4,4,5,5-Me_4	381/2.3	
26	4,4,6,6-Me_4	360/1.3	1.4488
27[a,b]	*r*-2-4,4,*c*-5,*t*-6-Me_4		
28	*r*-2-4,4,*t*-5,*t*-6-Me_4	353 – 355/1.3	1.4483
29	*r*-2-4,4,*t*-5,*c*-6-Me_4		
30	*r*-2-4,*c*-5,4*c*-6-Me_4		
31[a,b]	*r*-2-*t*-4,5,5,*t*-6-Me_4		
32[d]	*r*-2-*c*-4,5,5,*c*-6-Me_4	353-361/1.1	1.4563
33	*r*-2-*c*-4,5,5,*t*-6-Me_4		
34	*r*-2-4,4,5,5,*t*-6-Me_5	393 – 396/2.3	1.4566
35	*r*-2-4,4,5,5,*c*-6-Me_5	355	
36[a,b]	*r*-2-4,4,*t*-5,6,6-Me_5	388 – 393/2.0	1.4508
37	*r*-2-4,4,*c*-5,6,6-Me_5		
38	4,4,5,5,6,6-Me_6	402	
39[b,e]	*r*-2-*c*-5-isoPr	371 – 372/2.1	1.4535
40	*r*-2-*t*-5-isoPr		1.4602
41	*r*-2-*c*-5-t-Bu	371 – 372/1.2	1.4608
42	*r*-2-*t*-5-t-Bu	309	
43[a,b]	*r*-2-*c*-5-Ph	423 – 427/0.9	1.5466
44	*r*-2-*t*-5-Ph		
45[b,e]	*r*-2-*t*-4-isoPr	365 – 367/1.6	1.4459
46	*r*-2-*c*-4-isoPr		1.4607
47	*r*-2-*t*-4-t-Bu	373 – 378/1.2	1.4638
48	*r*-2-*c*-4-t-Bu	312	
49	*r*-2-*t*-4-Ph	408 – 413/1.1	1.5488
50	*r*-2-*t*-4-t-Bu-,*c*-4-Me	388 – 390/1.3	1.4543

[a] Boiling point and refractive index determined for a mixture of isomers. [b] Isomers were separated with a preparative gas chromatograph. [c] M.p. 363 K. [d] This isomer was not isolated. [e] Boiling point determined for a mixture of isomers.

Table 2. 1,3-Alkanediols prepared in this study.

Diol	B.p./K kPa^{-1} or m.p./K	n_4^{293}	Yield/%	Method[b] of preparation
1,3-propanediols				
2-Me-	373–374/0.9	1.4420	35	R
2-isoPr-	383–388/09	1.4495	82	R
2-t-Bu-	330		67	R
2-Ph-	443/1.3	1.5468	46	R
3-Ph-	437/0.8	–[a]	60	R
1,3-butanediols				
2-Me-	375-379/0.9	1.4500	63	R
3-Me-	363-365/0.9	1.4423	40	R
2,2-diMe-	375/1.1	1.4497	55	R
2,3-diMe-	369/0.9	1.4455	66	R
2,2,3-triMe	400		86	R
1,3-pentanediols				
4-Me-	385–388/1.2	1.4486	77	R
4,4-diMe-	333–334		87	R
3,4,4-triMe	333		99	R
2,3-pentanediols				
2,3-diMe-	362–367/0.9	1.4469	63	G
2,4-diMe-	365/1.2	1.4335	59	G
3,3-diMe	378/0.9	1.4498	55	R
2,3,3-triMe-	358		90	G
2,3,4-triMe-	355–357/0.9	1.4350	44	G
2,3,3,4-tetraMe	340–341		46	G

[a] Refractive index not determined (a viscous liquid). [b] R = reduction. G = Grignard reaction.

too low to cause the equilibration. Besides pyridine, other amines can also be used. The efficiency of an amine and the proportion of isomer *e* depend on the pK_a-value: the amine with higher pK_a gives higher proportion of isomer *e*. Since protonation is faster than the equilibration the latter method is kinetically controlled when the pyridine–diol ratio is eight or more.

The 2-oxo-1,3,2-dioxathianes presented in Table 1 were prepared using the latter of the above methods. The pyridine–diol ratio was generally close to two. When *cis*-4-methyl (*3*), *trans*-5-methyl (*5*), *cis*-4,*cis*-5-dimethyl (*10*) and *cis*-4,*trans*-5-dimethyl (*11*) and *cis*-4,*cis*-6-dimethyl (*13*) derivatives were prepared the amount of pyridine was ten times the amount of diol. In each case the method used appared to be an easy and excellent way to prepare the title compounds. Since the proportion of isomers *a* and *e* can be controlled by adding amine to the reaction mixture, the reaction gave not only a good yield of 2-oxo-1,3,2-dioxathianes but also the highest yield of the less stable isomer *e*.

EXPERIMENTAL

Materials. 2-Oxo-1,3,2-dioxathianes were prepared by adding dropwise, with stirring and external cooling, thionyl chloride (0.013–0.061 mol) in anhydrous benzene (10 cm^{-3}) to the solution of diol (0.012–0.055 mol) and pyridine (0.024–0.110 mol; see also text) in anhydrous benzene. The reaction was allowed to run 1–3 h. After filtering off the hydrochloride formed and neutralizing the traces of hydrogen chloride with dilute bicarbonate solution the organic layer was separated, washed with water and dried over $MgSO_4$. The solvent was evaporated and the product was distilled under reduced pressure. The stereoisomers were separated by distillation or with a preparative gas chromato-

graph using XE-60 and Carbowax 20 M columns. The yields varied generally from 60 to 75 %. The characterization of the compounds was performed by gas chromatography and ^{1}H NMR spectroscopy,[1,2] partly also by mass spectroscopy.[4]

The starting materials, 1,3-alkanediols, were prepared by $LiAlH_4$-reduction or by the Grignard reaction from 1,3-alkanediones or ethyl 3-hydroxyalkanoates using the methods described earlier.[5]

Apparatus. The refractive indices were determined with an Abbe refractometer at 293 K. The GLC analyses were performed with a Perkin Elmer F 11 analytical gas chromatograph using columns containing 10 % Carbowax 20M and 5 % XE-60 on Chromosorb G (60/80 mesh). The stereoisomers were separated with a Perkin Elmer F 21 preparative gas chromatograph equipped with the columns containing 10 % Carbowax 20M and XE-60 on Chromosorb G (60/80 mesh). The ^{1}H NMR spectra were recorded with a Jeol PMX-60 spectrometer at 303 K using 10 % (w/v) CCl_4-solutions and TMS as internal standard.

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2-Oxo-1,3,2-dioxathianes. II. Determination of the S=O Group Configuration by IR Spectroscopy

TERTTU VIRTANEN, KALEVI PIHLAJA and HANNU NIKANDER*

Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku 50, Finland

The IR spectra of 2-oxo-1,3,2-dioxathiane and several alkyl-substituted 2-oxo-1,3,2-dioxathianes were recorded and used for the configurational assignment of the S=O group. A very strong absorption at *ca.* 1190 cm^{-1} is characteristic of an axial S=O group whereas a strong absorption at *ca.* 1240 cm^{-1} indicates an equatorial S=O group. The intermediate value, which earlier has been assigned to the S=O group of twist conformations, is more likely due to the mixed C–C(–H) stretch vibrations.

2-Oxo-1,3,2-dioxathiane has been shown to exist in a chair conformation with the S=O group axially oriented.[1-6] An axial S=O group has been found to be 8–15 kJ mol^{-1} more stable than an equatorial S=O group owing largely to the dipole–dipole interactions.[2,7-10] Substituted 2-oxo-1,3,2-dioxathianes exist preferentially in a chair conformation with the S=O group axially or equatorially oriented or as a mixture of two chair conformations.[7]

The configurational and conformational analysis with the aid of the IR spectra of 2-oxo-1,3,2-dioxathianes is concentrated in the use of the stretching vibration, ν(S=O), of the S=O bond. With the exception of this vibration band the positions of the main bands are less useful for the assignment of the ring conformation or configuration (Table 1).[3,11] The S=O stretching vibration has been shown to be sensitive to the environment of the S=O bond[12] and only very small changes in the S=O bond environment are needed to change the stretching frequencies by 0–50 cm^{-1}. The frequency ranges observed in this study for the stretching vibrations are fairly narrow: 1188–1200 cm^{-1} for axial S=O groups and 1238–1247 cm^{-1} for

*To whom correspondence should be addressed.

Table 1. Group frequencies for 2-oxo-1,3,2-dioxathianes in the region of 1400–500 cm^{-1}.

Frequency range/cm^{-1}	Assignation of vibration
1400–1250	C–H vibration (CH_2-wag, as. CH_3-deformation, CH_3-rocking)
1250–1235	S=O stretch (an equatorial S=O group)
1235–1212	C–C(–H) stretch
1212–1200	CH_3 rock
1200–1185	S=O stretch (an axial S=O group)
1180–1100	C–H bend or deformation
1100–1060	C–O stretch
1050–1010	C–C stretch
970–900	Ring stretch
890–850	S–O–C stretch
750–670	S–O stretch (symm. and asymm.)
540–520	S=O deformation

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Table 2. IR frequencies of 2-oxo-1,3,2-dioxathiane and its methyl-substituted derivatives observed in the region of 1300 – 1100 cm^{-1} (values are in cm^{-1}, solvent CCl_4).

Compound		S=O(ax)	CH_3	C–C(–H)	S=O(eq)	C–H
1		1193 (vs)		1232 (m)		1250 (w)
2	2a4e	1193 (vs)		1225 (w)		
3[a]	2a4a⇌2e4e 16 % 84 %	1194 (w)		1225 (w)	1244 (s)	
4	2a5e	1194 (vs)		1227 (w)		1251 (w)
5	2a5a	1194 (vs)		1226 (w)		1250 (w)
6[a]	2e4e4a⇌2a4a4e	1195 (s)	1200 (vs)	1224 (w)	1244 (w)	1251 (m)
7	2a5a5e	1192 (vs)	1205 (s)	1220 (w)		1250 (w)
8	2a4e5e	1190 (vs)		1230 (w)		1250 (w)
9	2a4e5a	1190 (vs)		1230 (w)		
10[a,b]	2e4e5a⇌2a4a5e 45 % 55 %	1190 (s)			1240 (s)	
11[a,b]	2a4a5a⇌2e4e5e 22 % 78 %					
12	2a4e6e	1193 (vs)		1219 (w)		1260 (w)
13[c]	2e4e6e	1193 (vw)		1220 (w)	1241 (vs)	
14[a]	2e4e6a⇌2a4a6e 30 % 70 %	1197 (s)		1220 (w)	1243 (w)	
15	2a4a4e5e	1194 (s)	1206 (vs)	1227 (w)		
16[a]	2a4a4e5a⇌2e4e4a5e 48 % 52 %	1195 (s)	1206 (vs)	1225 (w) 1232 (m)	1245 (m)	
17[d]	2a4a4e6e	1189 (s)	1209 (vs)	1232 (m)		
18[e]	2e4a4e6e	1188 (w)	1207 (vs)		1238 (s)	
19	2a4e5a5e	1195 (vs)		1216 (m)		1250 (w)
20[a]	2e4e5e5a⇌2a4a5a5e 49 % 51 %	1194 (s)		1217 (m)	1247 (m)	1255 (w)
21	2a4e5e6e	1191 (s)		1216 (m)		
22	2a4e5a6e	1195 (s)		1218 (m)		1255 (w)
23[a]	2e4e5a6a⇌2a4a5e6e 13 % 87 %	1196 (s)		1219 (m)	1244 (w)	1255 (w)
24[a]	2e4e5e6a⇌2a4a5a6e 32 % 68 %	1195 (s)		1222 (m)	1244 (m)	1255 (w)
25[a]	2e4e4a5e5a⇌2a4a4e5a5e 24 % 76 %	1190 (s)	1206 (s)	1225 (m)	1242 (m)	1260 (w)
26[f]	2a4a4e6a6e	1200 (s)	1200 (s)	1225 (m)		1256 (w)
27[g]	2a4a4e5e6e					
28[g]	2a4a4e5a6e	1190 (s)	1212 (s)	1230 (m)	1246 (m)	
29[g]	2e4a4e5e6e					
30[g]	2e4a4e5a6e					
31	2a4e5a5e6e	1190 (s)	1202 (s)	1220 (m)		
32[h]	2e4e5a5e6e	–	–	–	–	
33[a]	2e4e5e5a6a⇌2a4a5a5e6e 21 % 79 %	1188 (s)	1200 (s)	1222 (m)	1245 (m)	
34	2a4a4e5a5e6e	1188 (s)	1205 (s)	1220 (w)		1255 (m)
35	2e4a4e5a5e6e		1205 (s)		1243 (m)	1250 (m)
36[i]	2e4a4e5e6a6e	1198 (s)	1202 (s)	1219 (w)	1243 (m)	1260 (m)
37[i]	2e4a4e5a6a6e					
38	2e4a4e5a5e6a6e		1203 (vs)	1220 (w)	1243 (s)	

[a] Not conformationally homogeneous; conformer population was determined by ^1H NMR spectroscopy using vicinal coupling constants and chemical shifts. [b] A mixture of isomers *10* and *11*. [c] Contains isomer *12* as impurity. [d] Contains isomer *18* as impurity. [e] Contains isomer *17* as impurity. [f] Exists in a more or less deformed chair conformation. [g] A mixture of isomers *27* – *30*. [h] Not measured. [i] A mixture of isomers *36* and *37*; isomer *37* exists in a deformed chair conformation.

equatorial S=O groups. The interaction between the axial lone-pair orbitals of the ring oxygen atoms and the anti-bonding orbitals of the axial S=O bond tend to weaken this bond thus rendering the vibration of the axial S=O bond to a lower wave number than the vibration of the equatorial S=O bond.[13]

Each 2-oxo-1,3,2-dioxathiane studied gives a characteristic IR spectrum, even though the spectra of isomeric derivatives are rather similar in the range of 1400–500 cm.$^{-1}$ Since only the S=O stretching vibration clearly depends on the configuration, the following inspection is restricted to the S=O stretching vibration region of 1180–1260 cm^{-1} (Tables 2 and 3).

The position of the very strong absorption due to an axial S=O group (range 1188–1200 cm^{-1}) shifts upon dilution to higher wave numbers in solvents of low polarity and to lower wave numbers in polar solvents. The change in the wave numbers in polar solvents. The change in the wave number is rather small, about 2–5 cm.$^{-1}$ An increase in the polarity and solvating power of the solvent has been stated to increase the induced polarity of the axial S=O bond, to decrease the bond order and hence to lower the stretching frequency concerned.[3] 4,4- and 5,5-dimethyl-substituted derivatives have bands both in the region of 1188–1200 cm^{-1} and 1200–1212 cm.$^{-1}$ The former is assigned to the S=O stretching and the latter to the methyl group vibrations, probably CH_3 rock.

A band in the region 1238–1247 cm^{-1} indicates the presence of an equatorial S=O group. The position of this band shows little or no variation with the solvent or solute concentration. Intensity changes with the change of solvent and upon dilution usually reflect alterations in the position of the conformational equilibrium. The intensity of the absorption at the higher wave number greatly increases at the expense of that at the lower wave number in a polar medium. This supports a solvation mechanism where the polar medium favours polar conformers, here the conformer with an equatorial S=O group.[2,14]

The band in the region of 1216–1232 cm^{-1} is probably due to C–C(–H) stretching vibrations[15,16] even though it has formerly been assigned to the S=O stretch of twist conformations.[2,13,17,18] It can be found as a sharp band or a shoulder of a band at 1200–1212 cm^{-1} for almost all the compounds studied, even for the non-substituted ring which has shown to be in an S=O-axial chair conformation.[1–6] Changing the solvent from non-polar to polar does not change the relative intensity of the band either although the twist form is

Table 3. IR frequencies of the S=O stretching vibration for isopropyl-, *tert*-butyl- and phenyl-substituted 2-oxo-1,3,2-dioxathianes (values are in cm^{-1}, solvent CCl_4).

Compound		S=O (ax)	S=O (eq)
39	2a5e-isoPr	1187 (vs)	
40[a]	2e5a-isoPr⇌2a5a-isoPr 8 % 92 %	1185 (s)	1247 (w)
41	2a5e-t-Bu	1185 (s)	
42[a]	2e5e-t-Bu⇌2a5a-t-Bu 25 % 75 %	1190 (s)	1240 (w)
43	2a5e-Ph	1185 (vs)	
44[a]	2e5e-Ph⇌2a5a-Ph 21 % 79 %	1185 (s)	1240 (w)
45	2a4e-isoPr	1185 (vs)	
46[a]	2a4a-isoPr⇌2e4e-isoPr 12 % 88 %		1200–1250 (vs)[b]
47	2a4e-t-Bu	1185 (vs)	
48[a]	2a4a-t-Bu⇌2e4e-t-Bu 13 % 87 %		1200–1250 (vs)[b]
49	2a4e-Ph	1185(s)	
50	2a4a-Me,4e-t-Bu	1185 (s)	

[a] Not conformationally homogeneous; conformer population was determined by ^{1}H NMR spectroscopy using vicinal coupling constants and chemical chifts. [b] A broad band.

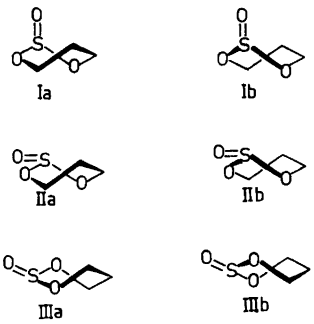

Fig. 1. Twist conformations of 2-oxo-1,3,2-dioxathiane.

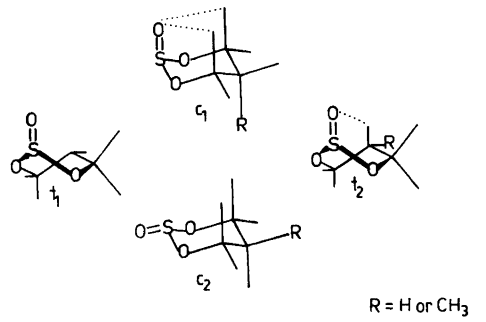

Fig. 2. Possible conformations of 4,4,6,6-tetramethyl derivatives studied.

assumed to be more polar than the chair conformation with an axial S=O group and the relative intensity of the band should increase.

2-Oxo-1,3,2-dioxathianes can, in principle, exist in three different twist forms: 2,5-, 1,4- and 3,6-twists (Fig. 1). When discussing the possible twist conformations for sterically crowded molecules one should remember that molecules which are so crowded as to be forced from a chair to a twist form may involve unfavourable interaction also in twist conformations. The axial orientation of the S=O group is favoured and hence assumed to considerably increase also the stability of twist forms *Ia* and *Ib* as compared to other twist forms. A methyl group (and any other alkyl group) is more hindered in a pseudoaxial position than in a pseudoequatorial or isoclinal position and avoids this position whenever possible.[19,20] For vicinal substituents three possible *cis*-arrangements have energetic drawbacks: $\psi_e-\psi_a$ (ψ = pseudo) and ψ_a-ic (ic = isoclinal) have an unfavourable pseudoaxial group and ψ_e-ic shows unfavourable torsional interaction. On the other hand, two conformations of trans-vicinal substituents may be energetically preferred: ψ_e-ψ_e with a dihedral angle of about 60° between the substituents and ψ_e-ic with a dihedral angle greater than 60°. In the light of this discussion and the calculated ΔH°_{CT}-value (the twist form has been estimated to be about 31 kJ mol^{-1} less stable thermodynamically than the chair form with an equatorial S=O)[7] it is easy to understand that an escape to a twist form does not necessarily relieve the steric compression in the polysubstituted derivatives.

4,4,6,6-Tetramethyl-substituted derivatives have a *syn*-axial interaction between 4- and 6-methyl groups, which could deform the ring. The deformation cannot be deduced directly by IR spectroscopy since the most favourable twist form would have a purely axial S=O group and would therefore give the normal S=O stretching in the region of 1188–1200 cm.$^{-1}$ The S=O stretching absorptions 1200 cm^{-1} and 1198 cm^{-1} for 4,4,6,6-tetramethyl (*26*) and 4,4,6,6,*trans*-5-pentamethyl (*36*) derivatives, respectively, correspond to axial S=O groups. Severe interaction between the axial 4- and 6-methyl groups and the axial S=O group greatly disfavours the S=O-axial chair conformation (c_1 in Fig. 2). Evidently these derivatives exist in a more or less deformed twist conformation (t_1). On the other hand 4,4,6,6,*cis*-5-pentamethyl (*37*) and 4,4,5,5,6,6-hexamethyl (*38*) derivatives exist in a S=O-equatorial chair conformation (c_2 in Fig. 2) since in these cases a twist conformation (t_2) cannot lead to a favourable relief of the steric interaction.

EXPERIMENTAL

The 2-oxo-1,3,2-dioxathianes studied were prepared earlier.[21] The characterization of the products was performed by gas chromatography and NMR spectra, and partly also by mass spectra. The isomers were separated by distillation or a preparative gas chromatograph using XE-60 and Carbowax 20 M columns.

The IR measurements were made on a Perkin Elmer 180 IR-spectrophotometer at 303 K using 4 % (w/v) CCl_4-solutions in a sealed AgCl cell of a fixed path length of 0.1 mm. The spectra for configurational assignment were recorded between 1400–400 cm^{-1} on a linearly expanded, calibrated scale. The reported values are considered to be accurate within 0.5 cm^{-1}.

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Short Communications

Selectivities of 4-Substituted Phenyl Radicals in Hydrogen, Deuterium and Bromine Atom Abstraction Reactions in Acetonitrile

MATS TILSET and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

We recently observed that relative rate constant ratios (k_H/k_{Br}) in reactions (1) and (2) were greater when Ar$^\cdot$ was 4-nitrophenyl than when the aryl

$$Ar^\cdot + S-H \xrightarrow{k_H} Ar-H + S^\cdot \quad (1)$$

$$Ar^\cdot + CH_mBr_n \xrightarrow{k_{Br}} Ar-Br + CH_mBr_{n-1} \quad (2)$$

radical was α-naphthyl reacting with the solvent, DMF, or with bromomethanes.[1] We found this somewhat surprising since the more electrophilic radical would be expected to preferentially attack the electron-rich halogen atoms. The comparison is not very meaningful since the structures of Ar$^\cdot$ differed considerably. We have now carried out competitive kinetic experiments on the atom abstraction reactions of a series of 4-substituted aryl radicals which resulted in clearly evident trends in the electrophilic – nucleophilic character of the radicals.

Results of reactions carried out in CH_3CN or CD_3CN are summarized in Table 1. The radicals reacting in (1) where S – H is the solvent were generated by the electron transfer reduction of the appropriate 4-substituted phenyldiazonium fluoroborate with iodide ion. The relative rate constants for reaction (1) compared to reaction (2) where CH_mBr_n was dibromomethane were obtained by GLC analysis of the resulting reaction mixtures as described earlier.[1] The data indicate that k_H/k_D decreases as the the aryl radical becomes less electrophilic. On the other hand, k_H/k_{Br} increases in the opposite sense, *i.e.* as the radical becomes more nucleophilic. The latter trend is even more evident in the k_D/k_{Br} ratios. The data were correlated using the Hammett relationship (3)[2] where c has no

$$\log(k_X/k_H) = \rho\sigma_p + c \quad (3)$$

significance. In all cases, the correlation coefficient was greater than 0.99.

The most interesting feature of the data is that $D-CD_2CN$ is much more prone to attack by the more nucleophilic radical, 4-methoxyphenyl than by electrophilic 4-nitrophenyl. The relative rate constant for the two reactions is equal to 6.7, as evident from the last column of Table 1. The corresponding relative rate constant for the reactions of the radicals with $H-CH_2CN$ is of the order of 2.5 (next to last column of Table 1). These relative reactivities then result in the deuterium kinetic isotope effect being very dependent upon the electrophilicity – nucleophilicity of the aryl radical.

Table 1. Deuterium kinetic isotope effects and selectivities in atom transfer reactions of 4-substituted phenyl radicals.[a]

Radical	k_H/k_D	$10^2k_H/k_{Br}$	$10^2k_D/k_{Br}$
4-Nitrophenyl	9.53	3.54	0.371
4-Chlorophenyl	5.50	6.35	1.16
4-Methoxyphenyl	3.53	8.70	2.47

[a] The radicals were generated by the reaction of equimolar amounts of the appropriate diazonium fluoroborate with Bu_4NI in solvent at 20 °C. The analysis procedure has been described.[1]

Table 2. Hammett equation correlations of the relative rate constants for atom abstraction reactions of 4-substituted phenyl radicals.[a]

Rate constant ratio	ρ[b]	r[c]
k_H/k_{Br}	−0.373	0.990
k_H/k_D	+0.411	0.999
k_D/k_{Br}	−0.786	0.996

[a] Data from Table 1. [b] The slope of the Hammett correlation using ρ_p from Ref. 2. [c] The correlation coefficient from *b*.

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The overall conclusion from the data in Tables 1 and 2 is that the more electrophilic the aryl radical is, the more favorable abstraction of Br from CH_2Br_2 is relative to abstraction of either H or D from acetonitrile. Nucleophilic radicals have a more pronounced tendency to abstract deuterium from acetonitrile than do electrophilic radicals.

The electrophilic–nucleophilic character of free radicals has been discussed in a number of instances.[3-5] However, we are not aware of any data on the reactions of 4-substituted phenyl radicals which so clearly show the effect of substituents on the relative nucleophilicity as that reported here. A number of Hammett correlations of the rates of halogen atom abstraction has been carried out.[6] However, these have involved substituent changes in the halogen compound[7-10] rather than in the radicals.

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Tetrabutylammonium Inorganocuprates(I)* – $Bu_4N^+CuCl_2^-$, $CuBr_2^-$, CuI_2^- and $Cu(CN)_2^-$

MARTIN NILSSON

Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

The dichlorocuprate(I) ion, $CuCl_2^-$, is important in the Sandmeyer [2] and Meerwein [3] reactions. It is also kinetically relevant in halogen exchange, especially that of 2-bromonitrobenzenes.[4,5] Similarly, cyanocuprate(I) ions are used in the displacement of diazonium groups by cyanide.[2] Sodium dicyanocuprate(I) has been used as a alternative to copper(I) cyanide for the replacement of aromatic or vinylic halogen by cyanide.[6]

The reactions may be summarised by a general equation for copper-promoted nucleophilic substitutions (*1*).[7,8] The equation also summarises several organocopper reactions.

Y is generally a soft atom or group, which forms a complex or a compound with copper(I), eqn. (1). The importance of the cuprate(I) ions mentioned above calls to mind the manifold uses of lithium diorganocuprates[9] in additions and in substitutions.

$$Ar-X+Y^- \xrightarrow{Cu^I, CuY \text{ or } CuY_2^-} Ar-Y+X^- \quad (1)$$

The increased use of phase transfer catalysis and the high reactivity of many anions in ion pairs with, for example, tetrabutylammonium (TBA) cation in suitable solvents[10,11] made it of interest to check the existence and behaviour of TBA dihalo- and dicyanocuprates(I).

Several TBA salts of transition metal anions have been described; in the present context, TBA dicyanoargentate(I) and TBA dicyanoaurate[12] as well as TBA perfluoroorganoaurates[13] are especially interesting. The $TBA^+PdCl_3^-$ has been used to catalyse the addition of organomercurials to α,β-enones in a phase transfer system.[14]

Tetrabutylammonium dichlorocuprate(I) was obtained in good yield in a water – dichloromethane system from tetrabutylammonium hydrogen sulfate, sodium chloride and copper(I) chloride. The cuprate was extracted into the organic phase while contaminating copper(II) remained in the aqueous phase. The salt was recrystallised from ethyl acetate and formed colourless crystals, m.p. 71 – 72 °C, which were rather stable in the air and sparingly soluble in water (analysis C,H,Cl,Cu,N). It was very soluble in haloalkanes, halogenoarenes, ketones *etc.*, moderately soluble in other arenes, and little soluble in alkanes.

Tetrabutylammonium dibromocuprate(I), m.p. 88 – 89 °C, was obtained when copper(I) bromide was dissolved in a tetrabutylammonium – dichloromethane solution. Recrystallisation from ethyl acetate gave beautiful crystals which took on a very faint lilac tint (analysis C,H,Br). Tetrabutylphosphonium bromide in dichloromethane dissolved at least two mol of copper(I) bromide, giving a compound, m.p. *ca.* 85 °C, with the approximate composition $Bu_4P\ Cu_2Br_3$, which was not stable, however; copper(I) bromide precipitated on attempted recrystallisation.

One mol of finely ground copper(I) iodide dissolved rather rapidly in a TBA iodide solution (one mol) giving a crystalline *TBA diiodocuprate(I)* on evaporation. On recrystallisation from ethyl acetate the salt had a strong tendency to precipitate as an oil, but from dilute solution colourless prisms m.p. 98 – 100 °C (analysis C,H,I) were obtained.

The preparation of *tetrabutylammonium dicyanocuprate(I)* gave some complications. Copper cyanide dissolved only slowly in tetrabutylammonium cyanide – dichloromethane. Mixing of an aqueous solution of sodium dicyanocuprate (from sodium cyanide and copper cyanide) with an equivalent amount of tetrabutylammonium hydrogen sulfate and sodium hydroxide in water gave a voluminous precipitate, which largely was dissolved in dichloromethane. The rapidly dried organic phase was evaporated and, on treatment with acetone, gave the desired TBA $Cu(CN)_2$ as a solid, which could be recrystallised from acetone (analysis C,H,Cu,N). The salt melted at 158 – 159 °C, giving off the characteristic odour of (butyl)isocyanide. The IR spectrum (KBr) showed a double cyanide band, 2190 and 2210 cm^{-1}, similarly to what is described[12] for potassium dicyanocuprate(I). Attempts to wash the crude cyanocuprate with water gave heavily hydrated crystals, which gradually gave off water from *ca.* 70 °C and could be dried at 120 °C, again giving evolution of isocyanide. During work-up and purification a higher-melting and sparingly soluble compound was obtained. The compound was also formed when dichloromethane solutions of TBA dicyanocuprate were dried, and also on attempted recrystallisation from acetonitrile. It melted at 249 – 252 C giving off the isocyanide odour. The composition, according to analysis (C,H,Cu,N), roughly corresponded to $Bu_4NCu(CN)_2 - BuNCCuCN$, but its IR spectrum gave only one peak in the cyanide region, at 2110 cm^{-1}.

*See Ref. 1.

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The dihalocuprates should provide possibilities of halogen exchange. Iodobenzene and TBA dichlorocuprate at *ca.* 180 °C slowly gave chlorobenzene, which could be distilled off during a day. A closer, kinetic study of the halogen exchange with activated halogenoarenes is under way.[15] The present reagents may provide new combinations for halogen exchange as compared to the methods employing copper(I) halide and complexing solvent or other ligands.[16-18]

Preliminary experiments were made on reactions of aromatic halides and TBA dicyanocuprate. With bromobenzene (at 150 °C), no bromide–cyanide exchange took place. Some isonitrile was evolved and GLC showed also the formation of tributylamine. The thermal stability of the cyanocuprate was limited, and on distillation (around 200 °C) decomposition giving tributylamine *etc.* was extensive. The reaction with iodobenzene and TBA dicyanocuprate also gave some isonitrile formation, but here benzonitrile was also formed. Further work is in progress, and it will be interesting to see whether the new reagent may provide the possibility of halogen–cyanide exchange under phase-transfer conditions.

The mechanisms of copper-catalysed reactions, organocopper and organocuprate reactions are still unclear. Single electron transfer mechanisms including formation of copper(II) species and also copper(III) species are presently favoured in the discussion.[18,19] There are, however, several other possibilities including two-electron transfers, copper acting as a nucleophile to provide copper(III) species, and also other possibilities of oxidative addition – reductive elimination.[20-24]

The TBA inorganocuprates may make it possible to investigate some copper-promoted reactions under different conditions than used previously. This may provide new information and also new preparative possibilites. For example, it seems as if the dihalocuprates in dichloromethane solution form CT complexes with electron-deficient substrates such as 1,3,5-trinitrobenzene and α,β-enones (*cf.* Ref. 25). Structural investigations, now under way,[26] may provide some information regarding bond lengths and thereby indications of the nucleophilicity of the central copper atom.

Experimental. The new compounds were analysed by Novo Microanalytical Laboratory, Bagsvaerd, Denmark. Halogen and cyanide exchange reactions were checked with an HP 5880 A gas chromatograph. Infrared spectra were recorded on a Perkin Elmer model 197 instrument.

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Acid-catalyzed Formation of Diazoalkanes. A One-pot Transformation of Alkylamines to Diazoalkanes

JAN M. BAKKE

Laboratory of Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

We recently reported results from the deamination of benzylamine and (α,α-$^2H_2$)benzylamine which indicated that three products were formed in the initial reaction between the amine and nitrosyl chloride. These were the *trans*- and *cis*-toluenediazohydroxides (*1* and *2*) together with toluenediazochloride (*3*). We proposed that diazotoluene was formed by water elimination from *cis*-toluenediazohydroxide in competition with its alkylation to α,α'-azoxytoluene.[1] We now present the results of experiments which show an improvement in the direct synthesis of diazoalkanes from the corresponding amines. The method may be of special value for preparation of specifically ^{15}N labeled diazoalkanes.

In the experiments described, nitrosyl chloride was reacted with an excess of amine.[1] The reaction mixture was therefore weakly basic during the reaction of the three intermediates. Under these conditions the formation of diazotoluene required several hours at -50 °C for completion. It occurred to us that the water loss from toluenediazohydroxide might be catalyzed by protonation of the hydroxyl group. The toluenediazonium ion (*4*) formed might then release a proton to form diazotoluene.

Acetic acid was therefore added to the reaction mixture prepared from benzylamine and nitrosyl chloride in diethyl ether at -75 °C. The pink color of diazotoluene became visible after a few seconds and its formation appeared to be complete within a minute. Gaseous ammonia was then added to neutralize the acetic acid. The yield of diazotoluene was determined by UV/VIS and IR spectroscopy to be 31% (based on nitrosyl chloride). This should be compared to the 16% yield obtained when the reaction was carried out without acidic catalysis.[1] The other products were benzyl chloride, benzyl alcohol and benzyl acetate. The variety of other products reported earlier was either absent or present only in trace amounts (Table 1). The reaction was tried with several other alkylamines and gave fair yields from those with primary alkyl groups but low or zero yields from those with secondary alkyl groups (Table 2). There is one notable exception, 9-fluorenylamine gave a 32% yield of 9-diazofluorene. This is understandable both from the ability of the 9-fluorenyl system to stabilize a negative charge and from its reluctance to accept positive charge.

When perchloric acid (130 mM, 70% in water) was tried as a catalyst no diazotoluene was formed. Instead an increase in the yield of benzyl alcohol was observed. If the acid was neutralized with ammonia after five min at -75 °C a substantial increase in the yield of dibenzylamine was found (Table 1). This

Table 1. Reaction of benzylamine (160 mM) with NOCl (40 mM) in diethyl ether at -75 °C.

Product	Yields in % of NOCl		Reaction conditions		
	a	*b*	*c*	*d*	*e*
Benzaldehyde	2±0.5	–	–	–	–
Benzyl chloride	31±2	16±1	18±1	17±1	14±1
Benzyl alcohol	15±1	10±1	64±2	41±2	10±1
Benzyl ethyl ether	0.1±0.05	–	–	–	–
Diazotoluene	16±1	31±1	–	–	23±1
Benzyl acetate	–	27±1	–	–	38±1
Dibenzyl ether	3±1	–	–	–	–
Dibenzylnitrosamine	6±1	–	–	–	–
α,α-Azoxytoluene	12±1	–	–	–	–
Dibenzylamine	3±1	1±0.5	7±1	18±2	2±1
N-Benzylidenebenzylamine	2±1	–	–	–	–

[a] Kept at -50 °C.[1] [b] Acetic acid (130 mM) added after 30 min at -75 °C, ammonia (80 mM) added 3 min later. [c] Perchloric acid (130 mM, 70% solution) added after 30 min at -75 °C. [d] As [c] but ammonia (80 mM) added 5 min after addition of acid. [e] (α,α-$^2H_2$)Benzylamine reacted as under condition.[b]

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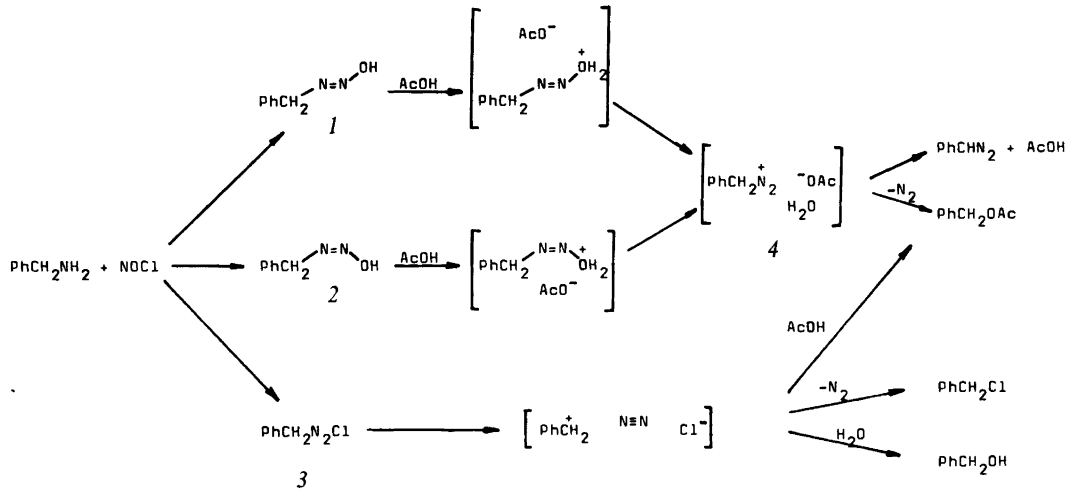
Table 2. Formation of diazoalkanes from the corresponding amines. Reaction conditions as in[b] in Table 1.

Amine reacted	Diazoalkane formed	Yields in % of NOCl
Benzylamine	Diazotoluene	31±1
(α,α-$^2H_2$)benzylamine	(α-^{2}H)Diazotoluene	23±1
Octylamine	Diazooctane	40±1
Methylamine	Diazomethane	22±1
2-Octylamine	None	0±0.3
1-Indanylamine	None	0±0.3
Diphenylmethylamine	Diphenyldiazomethane	10±1
9-Fluorenylamine	9-Diazofluorene	32±1

suggests that the toluenediazonium ion (*4*) was still present in the solution and shows that benzylamine could act as a nucleophile to give dibenzylamine but not as a base which could abstract a proton to give diazotoluene even if it is a stronger base than the acetate ion. In the reaction catalyzed by acetic acid the proton abstraction must therefore have taken place simultaneously with or shortly after the departure of the protonated hydroxyl group but before the acetate ion had left the ion pair (Scheme 1). The reaction of (α,α-$^2H_2$)benzylamine with nitrosyl chloride followed by addition of acetic acid gave results in accordance with this (Table 1). A decrease in the yield of diazotoluene was observed but no increase in the yield of benzyl alcohol, benzyl chloride or dibenzylamine. The only increase in yield was observed for benzyl acetate as would be expected from Scheme 1, where diazoalkane formation competes with decomposition of the diazonium ion to give the acetate. It is thus clear that in this case, as it was without acid catalysis,[1] diazotoluene was formed from a different intermediate than were benzyl chloride and benzyl alcohol.

Experimental. The technique for the deamination reactions has been described.[1] The acid was added after 30 min at −75 °C. The color of the diazoalkane appeared after a few seconds and no further increase in intensity of the color could be seen after *ca.* 1 min. Gaseous ammonia was then added with a syringe through the rubber septum. The reaction mixture was filtered and the amount of diazoalkane was determined by IR (band at *ca.* 2060 cm^{-1}) and UV/VIS spectroscopy. The extinction coefficients of diazotoluene were determined from the experiments with (α,α-$^2H_2$)benzylamine. A portion (10 ml) of the reaction mixture (50 ml) was filtered and its absorbances at 2060 cm^{-1} and 490 nm were determined. To the remainder of the mixture excess acetic acid was added. The amount of benzyl acetate was determined by GLC analysis and the ratio of (α-^{2}H)benzyl acetate − (α,α-$^2H_2$)-benzyl acetate was



Scheme 1.

determined by MS (AEI 90). Assuming that (α-^{2}H)benzyl acetate was formed quantitatively from (α-^{2}H)diazotoluene and that it was formed in that way only, $\varepsilon_{490}=28$ M^{-1} cm^{-1} and $A_{2060}=8.5\times10^4$ M^{-1} cm^{-2} (area of band determined by method I of Ramsey)[2] were found. Both these values are higher than those reported earlier ($\varepsilon_{490}=22$ M^{-1} cm^{-1} and $A_{2060}=5.4\times10^4$ M^{-1} cm^{-2}).[3,4] Diazooctane,[5] diphenyldiazomethane[6] and diazofluorene[3] were determined by known spectral extinction coefficients. The other compounds were determined by GLC analyses (Carlo Erba 4160, 15 m SE 54 WCOT column, Hewlett Packard 3390A integrator, *p*-nitrotoluene as internal standard).

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Comparison of the Thermal Stability of Acid Phosphatase Conjugated with Ficoll and with Modified Ficolls

HEIDI KILL BLOMHOFF and
TERJE B. CHRISTENSEN

Institute of Biochemistry, Postbox 1041, Blindern, University of Oslo, Oslo, Norway

The thermal stability of proteins may be enhanced by covalent attachment to carbohydrate. This applies to naturally occurring glycoproteins [1,2] as well as to synthetic conjugates.[3,4] Little evidence has been presented to explain the reason(s) for the stabilising effect although Marshall [3] has suggested that synthetic conjugates are stabilised due to internal, covalent, crosslinks formed by carbohydrate.

The present experiments were carried out in order to obtain further insight into the causes of stabilisation and the results showed that partial methylation of Ficoll prior to conjugation with acid phosphatase reduced the stabilising effect. This was not the case for partial acetylation and the results suggest that the hydrophilic nature of the polysaccharide may be of importance for the stabilisation.

Experimental. Wheat germ phosphatase (EC 3.1.3.2) was obtained from Koch Light Laboratories, cyanogen bromide and dimethyl sulfate from Fluka AG, acetic anhydride from Merck AG, Ficoll-70 from Pharmacia Fine Chemicals and sodium metaperiodate from Riedel-de-Haen AG.

Methylation of Ficoll was carried out according to the classical Haworth procedure.[5] 2 g Ficoll was dissolved in 10 ml water, dimethyl sulfate added at a rate of 4 ml/h and 30 % sodium hydroxide at the rate of 8 ml/h. The first sample was withdrawn after 5 min and the second after 15 min. The temperature was 20 °C. The samples were worked up in the usual way, dialysed against distilled water and freeze dried.

Acetylation was effected by dissolving 5 g Ficoll in 35 ml acetic anhydride at 20 °C. After 5 min the first sample was taken and the temperature raised to 35 °C. The second sample was taken after 3 min at this temperature. The reagent was removed by dialysis at 10 °C, first against tap water and then against distilled water before the samples were freeze dried.

The approximate degree of substitution in Ficoll was determined by comparing the uptake of periodate by the substituted and the unsubstituted polysaccharide. Increased degree of substitution lowers the content of vicinal hydroxyl groups and in turn the susceptibility to periodate oxidation. The consumption of periodate in the oxidation reaction was determined from the absorbance at 223 nm.[6] The degree of substitution in the methylated and acetylated Ficolls were determined to be: $MeFicoll_1$ 19 %, $MeFicoll_2$ 45 %, $AcFicoll_1$ 34 % and $AcFicoll_2$ 55 %.

Conjugation of acid phosphatase with polysaccharide was carried out by the cyanogen bromide method.[7] 50 mg solid cyanogen bromide was added to a solution of 100 mg polysaccharide in 20 ml dilute sodium hydroxide, pH 11.0. After the activation of the polysaccharide, the coupling reaction took place at 0 °C in 0.1 M borate buffer, pH 8.0, for 18 h. 10 mg enzyme was used.

Acid phosphatase was assayed by a method based on the method of Verjee [8] using *p*-nitrophenyl phosphate as substrate. The reaction was stopped by addition of 0.25 M sodium carbonate solution and the absorbance read at 400 nm.

Three methods were used to test if the conjugation reaction had taken place to a reasonable extent. The first one was based on the solubility of the enzyme in 6 % (w/v) perchloric acid. Conjugation with polysaccharide caused a marked increase in the solubility. The second test was based on the failure of the polysaccharide to migrate on electrophoresis unless attached to acid phosphatase. Slabs of 5 % polyacrylamide were used with 0.2 M phosphate, pH 7.2. Duplicate tracks were run of each sample, one being stained for protein by Coomassie brilliant blue, the other for carbohydrate by the periodate-Schiff method.[9]

The third method involved determination of molecular weight by sedimentation equilibrium in the analytical ultracentrifuge. Registration was by photoelectric scanner at 280 nm. The partial specific volume of acid phosphatase was assumed to be 0.72 ml/g and that of the conjugates 0.68 ml/g.

Results and discussion. Judging by the increases in molecular weight and in perchloric acid solubility it is evident that reasonable conjugation has been achieved although it is not complete (Table 1). Conjugation was also confirmed by the results of electrophoresis which showed that polysaccharide co-migrated with protein in the conjugate preparations but not in the controls where polysaccharide was mixed with the enzyme.

The results of the thermal inactivation experiments are shown in Table 2. Acetylated and unsubstituted Ficolls induced stability on conjugation with acid phosphatase whereas the methylated Ficolls had little effect.

A control experiment showed that the presence of 50 mg/ml of unconjugated and unsubstituted Ficoll did not enhance the thermal stabilibity at 60 °C. We shall consider some possible explanations of the

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Table 1. Characterisation of acid phosphatase and the conjugates.

Preparation	Average molecular weight[a] (Daltons)	Solubility in 6% perchloric acid (%)	Relative specific activity (%)
Acid phosphatase	30 000	34	100
Acid phosphatase – Ficoll	55 000	84	87
Acid phosphatase – $MeFicoll_1$	66 000	74	84
Acid phosphatase – $MeFicoll_2$	82 000	74	88
Acid phosphatase – $AcFicoll_1$	117 000	86	87
Acid phosphatase – $AcFicoll_2$	105 000	86	89

[a] Unconjugated polysaccharide in the preparations did not influence the results because optical registration was at 280 nm.

Table 2. Thermal inactivation of acid phosphatase and the conjugates. Heating was carried out at 60 °C in 0.2 M acetate, pH 5.0, at a protein concentration of 0.1 mg/ml. Duplicate samples were taken at intervals, frozen down and assayed later for enzyme activity. The results quoted are the mean of duplicate assays. The ratio (by weight) of polysaccharide to protein was always 10, whether the two were conjugated or mixed.

Time at 60 °C (min):	Remaining enzyme activity (%)			
	0	30	60	120
Control preparations				
Phosphatase	100	20	11	3
Phosphatase + Ficoll	100	19	11	4
Phosphatase + $AcFicoll_1$	100	23	12	5
Phosphatase + $MeFicoll_1$	100	23	13	5
Conjugate preparations				
Phosphatase-Ficoll	100	41	26	18
Phosphatase-$AcFicoll_1$	100	37	25	17
Phosphatase-$AcFicoll_2$	100	37	25	16
Phosphatase-$MeFicoll_1$	100	28	16	8
Phosphatase-$MeFicoll_2$	100	27	15	6

stabilising effect in relation to our results. In the discussion below, *intramolecular* crosslink signifies that the polysaccharide forms a bridge within one protein molecule. *Intermolecular* crosslink signifies a bridge between two protein molecules.

Intramolecular, covalent, crosslinks. Marshall [3] has suggested that synthetic protein-polysaccharide conugates owe their enhanced stability to the presence of intramolecular, covalent, crosslinks formed by the polysaccharide. This explanation, however, is not valid for natural glycoproteins nor does it seem to fit our results. Thus the partially acetylated and methylated Ficolls should be able to form crosslinks to about the same extent. Yet, only the methylated Ficolls failed to induce stability.

Marshall's hypothesis seems plausible and may well be true in many cases, but there must also be other causes for the stabilising effect.

Intramolecular, non-covalent, crosslinks. Another possibility is that the bound carbohydrate can establish close association with the protein surface by forming a number of hydrogen bonds with hydrophilic amino acid sidechains. This might be considered as intramolecular, non-covalent cross-linking and could conceivably lead to enhanced thermal stability. An effect of this type would be similar to the well-known substrate-induced stabilisation of enzymes caused by non-covalent binding of the substrate at the active site of the enzyme.

Intermolecular, covalent crosslinks. When proteins are linked to polysaccharides there will normally be two or more potential sites of attachment in all polymer molecules. Aggregates consisting of several molecules of each kind may therefore arise (superpolymers) and the enhanced thermal stability be due to the presence of intermolecular, covalent bonds.[3] However, we consider this an unlikely explanation of our results since the average molecular weights of the conjugates were rather low (Table 1). This indicates the presence of only small amounts of superpolymers.

Prevention of aggregation. Glycosylation of proteins may reduce the tendency to aggregate at elevated temperature[1,10,11] and the observed stability could possibly be due to this effect. To test this, the preparations were analysed for molecular weight by sedimentation equilibrium before and after heating at 60 °C for 2 1/2 h. Only small increases were observed and the different conjugate preparations showed similar behaviour. We therefore consider prevention of aggregation to be an improbable explanation of our results.

Effects on protein hydration. Both hydration[1] and dehydration[4] have been suggested as causes of thermal stabilisation of proteins, and we feel rather undertain as to the importance of this parameter for proteins in solution. However, it seems plausible that a bound polysaccharide in close association with the protein surface might affect the hydration in local areas and possibly the thermal stability. An effect of this nature should be sensitive to the introduction of hydrophobic groups in the polysaccharide and is thus compatible with our results.

Chemical alteration of amino acid sidechains. The cyanogen bromide method of coupling involves free amino groups in the protein and the alteration of these groups might affect the thermal stability. However, this change in the protein is the same whether the polysaccharide is methylated, acetylated or unsubstituted and could not adequately explain the results obtained.

Having considered various possible explanations for the stabilising effect of bound carbohydrate, we believe "crosslinking" by hydrogen bonds between polysaccharide and protein to be the most probable one. It is also possible that effect on protein hydration are important. Both explanations are compatible with the experimental results.

Further experiments should be carried out using other enzymes and other modified polysaccharides in order to test if our observations have general validity.

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The Correlation of Kinetic Isotope Effects with Reaction Orders to Establish the Mechanism of the Hydroxylation of Thianthrene Cation Radical in Acetonitrile

VERNON D. PARKER [a] and
OLE HAMMERICH [b]

[a] Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and
[b] Department of General and Organic Chemistry, the H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

We find that in the presence of 2,6-lutidine (L), the hydroxylation of the thianthrene (Th) cation radical in acetonitrile (AN) is first order in water. This result is in sharp contrast to those reported in neutral AN in which case the reaction was observed to be third order in water.[1] We have been able to verify an approximate third order in H_2O under some conditions but find the reaction order complex and not to fit into a reasonable simple mechanistic scheme. It has been pointed out that the mechanism proposal of Evans and Blount [1] is inconsistent with their experimental rate law.[2] We have now been able to clarify the mechanism by correlating deuterium kinetic isotope effects (k_H/k_D) with reaction orders.

$$2Th^{\cdot+} + H_2O \rightarrow ThO + 2H^+ + Th \quad (1)$$

The kinetics of the hydroxylation of $Th^{\cdot+}$ which has the stoichiometry shown in eqn. (1), were studied by derivative cyclic voltammetry [3] using the reaction order approach.[4] The essence of the method is that $v_{\frac{1}{2}}$, the voltage sweep rate necessary for the derivative peak ratio to equal 0.500, is directly proportional to the apparent rate constant. Any changes in reaction conditions, such as substrate concentration (C_A) or isotopic content of the reactants, which affect the reaction rate are directly reflected in $v_{\frac{1}{2}}$.[4] The data shown in Table 1 are from six different reaction series in which C_A was varied in the range 0.5 to 2.0 mM. The data reported are for C_A equal 1.0. The basic buffer consisted of $CF_3CO_2^-LH^+$ (3.3 mM) and L (7.5 mM) while the acidic buffer contained $CF_3CO_2^-LH^+$ (3.4 mM) and CF_3CO_2H (12.9 mM). The k_H/k_D and reaction orders ($R_{A/B}$) are listed in Table 2. The $R_{A/B}$ reflect both the contribution of $Th^{\cdot+}$ (B) and Th (A) to the overall reaction rate.[4]

Table 1. Derivative cyclic voltammetry kinetics of the hydroxylation of the thianthrene cation radical in acetonitrile.

X	C_X/M	Conditions	$v_{\frac{1}{2}}$/V s^{-1}
H_2O	2.09	Neutral	91.8
D_2O	2.09	Neutral	9.64
H_2O	1.11	Basic buffer	26.2
D_2O	1.11	Basic buffer	12.6
H_2O	1.11	Acidic buffer	102.9
D_2O	1.11	Acidic buffer	101.0

The data are consistent with a mechanism consisting of steps (1) to (4) where we do not specify

$$Th^{\cdot+} + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Th^{\cdot}-\overset{+}{O}H_2 \quad (1)$$

$$Th^{\cdot}-\overset{+}{O}H_2 + Base \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Th^{\cdot}-OH + BaseH^+ \quad (2)$$

$$Th^{\cdot}-OH + Th^{\cdot+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} Th^{+}-OH + Th \quad (3)$$

$$Th^{+}-OH \xrightarrow{k_4} ThO + H^+ \quad (4)$$

the base, but this may be H_2O, L or $CF_3CO_2^-$. Under neutral conditions $R_{A/B}$ approaches 1. This is consistent with the orders in $Th^{\cdot+}$ and Th being 2 and -1, respectively. The rate law for this extreme case is (5). The large value of k_H/k_D under these

$$Rate = 2k_4K_1K_2K_3[Th^{\cdot+}]^2[H_2O][Base]/[Th][BaseH^+] \quad (5)$$

conditions arises from both (2) and (4). We point out that the observed value of $R_{A/B}$ was 1.3 indicating that the limit represented by rate law (5) was not observed. We also do not specify a base in reaction (4) and this is because the kinetics indicate first order in both L and H_2O. Reaction (4) may be subject to general base catalysis where the solvent can also act as the base.

Table 2. Correlation of reaction orders with kinetic isotope effects observed during the hydroxylation of the thianthrene cation radical in acetonitrile.

Reaction conditions	k_H/k_D	$R_{A/B}$
Neutral	~10	1.3
Basic buffer	2.1	1.75
Acidic buffer	1.0	2.0

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In the acidic buffer k_H/k_D was 1 and $R_{A/B}$ was 2. Under these conditions the reaction order in $Th^{\cdot+}$ is apparently 2 and that for Th is 0. The rate law consistent with the data is (6). Thus, in acidic buffer

$$\text{Rate} = 2k_3K_1K_2[Th^{\cdot+}]^2[H_2O][\text{Base}]/[\text{BaseH}^+] \quad (6)$$

reaction (2) can be considered in equilibrium giving rise to a small value of k_H/k_D and the electron transfer step (3) is rate limiting.

The reactions carried out in basic buffer represent the intermediate case between the two extremes outlined above with observed values of k_H/k_D of about 2 and $R_{A/B}$ about 1.75. This situation can be described by rate law (7). The fractional reaction

$$\text{Rate} = 2k_4K_1K_2K_3[Th^{\cdot+}]^2[H_2O][\text{Base}]/[\text{BaseH}^+](k_4/k_{-3} + [\text{Th}]) \quad (7)$$

order is due to the competition of back reaction (3) with product forming reaction (4). The reactions in the basic buffer are considerably slower than those in either neutral solution or acidic buffer. This means that trifluoroacetate is a more effective base in reaction (2) than is 2,6-lutidine.

It seems highly unlikely that the presence of L in low concentrations can have a drastic effect on the overall mechanism of the reaction. It is more likely that in the absence of L the overall mechanism is the same but that the role of water is complex. For example, if Base in step (2) is H_2O and H_2O is implicated in step (4) an approximate third order in water could be expected under conditions outlined above where either rate law (5) or (7) applies. The work reported here has bearing on related studies[1,2,5-9] and the new approach, that of correlating kinetic isotope effects with reaction orders, may prove to be of value in clarifying other complex mechanisms of the reactions of ion radicals.

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Synthesis of a Crown Ether with D_2 Symmetry

MARIANNE FRANTSI, GÖRAN LINDSTEN, and OLOF WENNERSTRÖM

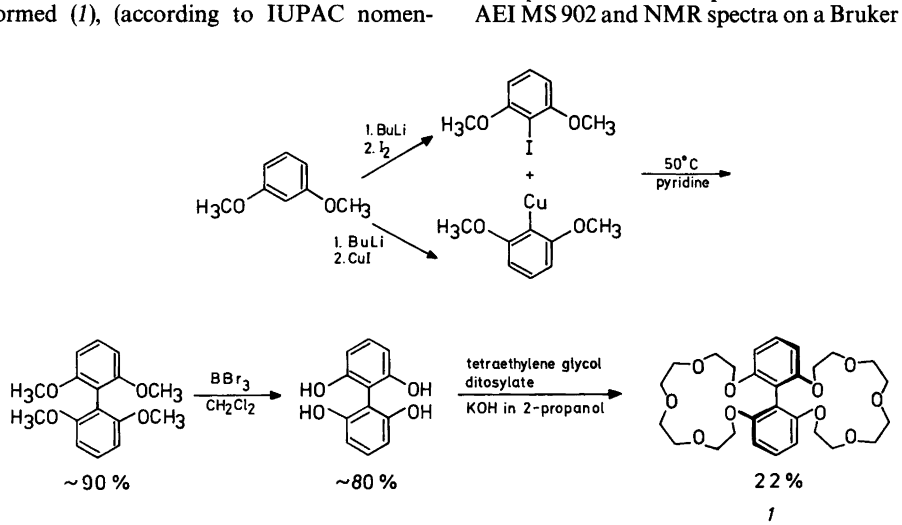
Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

The rapid development of crown ether chemistry and the increasing interest in chiral crown ethers have encouraged us to report a facile synthesis of a new chiral crown ether from 2,2',6,6'-tetrahydroxybiphenyl. Crown ethers with D_2 symmetry are chiral, several examples of chiral crown ethers being known.[1] Recently, Rebek Jr. and coworkers have reported allosteric effects in a crown ether from 2,2',6,6'-tetra(hydroxymethyl)biphenyl.[2]

2,2',6,6'-Tetramethoxybiphenyl can conveniently be prepared from 2,6-dimethoxyiodobenzene and 2,6-dimethoxyphenylcopper.[3] The cleavage of the methyl ether bonds has previously been performed with hydrobromic acid.[4] We found that neither hydrobromic nor hydroiodic acid gave satisfactory yields, as ring closure reactions gave dibenzofurane derivatives at rates comparable with that of methyl ether bond cleavage. Instead, boron tribromide is the reagent of choice for the reaction, giving a near-quantitative yield of 2,2',6,6'-tetrahydroxybiphenyl. The two oligoethylene glycol chains were attached by refluxing the tetrahydroxybiphenyl with sodium hydroxide and tetraethylene glycol ditosylate in 2-propanol for 48 h. The structure of the crown ether formed (*1*), (according to IUPAC nomenclature; 2,5,8,11,14,20,23,26,29,32-decaoxatetracyclo[31.3.1.1^{15,19}.0^{37,38}]octatriaconta-1(37),15,17,19(38),33,35-hexaene), was determined from its mass and ^{1}H NMR spectra (270 MHz). The latter showed a distinct ABCD pattern for half of the methylene protons, interpreted as being due to restricted rotation around the phenyl bond on the NMR time scale. Recently, a reinvestigation of the rotational barriers in some 2,2',6-tri- and 2,2',6,6'-tetramethoxybiphenyls has revealed that the barriers are considerably higher than previously assumed.[5] Whether the enentiomers of the crown ether can be isolated or not remains to be proven. The ABCD pattern is also compatible with an isomer having the chains linked 2,6 and 2',6'. However, reaction between 2-acetylresorcinol and tetraethylene glycol ditosylate gave a neglible yield of crown ether.[6] We therefore consider it very unlikely that a 2,6-type isomer should form in 22 % yield, a conclusion which is supported by inspection of CPK-models.

The crown ether (*1*) forms complexes with simple cations. The complex constants were determined by extraction of picrates from water to a chloroform solution.[7] The prerequisite for this method is that the crown ether is insoluble in water so that extraction of crown ether from chloroform to water can be neglected. The method also neglects the influence of water on the formation of the complex.[8] In the calculation of the constants, independent complexation by the two crown ether groups in the molecule was assumed, *i.e.* no allosteric effects. As expected from the ring size, a small preference for sodium ($K = 54 \times 10^3$ M^{-1}) over potassium ($K = 23 \times 10^3$ M^{-1}) and ammonium ($K = 21 \times 10^3$ M^{-1}) was observed.

Experimental. Mass spectra were recorded on an AEI MS 902 and NMR spectra on a Bruker WH 270



Scheme 1. Synthesis of crown ether *1*.

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instrument. Absorbances for complex constant measurements were determined on a Varian Cary 210 spectrophotometer.

2,2',6,6'-Tetramethoxybiphenyl. To a stirred solution of 1,3-dimethoxybenzene (44 g, 0.32 mol) in dry ether (500 ml) *n*-butyllithium (200 ml, *ca.* 15 % in hexane, Merck) was added *via* syringe. The mixture was stirred overnight under nitrogen at room temperature. Copper(I) iodide (61 g, 0.32 mol), dried overnight in an oven at 110 °C, was added in portions, and the mixture was stirred at room temperature for another 2 h. 2,6-Dimethoxyiodobenzene (66 g, 0.25 mol) was then added in portions together with dry pyridine (600 ml). The mixture was heated to 50 °C, and most of the diethyl ether was distilled off. The dark solution was stirred at 50 °C for three days under nitrogen. The product mixture was poured onto ice and acidified with hydrochloric acid (4 M). The solid residue was collected by filtration, dried and recrystallised from dichloromethane – ethanol to give 2,2',6,6'-tetramethoxybiphenyl (70 g, 90 %, m.p. 174 – 175 °C).

2,2',6,6'-Tetrahydroxybiphenyl. 2,2',6,6'-Tetramethoxybiphenyl (18 g, 65 mmol) was dissolved in dichloromethane (250 ml) and cooled to −78 °C. Boron tribromide (25 ml) dissolved in dichloromethane (150 ml) was added, and the stirred solution was allowed to reach room temperature slowly during 5 h. Water was added carefully to the reaction mixture, followed by ether. The acidic water phase was extracted several times with ether, sodium chloride was added and the mixture was again extracted with ether. The combined ether solutions were dried and the solvent distilled off. The residue slowly crystallised from ethanol to give 2,2',6,6'-tetrahydroxybiphenyl (11.5 g, 78 %, m.p. 191 – 192 °C after careful drying to remove ethanol).

^{1}H NMR (270 MHz, acetone-d_6): δ 6.48 (4 H, d, *J* 8.2 Hz), 7.03 (2 H, t, *J* 8.0 Hz), 7.46 (4 H, broad s).

MS (50 eV): *m/e* 218 (M^+, 100 %), 200 (39), 173 (15), 150 (15), 147 (16), 122 (18), 115 (16). Mol.wt., obs. 218.058, calc. for $C_{12}H_{10}O_4$ 218.058.

Crown ether (1). 2,2',6,6'-Tetrahydroxybiphenyl (0.50 g, 2.3 mmol) and sodium hydroxide (0.50 g, 12 mmol) were added to 2-propanol (65 ml) which was then heated to reflux. After 30 min tetraethylene glycol ditosylate (2.43 g, 48 mmol) suspended in a small amount of 2-propanol was added, and the mixture was refluxed for 48 h. The reaction mixture was cooled, and the precipitate (sodium tosylate, 1.7 g) was filtered off and washed with dichloromethane and acetone. The solvent was removed by distillation from the combined solutions and the residue slowly crystallised from ethanol to give the crown ether *1* (0.27 g, 22 %, m.p. 140 – 144 °C).

^{1}H NMR (270 MHz, $CDCl_3$): δ 3.53 (16 H, m), 3.67 (8 H, m), 3.96 (4 H, m), 4.11 (4 H, m), 6.63 (4 H, d, *J* 8.5 Hz), 7.18 (2 H, t, *J* 8.2 Hz).

MS (50 eV): *m/e* 535 (31), 534 (M^+, 100 %), 227 (33), 226 (18), 201 (13), 200 (16), 199 (16), 197 (11), 89 (13), 87 (12). Mol.wt., obs. 534.244, calc. for $C_{28}H_{38}O_{10}$ 534.246.

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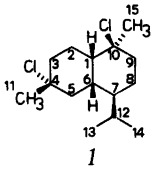
The Structure of Muurolene Dihydrochloride and its Transformation to Cadinene Dihydrochloride

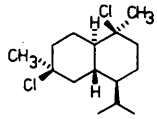
ANNA-KARIN BORG-KARLSON,[a] TORBJÖRN NORIN,[a] ANNE-MARIE PILOTTI,[b] THEODOR STEFANIDES,[b] ANNE-CHARLOTTE SÖDERHOLM[b] and DONALD WIJEKOON[a]

[a] Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden and [b] Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Muurolene dihydrochloride (*1*) is a useful and characteristic derivative for the identification and characterization of sesquiterpenes of the muurolane type.[1,2] It shall be noted that the muurolene dihydrochloride (*1*) is formed together with cadinene dihydrochloride (*2*)[3,4] in the hydrochlorination reaction of a muurolene derivative.[1,2] There are also indications for a conversion of *1* to *2* under the conditions used for the reaction.



1



2

The structure of cadinene dihydrochloride (*2*) was settled already in 1958.[3,4] The structure of muurolene dihydrochloride (*1*) has still not been determined although the *cis*-ring junction of the decalin system follows from a chemical transformation of *1* to ε-muurolene of a known configuration.[5] In this communication we report a structure determination of muurolene dihydrochloride (*1*) by X-ray diffraction methods.* We also report that muurolene dihydrochloride (*1*) almost quantitatively is converted to cadinene dihydrochloride (*2*) by treatment with hydrogen chloride in ethanol.

Muurolene dihydrochloride (*1*) crystallizes in space group $P2_1$ with two molecules in the asymmetric unit. The cell dimensions are $a = 13.699(5)$, $b = 11.633(4)$, $c = 9.855(2)$ Å and $\beta = 95.08(4)°$. Intensity data were recorded on a Philips 1100 computer-controlled diffractometer with Cu-radiation. Out of the 3220 reflexions collected ($\theta \leq 65°$), 2107 were considered to be significant $I_{net} \leq 3\sigma(I_{net})$ and were used in the subsequent refinements. Correction for intensity loss due to deterioration was made as a linear function of time. The structure was solved by a combination of the heavy atom technique and direct methods.[6] It was refined to an R value of 0.135 using the block-diagonal least-squares method with unitary weights. No hydrogens have been included in the calculations. The final parameters for the non-hydrogen atoms are listed in Table 1.

The rather low accuracy in the crystal structure determination is partly due to deterioration and to difficulties in finding crystals suitable for the investigation. The diffraction peaks of the crystal

*The structure *1* of muurolene dihydrochloride was recently reported by Dr. V. A. Barkhash (Institute of Organic Chemistry, Siberian Division of the Academy of Sciences, Novosibirsk 90, USSR) in a lecture of the 9th Conference on Isoprenoids, Prague, September 1981. [*cf.* Gatilov, Yu. V., Osadchii, S. A. and Dubovenko, Zh. V. *Khim. Prir. Soedin.* (1981) 52; *Chem. Abstr.* 95 (1981) 98046].

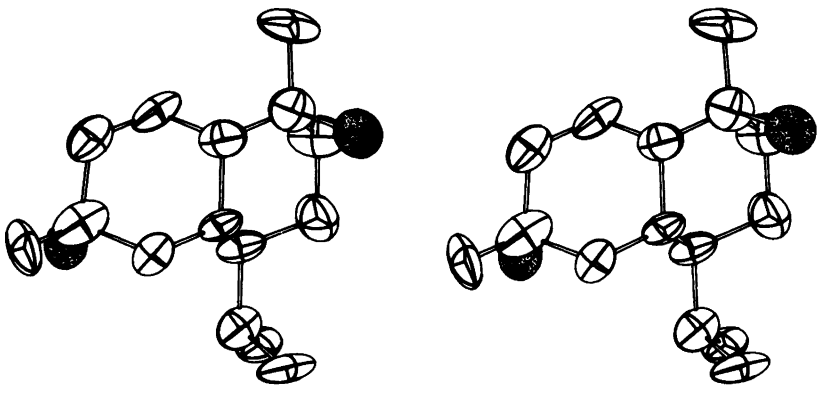


Fig. 1. An ORTEP drawing of one of the independent molecules. The chlorine atoms are shaded.

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Table 1. Positional and thermal parameters for the non-hydrogen atoms in the two molecules. Values are $\times 10^3$ for the coordinates and $\times 10^2$ for the U's. E.s.d.'s are given within parantheses. The U-values refer to the temperature factor expression $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{23}klb^*c^*+2U_{13}hla^*c^*+2U_{12}kha^*b^*)]$.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	317(2)	215(3)	1036(4)	4(2)	5(2)	5(2)	0(2)	0(2)	0(2)
C2	410(3)	148(4)	1043(4)	8(3)	5(3)	8(3)	0(3)	1(2)	0(2)
C3	440(3)	128(4)	889(4)	12(3)	6(4)	7(3)	−3(3)	2(3)	0(3)
C4	353(3)	62(4)	807(4)	10(3)	6(3)	5(3)	0(3)	1(3)	−4(3)
C5	253(2)	116(4)	815(4)	2(2)	11(4)	6(2)	−1(3)	0(2)	1(2)
C6	224(3)	151(3)	964(4)	5(2)	5(2)	7(2)	0(2)	2(2)	2(2)
C7	190(3)	43(3)	1044(4)	9(3)	3(2)	8(3)	0(2)	4(3)	1(2)
C8	162(4)	98(5)	1189(4)	11(4)	11(5)	6(3)	−1(3)	5(3)	0(4)
C9	258(4)	154(4)	1265(4)	12(4)	7(3)	4(2)	1(2)	2(3)	−1(3)
C10	298(3)	258(3)	1188(4)	8(3)	3(2)	9(3)	−1(2)	3(3)	2(3)
C12	100(3)	−14(4)	968(4)	6(2)	8(3)	9(3)	−2(3)	1(2)	−6(2)
C14	76(4)	−122(5)	1052(5)	5(4)	14(5)	11(4)	−3(4)	−1(3)	−3(4)
C13	12(3)	58(5)	936(5)	3(3)	8(4)	19(6)	2(4)	−2(3)	0(3)
C11	380(4)	47(4)	652(4)	13(5)	8(3)	6(3)	1(3)	2(3)	−3(4)
C15	383(4)	308(4)	1258(4)	13(4)	7(3)	6(3)	−5(3)	−1(3)	−2(3)
Cl1	355(1)	−89(1)	871(2)	11(1)	7(1)	10(1)	−3(1)	3(1)	0(1)
Cl2	194(1)	362(1)	1156(1)	8(1)	6(1)	11(1)	−1(1)	1(1)	2(1)
C'1	198(2)	592(3)	641(3)	5(2)	12(3)	5(2)	1(2)	0(2)	−1(2)
C'2	158(3)	694(3)	719(3)	10(3)	9(3)	5(2)	−2(2)	4(2)	−1(2)
C'3	55(2)	728(3)	664(3)	7(2)	8(3)	8(3)	0(2)	3(2)	−1(2)
C'4	42(3)	769(3)	505(4)	11(3)	6(2)	8(3)	−2(2)	4(2)	−4(2)
C'5	100(2)	679(3)	424(3)	4(2)	6(2)	6(2)	−2(2)	1(2)	−2(2)
C'6	200(2)	638(3)	481(3)	6(2)	7(2)	3(2)	−2(2)	1(2)	−1(2)
C'7	290(2)	720(3)	470(3)	6(2)	7(2)	4(2)	0(2)	2(2)	−3(2)
C'8	393(2)	660(4)	522(3)	6(2)	10(3)	9(2)	1(3)	0(2)	1(3)
C'9	384(2)	621(3)	674(3)	7(2)	12(4)	7(2)	1(2)	−1(2)	−5(2)
C'10	296(3)	544(3)	696(4)	11(3)	5(2)	6(3)	2(2)	0(2)	1(2)
C'12	297(2)	232(3)	321(3)	9(2)	5(2)	7(2)	−1(2)	3(2)	−1(2)
C'14	384(2)	153(3)	318(3)	8(2)	6(2)	6(2)	1(2)	3(2)	−1(2)
C'13	301(3)	330(3)	214(3)	14(4)	9(3)	3(2)	0(2)	3(3)	−3(3)
C'11	57(2)	783(3)	451(4)	4(2)	10(3)	13(4)	2(3)	0(2)	2(2)
C'15	296(4)	481(3)	857(3)	24(5)	3(2)	5(2)	1(2)	1(3)	1(3)
Cl'1	109(1)	907(1)	505(1)	10(1)	7(1)	9(1)	1(1)	3(1)	2(1)
Cl'2	311(1)	408(−)	613(1)	13(1)	5(1)	9(1)	0(1)	1(1)	1(1)

used showed a small splitting; however, intensities were measured as sums of the two components.

A stereoscopic view[7] of one of the molecules in the asymmetric unit is shown in Fig. 1. The bond lengths and angles of the two independent molecules appear to be normal, within the rather low accuracy. The two molecules in the asymmetric unit are similar within estimated standard deviations.

It is interesting to note the strong steric interaction between the axially oriented chlorine atom on C(4) and the carbon atom C(7). It is therefore not surprising to find that muurolene dihydrochloride (*1*) is not stable upon treatment with hydrogen chloride in ethanol and that it is transformed into the more stable cadinene dihydrochloride (*2*). This transformation involves inversions at C(1) and C(4).

The transformation may proceed *via* a nucleophilic S_N2 replacement or a dehydrochlorination/hydrochlorination reaction leading to an inversion at C(4) and a dehydrochlorination −hydrochlorination involving the C(1)−C(10) positions to invert the C(1)-position.

Conversion of muurolene dihydrochloride (1) to cadinene dihydrochloride (2). Muurolene dihydrochloride (0.8 g; m.p. 84−86 °C; $[\alpha]_D^{22}-13.9°$, *c* in $CHCl_3$ 1.5)[2] was dissolved in ethyl alcohol (99.5%, 2 ml). To this solution hydrochloric acid (6N, 3 drops)

was added and the mixture was warmed on a water bath (80 °C) for 5 min. The excess ethyl alcohol was then removed by applying reduced pressure. The concentrated solution was cooled thoroughly to obtain a white solid. This solid was filtered and recrystallized from light petroleum (b.p. 40 – 60 °C). The yield of colourless needles of cadinene dihydrochloride (*2*) was almost quantitative: m.p. 117 – 119 °C; $[\alpha]_D^{22}$ −36° (*c* in $CHCl_3$ 2.0).

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N-Desmethyl Analogues of (+)-4-Dimethylamino-2,α-dimethylphenethylamine. Synthesis and Configurational Relationships

LENNART FLORVALL and MAJ-LIZ PERSSON

Department of CNS-Medicinal Chemistry, Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

The resolution of 4-dimethylamino-2,α-dimethylphenethylamine (*1*) and the synthesis of the 4-methylamino- and 4-amino-analogues *2* and *8* are described. The dextrorotatory isomer *(+)2* was prepared from *(+)1* by oxidative *N*-monodemethylation. The configurational relationships of *(+)2* and the dextrorotatory isomer of *8* was established from the corresponding carbamates, which both on reduction yielded an identical compound, (+)-4-methylamino-2,α,*N*-trimethylphenethylamine dihydrochloride [*(+)3*]. In connection with the preparation of *(+)3*, the synthesis and selective ethoxycarbonylating properties of a new type of mixed anhydride agent, are reported.

Recently the title compound, 4-dimethylamino-2,α-dimethylphenethylamine, has been shown to be a reversible and selective inhibitor of the A form of the enzyme monoamine oxidase (MAO).[1] In the course of the pharmacokinetic investigation of the dextrorotatory enantiomer of this compound it was found that the main metabolic pathway was *N*-demethylation at the aniline nitrogen.[2] At that time access to the optical isomers of these metabolites became desirable. The sequences of reactions leading to the synthesis of the target compounds are presented in Scheme 1.

The preparation of racemic 4-dimethylamino-2,α-dimethylphenethylamine was readily effected by a standard procedure involving reduction of the corresponding nitrostyrene with lithium aluminum hydride.[1] The racemic amine was conveniently resolved by use of L(+)-tartaric acid. The optical purity of the obtained isomer *(+)1* was checked by GLC analysis of the (−)-camphanic amide. Oxidative *N*-monodemethylation of *(+)1* by means of lead tetraacetate in acetic anhydride yielded *(+)2*. The method has been used previously for the selective *N*-demethylation of simple substituted *N*,*N*-dimethylanilines *e.g.* *N*,*N*-dimethyl-*p*-toluidine.[3]

In the work on the synthesis of (+)-4-methylamino-2,α,*N*-trimethylphenethylamine *(+)3* from *(+)2*, it was found that the potential intermediate, ethyl *N*-(4-methylamino-2,α-dimethylphenethyl)-carbamate, could be obtained by selective ethoxycarbonylation of *(+)2* with a new mixed anhydride, *S*-ethoxycarbonyl 4-methyl-1-piperazinecarbodithioate. The carbamate was then converted to the required amine *(+)-3* by the reduction with lithium aluminum hydride (Method A). The mixed anhydride used was initially prepared in connection with a study on biologically active dithiocarbamates, but was rejected owing to its instability.[4] The compound is easily prepared from sodium or potassium 4-methyl-1-piperazinecarbodithioate and ethyl chloroformate. The synthesis of some *S*-alkoxycarbonyl analogues is described. Examples of related anhydrides are reported in the literature, but no references were found to the potential alkoxycarbonylating properties of these compounds.[5,6] In an attempt to prepare thiosemicarbazides from *S*-ethoxycarbonyl *N*,*N*-dimethyldithiocarbamate and hydrazines only hydrazinium dimethyldithiocarbamates could be isolated.[7]

The key intermediate to compound *8*, 4-amino-2-methylbenzaldehyde (4), was prepared essentially as described in the literature from 1,2-dimethyl-4-nitrobenzene by the reaction with aqueous alcoholic sodium polysulfide.[8] The previous procedure involving steam distillation was simplified in that the compound was isolated by means of extraction. Initial attempts to condense aldehyde *4* with nitro-

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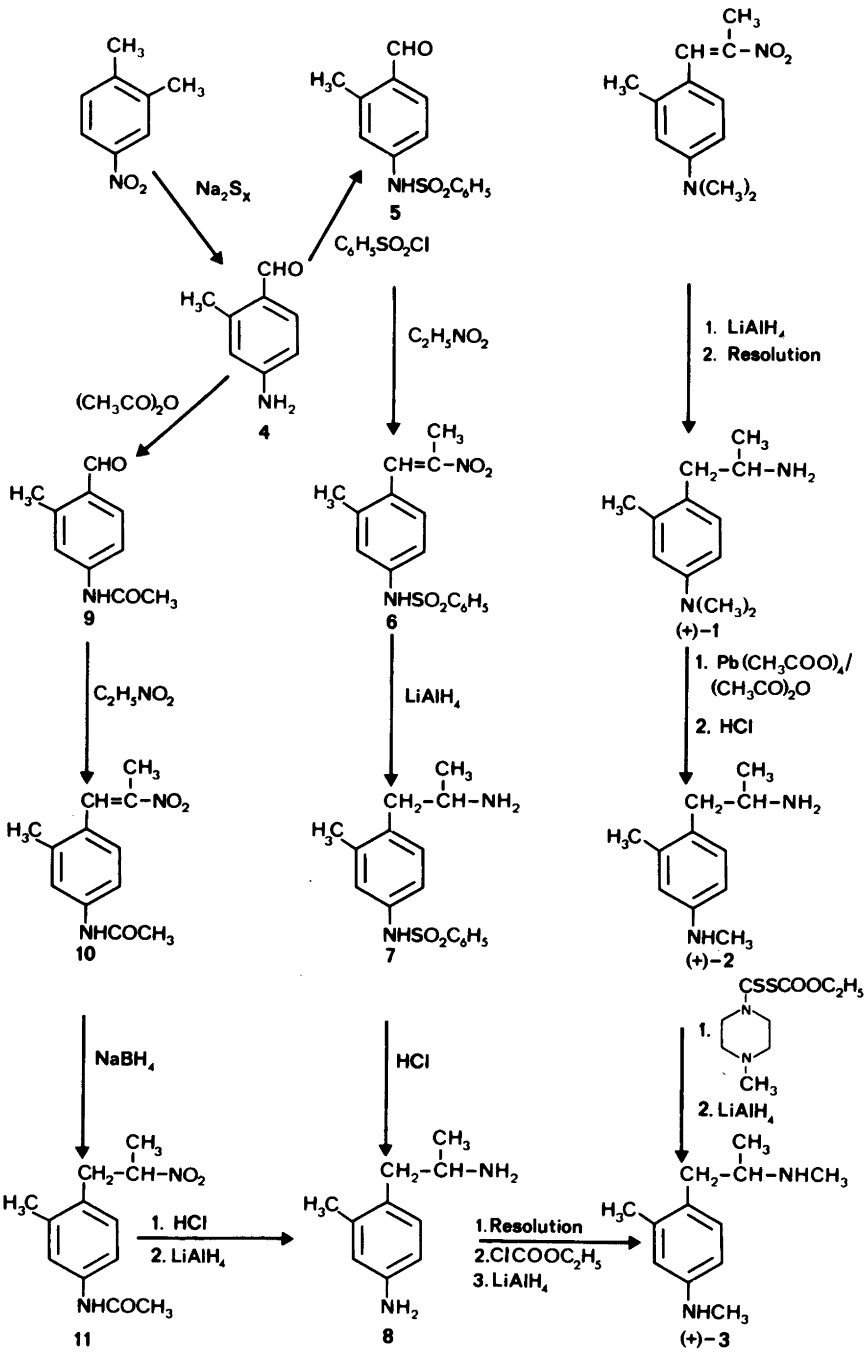
Na2Sx
C6H5SO2Cl
(CH3CO)2O
C2H5NO2
LiAlH4
NaBH4
HCl
1. HCl
2. LiAlH4
1. Resolution
2. ClCOOC2H5
3. LiAlH4
1. LiAlH4
2. Resolution
1. Pb(CH3COO)4/
(CH3CO)2O
2. HCl
2. LiAlH4
4
5
6
7
8
9
10
11
(+)-1
(+)-2
(+)-3

Scheme 1.

ethane resulted in the formation of polymeric products. To eliminate this tendency of self-condensation, the amino group of *4* was blocked by acetylation with acetic anhydride yielding compound *9*. Alternatively reaction of *4* with benzenesulfonyl chloride in the presence of pyridine gave the sulfonamide *5*. Condensation of *9* and *5* with nitroethane in ethanol, using ammonium acetate as a catalyst, yielded the corresponding nitrostyrenes *10* and *6*. Compound *6* was then reduced with lithium aluminium hydride to the intermediate sulfonamide *7*. In the next step the hydrolysis of *7* with hydrochloric acid yielded compound *8* (Method B). The preparation of *(+)-8* was achieved from *6* without isolating the intermediate sulfonamide *7*. The racemic amine *8* was readily resolved by means of L(+)-tartaric acid.

The acid hydrolysis of the acetanilide *10* resulted in tar formation. Attempts to convert *10* into the 4-acetamido analogue of *8* by means of selective reduction with lithium aluminium hydride were unsuccessful, the acetamide group of *10* being reduced at the same time. Thus, the synthesis of *8* from *10* was achieved in a two-step procedure. In the first step the double bond of *10* was selectively reduced with sodium borhydride. The obtained acetanilide *11* was then deacetylated with hydrochloric acid to give the intermediate compound 1-(4-amino-2-methylphenyl)-2-nitropropane, which on reduction with lithium aluminium hydride, yielded *8* (Method C).

Ethoxycarbonylation of the optical isomer *8(+)* with ethyl chloroformate followed by reduction of the intermediate di-carbamate with lithium aluminium hydride yielded *(+)-3* (Method D). The compound was identical with that prepared according to Method A.

EXPERIMENTAL

Melting points were determined in an electrically heated metal block, using calibrated Anschütz thermometers. Optical rotation were measured with a Perkin-Elmer Model 41 polarimeter and ^{1}H NMR spectra were recorded with a Varian A-60 A or a Bruker WP 200 NMR spectrometer. The analyses were performed by the Department of Analytical Chemistry at the University of Lund, Sweden. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4 % of the theoretical values. The absence of precursors in the prepared compounds was established by GLC using a Perkin-Elmer 3920 gas chromatograph equipped with flame ionization detector.

(+)-4-Dimethylamino-2,α-dimethylphenethylamine (+)-hydrogen tartrate [(+)-1]. A solution of 98.4 g (0.45 mol) of 4-dimethylamino-2,β-dimethyl-β-nitrostyrene in 250 ml of dry tetrahydrofuran was added dropwise while stirring and cooling in ice-water to 50.0 g (1.3 mol) of lithium aluminium hydride in 950 ml of dry ether. The mixture was stirred overnight at room temperature. Then 300 ml of saturated sodium sulfate solution was added dropwise with vigorous stirring and cooling. The mixture was filtered and the solid washed with ether. The filtrate and washings were dried over anhydrous sodium sulfate. The solvent was removed by evaporation and the residual crude base was dissolved in 500 ml of 95 % methanol. The solution was added to 75.0 g (0.5 mol) of L(+)-tartaric acid dissolved in 500 ml of 95 % methanol. The solution was allowed to stand for 18 h at room temperature. The solid was filtered, washed with ether and air-dried, giving 89.5 g of fluffy crystals. Four recrystallizations in a like manner (3 × 1000 ml 95 % methanol + 500 ml 95 % methanol) gave 22.5 g (29 %) of the pure compound, m.p. 191 – 192 °C. $[\alpha]_D^{20} = +27.2°$ ($c = 0.1$, H_2O). $[\alpha]_D^{20}$ of the base = $+33.7°$ ($c = 2$, EtOH). Anal. $C_{16}H_{26}N_2O_6$: C,H,N,O. NMR: (base, $CDCl_3$): 7.0 (dd, 1, phenyl), 6.5 (dd, 2, (overlap, phenyl), 3.1 (m, 1, methine), 2.9 (s, 6, *N,N*-dimethyl), 2.5 (dq, 2, methylene) 2.3 (s, 3, 2-methyl), 1.3 (s, 2, amino), 1.1 (d, 3, α-methyl).

The optical purity was checked in the following manner. An appropriate amount of the tartrate (10 – 50 mg) was dissolved in 2 ml of dry pyridine and a molar excess (300 %) of (−)-campanic acid chloride added. The mixture was incubated at 50 °C for 10 min and the solution was subjected directly to GLC analysis. A 2 m × 3 mm glass column was used, with 3 % OV-17 as liquid phase and 100 – 120 mesh Gas Chrom Q as support. Samples were injected at a column temperature of 230 °C with a nitrogen flow rate of *ca.* 30 ml/min. Under these conditions retention times of *ca.* 30 min were observed, with 1 – 2 min separation between isomer peaks. Isomer purities of >95 % (peak heights) were found by this technique.

(+)-4-Methylamino-2,α-dimethylphenethylamine (+)-hydrogen tartrate [(+2)]. To 68.4 g (0.2 mol) of *(+)1* was added with stirring 400 ml of 10 % NaOH. The resulting mixture was extracted with ether. The extract was dried over sodium sulfate, filtered and the ether was evaporated. To the residue, 37.6 g oil, 120 ml of acetic anhydride was added dropwise while stirring. The resulting mixture was refluxed for 10 min and then cooled down to room temperature. To the stirred solution was added dropwise while cooling in tap water a solution of 115.0 g (0.22 mol) of 85 % lead tetraacetate

in 500 ml of chloroform. After standing overnight at room temperature the precipitated lead acetate was filtered off and washed with chloroform. The filtrate and washings were combined and extracted with 3 × 100 ml of water. The chloroform was evaporated and to the residue, 55.0 g of brown oil, was added 100 ml of concentrated hydrochloric acid and 100 ml of water. The mixture was refluxed overnight and then evaporated to half its volume and made basic with NaOH. Extractions with ether and drying of the extract with sodium sulfate gave, after evaporation of the solvent, 32.3 g of a brown oil. The product was distilled at reduced pressure giving 18.3 g of a fraction boiling at 141–145 °C/2 mm. The obtained base was dissolved in 400 ml of ethanol. The solution was added to 16.5 g (0.11 mol) of L(+)-tartaric acid dissolved in 400 ml of ethanol. The mixture was left overnight at room temperature. The precipitated solid was collected and washed with ethanol and ether. Yield 31.0 g. M.p. 169–170 °C. The salt was recrystallized from 1000 ml of 98 % ethanol, giving 27.8 g (42 %) of *(+)2*. M.p. 170–171 °C $[\alpha]_D^{20} = +30.3°$ ($c=1$, H_2O). Anal. $C_{15}H_{24}N_2O_6$: C,H,N,O.

(+)-4-Methylamino-2,α,N-trimethylphenethylamine dihydrochloride [(+)3]. *Method A.* To 7.5 g (0.023 mol) of *(+)2* was added 40 ml of 10 % NaOH. The mixture was extracted with 2 × 100 ml of ether. The extract was dried over sodium sulfate and was added dropwise while stirring and cooling in ice to a solution of 5.8 g (0.023 mol) of fresh prepared *S*-ethoxycarbonyl 4-methyl-1-piperazinecarbodithioate. The mixture was stirred for 18 h at room temperature. The precipitated 4-methyl-1-piperazinedithiocarboxylic acid (4.0 g, 97 %) was filtered off and washed with ether. The filtrate and washings were combined and evaporated. The residue, 6.1 g of colourless oil, was dissolved in 100 ml of dry ether. The solution was added dropwise while stirring to 7.6 g (0.2 mol) of lithium aluminium hydride in 200 ml of dry ether. The mixture was refluxed for 5 h. After the addition of 25 ml of saturated sodium sulphate the mixture was filtered. The filtrate was dried with anhydrous sodium sulfate and treated with dry hydrogen chloride in ether. The precipitate was recrystallized from ethanol–isopropyl ether. Yield 4.7 g (77 %). M.p. 218–219 °C $[\alpha]_D^{20} = +9.6°$ ($c=1$, H_2O).

S-Ethoxycarbonyl 4-methyl-1-piperazinecarbodithioate. To a stirred suspension of 20.0 g (0.1 mol) of sodium 4-methyl-1-piperazinecarbodithioate in 200 ml of ether was added gradually while stirring and cooling in ice a solution of 9.5 ml (0.1 mol) of ethyl chloroformate in 25 ml of ether. The mixture was stirred overnight at room temperature and filtered. The filtrate was evaporated and the residue was crystallized from ether–lightpetroleum yielding 13.5 g (54 %) of yellow crystals melting at 44–45 °C. Anal. $C_9H_{16}N_2O_2S_2$: C,H,N,S. The compound was unstable and liquified at room temperature within a few weeks. When stored in a refrigerator no signs of decomposition were found for a period of one year.

The following compounds were obtained in a similar way. *S*-Isopropoxycarbonyl 4-methyl-1-piperazinecarbodithioate, yield 30 %, m.p. 60–61 °C. Anal. $C_{10}H_{18}N_2O_2S_2$: C,H,N,S. *S*-Benzyloxycarbonyl 4-methyl-1-piperazinecarbodithioate, yield 11 %, m.p. 90–91 °C. Anal. $C_{14}H_{18}N_2O_2S_2$: C,H,N,S.

In a model reaction a solution of 7.9 g (0.05 mol) of *N*-ethoxycarbonylpiperazine in 50 ml of ether was added by portions to a stirred solution of freshly prepared *S*-ethoxycarbonyl 4-methyl-1-piperazinecarbodithioate in 100 ml of ether. The mixture was stirred overnight at room temperature. The precipitate was filtered off and washed with ether giving 8.8 g (99.5 %) of *N*-methyl-*N'*-dithiocarboxylic acid, m.p. 185 °C (subl.). The melting point was undepressed on admixture with an authentic sample of *N*-methyl-*N'*-dithiocarboxylic acid. Anal. $C_6H_{12}N_2S_2$: C,H,N,S. The filtrate and washings were combined and the ether was evaporated yielding 11.4 g (99 %) of *N,N'*-diethoxycarbonylpiperazine melting at 43–45 °C (lit.[9] m.p. 44–45 °C). An analytical sample, recrystallized from ligroin, melted at 46–46.5 °C. Anal. $C_{10}H_{18}N_2O_4$: C,H,N,O.

4-Amino-2-methylbenzaldehyde (4). 108.8 g (0.72 mol) of 1,2-dimethyl-4-nitrobenzene was dissolved in 600 ml of ethanol and added slowly while heating at reflux temperature to a stirred solution of 54 g of sodium hydroxide, 160 g of sodium sulphide nonahydrate and 30 g of sulfur in 1.0 l of water. The solution was boiled under reflux for 3 h and the ethanol was evaporated at reduced pressure. The mixture was extracted with chloroform. The extract was dried with sodium sulphate and the chloroform was evaporated. The residual yellow oil was crystallized from toluene–ligroin yielding 51.7 g (53 %) of *4*. M.p. 83–84 °C (Lit.[8] m.p. 83–84 °C).

4'-Formyl-3'-methylbenzenesulfonanilide (5). To a stirred solution of 51.9 g (0.38 mol) of *4* in 200 ml of tetrahydrofuran and 80 ml of pyridine was added by portions 75 ml (0.59 mol) of benzenesulfonyl chloride. After stirring for 1 h at room temperature 1.5 l of ice-water was added. The precipitate was filtered off and washed with water giving 99.8 g (95 %) of *5*. M.p. 156–158 °C. An analytical sample, melting at 161–62 °C was obtained by recrystallization of this material from aqueous EtOH. Anal. $C_{14}H_{13}NO_3S$: C,H,N,O,S.

3'-Methyl-4'-(2-nitropropen-1-yl)benzenesulfonanilide (6). A mixture of 142 g (0.51 mol) of *5*, 65 ml (0.9 mol) of nitroethane, and 10 g of ammonium

acetate in 1.2 L of ethanol was heated under reflux for 3 h. To the hot solution was added 1 l of water and the mixture was cooled in ice. The precipitate was filtered off and washed with water yielding 120.8 g (71 %) of the crude compound melting at 135–137 °C. An analytical pure sample (76.2 g) was obtained by recrystallization of 87.0 g of this material from 1 l of 75 % EtOH. M.p. 138–139 °C. Anal. $C_{16}H_{16}N_2O_4S$: C,H,N,O,S.

4'-(2-Aminopropyl)-3'-methylbenzenesulfonanilide (7). A solution of 22.0 g (0.066 mol) of *6* in 130 ml of tetrahydrofuran was added dropwise while stirring to a mixture of 7.9 g (0.21 mol) of lithium aluminium hydride in 200 ml of dry ether. The reaction mixture was refluxed for 1.5 h. After dropwise addition of 25 ml of a saturated sodium sulfate solution while stirring and cooling, the mixture was filtered. The filter cake was extracted with 4 × 200 ml of boiling ethanol. The combined extracts were acidified with hydrogen chloride in ether. Concentration of the solution to 200 ml and the addition of 100 ml of isopropyl ether gave a small amount of precipitation which was filtered off. The filtrate was evaporated and the residue recrystallized from ethanol–ether giving 10.7 g (48 %) of the hydrochloride. M.p. 217–219 °C. The hydrochloride was dissolved in water and converted into the free base by the addition of NaOH to a pH-value of ~7. The compound was extracted with methylene chloride and recrystallized from ethanol–light petroleum. M.p. 121.5–122.5 °C.

Anal. $C_{16}H_{20}N_2O_2S$: C,H,N,O,S.

4-Amino-2,α-dimethylphenethylamine dihydrochloride (8). Method B. A mixture of 0.5 g (0.0016 mol) of 7 and 3.5 ml of 25 % hydrochloric acid was refluxed for 5 h. The solution was extracted with ether and the aqueous layer was made alkaline with NaOH. Extraction with ether and acidifying of the sodium sulfate dried extract yielded the crude hydrochloride. The salt was filtered off and recrystallized twice from ethanol–isopropyl ether giving 0.20 g (53 %) of *8*. M.p. 296–298 °C (D). Anal. $C_{10}H_{16}N_2 \cdot 2\,HCl$: C,H,Cl,N.

4-Formyl-3-methylacetanilide (9). To 1.0 g (0.0074 mol) of compound *4* was added 10 ml of acetic anhydride. After stirring at room temperature for 2 h the reaction mixture was poured into ice-water. The obtained precipitate was filtered off and washed with water. Yield 1.3 g (99 %). M.p. 109–112 °C. An analytical sample was obtained by recrystallization of this material from aqueous ethanol. M.p. 112.5–113.5 °C. Anal. $C_{10}H_{11}NO_2$: C,H,N,O.

3-Methyl-4-(2-nitropropen-1-yl)acetanilide (10). A mixture of 34.4 g (0.19 mol) of *9*, 22.5 g (0.3 mol) of nitroethane and 20 g of ammonium acetate in 200 ml of ethanol was heated under reflux while stirring for 5 h. To the hot solution was added 300 ml of water. After cooling the obtained precipitate was filtered off and recrystallized from aqueous ethanol yielding 17 g (38 %) of *10*. M.p. 130–31 °C. Anal. $C_{12}H_{14}N_2O_3$: C,H,N,O.

3-Methyl-4-(2-nitropropyl)acetanilide (11). To a stirred solution of 20.0 g (0.085 mol) of *10* in 600 ml of ethanol was added by portions 5.7 g (0.15 mol) of sodium borohydride. Stirring was continued for 4.5 h when a portion of 1.0 g of sodium borohydride was added. After 0.5 h the mixture was diluted with 100 ml of water and made acidic with acetic acid to pH 4. The ethanol was evaporated and aqueous ammonia was added to pH 10. The mixture was extracted with 3 × 500 ml of chloroform. The extract was washed with water, dried with sodium sulfate and the solvent was evaporated. The residue was recrystallized from ethanol–light petroleum yielding 17.0 g (85 %) of crude *11*, melting at 79–81 °C. An analytical sample was obtained from a repeated crystallization. M.p. 88.5–90 °C. Anal. $C_{12}H_{16}N_2O_3$: C,H,N,O.

4-Amino-2,α-dimethylphenethylamine dihydrochloride (8). Method C. A solution of 11.6 g (0.050 mol) of compound *11* was dissolved in a mixture of 200 ml of ethanol and 40 ml of concentrated hydrochloric acid. The stirred solution was refluxed for 2 h and was then evaporated. Saturated sodium hydrogen carbonate solution was added to the residue and the mixture was extracted with chloroform. The extract was dried with sodium sulphate and the solvent was evaporated. The evaporation of the solvent gave 9.9 g of crude 1-(4-amino-2-methylphenyl)-2-nitropropane as an oil. A solution of 4.99 g (0.025 mol) of the oil above in 25 ml of dry ether was added while stirring to 3.3 g (0.087 mol) of lithium aluminium hydride in 300 ml of dry ether. The mixture was refluxed for 3.5 h and 25 ml of saturated sodium sulfate solution was added. The filtrate was dried over sodium sulfate and the ether was evaporated. The residual oil was dissolved in 225 ml of ethanol and 5.0 g (0.04 mol) of oxalic acid dihydrate was added. The precipitate was collected and recrystallized from 300 ml of aqueous ethanol giving 3.0 g (40 %) of the oxalate. M.p. 184–185 °C. Anal. $C_{10}H_{16}N_2 \cdot 1.5\,C_2H_2O_4$: C,H,N,O.

The melting point of the dihydrochloride, 297–298 °C (D), was undepressed on admixture with the product prepared by Method B.

A little amount of the crude intermediate compound, 1-(4-amino-2-methylphenyl)-2-nitropropane, was dissolved in ether and a slight excess of toluene-4-sulfonic acid was added. The precipitated sulfonic acid salt melted at 177–178 °C. Anal. $C_{10}H_{14}N_2O_2 \cdot C_7H_8O_3S$: C,H,S,O.

(+)-4-Amino-2,α-dimethylphenethylamine (+)-hydrogen ditartrate [(+)8]. 76.2 g (0.23 mol) of compound *6* was reduced with 31.0 g of lithium aluminium hydride according to the directions given for 7. The combined ethanol extracts were

evaporated and the residue (69.3 g) was dissolved in 200 ml of concentrated hydrochloride acid and 200 ml of water. The solution was refluxed overnight, concentrated to half its volume and then diluted with water to 800 ml and extracted with ether. The water layer was alkalized with sodium hydroxide and extracted with 3 × 300 ml of ether. The extracts were dried with sodium sulfate and the ether was evaporated. The residue, 26.0 g oil, was dissolved in 100 ml of ethanol and the solution was added to 48.0 g (0.32 mol) of L(+)-tartaric acid in 300 ml of ethanol. The mixture was cooled in ice-water and the obtained precipitate was filtered off giving 60.8 g of crude tartrate. M.p. 146–150 °C. The salt required three recrystallizations from 80% methanol (1500 ml + 2 × 1000 ml) before constant physical properties were attained. Yield 22.7 g (43 %). M.p. 152–155 °C. $[\alpha]_D^{20} = +26.3°$ ($c = 1$, H_2O). Anal. Found: C 45.81; H 6.34; N 5.58; O 41.78. Calc. for $C_{10}H_{16}N_2 \cdot 2C_4H_6O_6$: C 46.55; H 6.08; N 6.03; O 41.34.

(+)-4-Methylamino-2,α,N-trimethylphenethylamine dihydrochloride [(+)3]. *Method D.* To a solution of 18.6 g (0.04 mol) of *(+)8* in 50 ml of water was added 25 ml of 45 % NaOH. The mixture was extracted with 2 × 150 ml of ether. The extract was evaporated and to the residual oil was added 200 ml of toluene and a solution of 12.0 g (0.3 mol) of NaOH in 150 ml of water. The mixture was stirred and cooled in ice-water while 30 ml (0.3 mol) of ethyl chloroformate was added by portions. The mixture was stirred for 2 h at room temperature and 200 ml of ether was added. The solvent layer was separated and dried with anhydrous sodium sulfate. The solvent was evaporated giving 11.8 g of the solid intermediate carbamate. The compound (11.8 g) was dissolved in 100 ml of tetrahydrofuran and added to 15.0 g (0.4 mol) of lithium aluminium hydride in 200 ml of ether according to the direction in Method A. The crude hydrochloric salt was recrystallized twice from ethanol–isopropyl ether giving 7.7 g (73 %) of *(+)3*. M.p. 218–219 °C. $[\alpha]_D^{20} = +9.3°$ ($c = 1$, H_2O). The melting point was undepressed on admixture with the compound prepared by Method A. The NMR spectrum of the compound was found to be identical with that obtained from the product prepared by Method A. Anal. $C_{12}H_{20}N_2 \cdot 2HCl$: C,H,Cl,N.

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Tobacco Chemistry. 55.* Three New Cembranoids from Greek Tobacco. The Stereochemistry of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-Cembratriene-4,6-diol

INGER WAHLBERG,[a] INGRID WALLIN,[a] CLAUDINE NARBONNE,[a] TOSHIAKI NISHIDA,[a] CURT R. ENZELL[a] and JAN-ERIC BERG[b]

[a] Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62 Stockholm, Sweden and [b] Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Three new diterpenoids have been isolated from tobacco. The first was shown to be (1*S*,2*E*,4*S*,6*R*,-7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*3*) by X-ray analysis and chemical correlation, the second was formulated as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triol (*4*) by spectral and chemical methods, while the third, (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,-12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*5*), previously described as a synthetic product, is now reported as a tobacco constituent.

The configuration at C-6 in (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*2*) has been resolved by chemical correlation with triol *3*.

The biogenesis of the two new triols (*3*,*4*) is discussed in the light of results obtained by singlet oxygen reactions.

More than twenty cembranoids have so far been isolated from the cuticular wax of different tobacco varieties. Most of these have a hydroxyl group at C-4 and are conveniently divided into two series, one comprising compounds having a 4*R*- and the other, compounds having a 4*S*-configuration. Additional oxygenation is commonly found at C-6, C-8, C-11 or C-12.[2]

Two diols, originally isolated by Roberts and Rowland in 1962[3] and characterized as (2*E*,7*E*,11ξ)-cembratriene-4,6-diols having different configurations at C-4[3] and possibly also at C-6,[4] are the major tobacco cembranoids. While the relative stereochemistry and absolute configuration of one of these have later been determined to be (1*S*,2*E*,-4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*1*) by X-ray analysis[4] and ozonolytic degradation,[5] the chirality at C-6 in the other diol (*2*) has remained unknown.

This structural uncertainty has now been resolved in conjunction with the structure determination of two new cembratrienetriols (*3*,*4*), which have been isolated from a wax extract of Greek tobacco. The present communication describes these results as well as the isolation of a third new tobacco constituent (*5*).

RESULTS

The first tobacco isolate (*3*), $C_{20}H_{34}O_3$, is a triol having two secondary hydroxyl groups (signals at δ 4.07, dd, and δ 4.67, ddd, both shifted downfield in the ^{1}H NMR spectrum of diacetate *6*) and one tertiary hydroxyl group (^{13}C NMR signal at δ 74.1 (s), *cf.* Table 1; OH-absorption in the IR spectrum of *6*). Furthermore, since the ^{1}H and ^{13}C NMR spectra were consistent with the presence of three double bonds, of which one is attached to an exocyclic methylene group, one is a di- and one a trisubstituted double bond, it followed that triol *3* is carbomonocyclic.

The occurrence of two methyl groups, one of which is vinylic and one attached to the fully substituted carbon atom carrying the tertiary hydroxyl group, and an isopropyl group demonstrated that the carbocyclic ring is fourteen-

*For part 54 see Ref. 1.

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Table 1. Carbon-13 chemical shifts and assignments for compounds *3*, *4*, *6* and *8*–*11*.[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
3	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
4	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[c]	18.0	30.0[c]
6[e]	48.2	129.5	138.3	72.0	47.8	69.5	124.4	140.2	30.4	30.4	75.5	147.7	34.5	30.1	31.9	19.2/	20.7	30.4	16.8	112.2
8[f]	49.5	128.0	138.0[b]	72.0	48.6	69.8	124.1	139.7	40.9	124.3	138.5[b]	73.7	40.1	26.1	30.2	18.0/	21.4	29.7[c]	18.3	29.4
9[g]	46.3	127.8	137.1	72.3	50.7	68.8	126.6	139.2	38.8	23.1	124.3	133.4	36.6	27.8	32.9	19.4/	20.7	29.6	16.1	14.8
10[h]	48.3	130.0	137.8	72.1	48.1	69.5	124.4	140.9	30.9	32.0	72.9	152.1	34.9	29.9	31.9	19.2/	20.7	30.7	16.6	110.5
11[i]	48.6	128.4	137.6[b]	72.3	48.0	69.9	123.9	140.3	41.5	125.7	138.2[b]	74.1	39.7	27.5[d]	29.9[c]	18.2/	21.3	29.8[c]	18.2	27.6[d]

[a] δ-Values in $CDCl_3$ relative to TMS. [b,c,d] Assignment may be reversed. [e] $OCOCH_3$ 170.4 and 169.8;$OCOCH_3$ 21.4 and 21.3. [f] $OCOCH_3$ 169.9;$OCOCH_3$ 21.4. [g] $OCOCH_3$ 170.2; $OCOCH_3$ 21.4. [h] $OCOCH_3$ 170.0;$OCOCH_3$ 21.4. [i] $OCOCH_3$ 169.8; $OCOCH_3$ 21.4.

membered and suggested that triol *3* is a cembratrienetriol.

Treatment of triol *3* with weakly acidified chloroform, which resulted in the formation of (1*S*,2*E*,4*S*,-6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*),[6] verified this assignment and allowed the formulation of triol *3* as a (1*S*,2*E*,4*S*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol. The geometry of the 7,8 double bond was determined to be *E* from the characteristic chemical shift value[7] of the C-19 methyl group, δ 16.0, in the ^{13}C NMR spectrum of triol *3*, thereby leaving the chirality at C-6 to be accounted for. An X-ray analysis of triol *3* using a direct phase determination procedure was therefore undertaken.

Triol *3* formed orthorhombic crystals of space group $P2_12_12_1$. The crystal data, obtained on a Philips PW 1100 diffractometer, were: $a=16.047$, $b=21.247$ and $c=6.229$ Å, $Z=4$. The present *R*-value including anisotropic thermal parameters for all non-hydrogen atoms is 0.122; location of the hydrogen atoms and further refinement being under way.[8] A stereoscopic view, which summarizes the X-ray results and demonstrates that triol *3* has a 6*R*-configuration, is shown in Fig. 1.

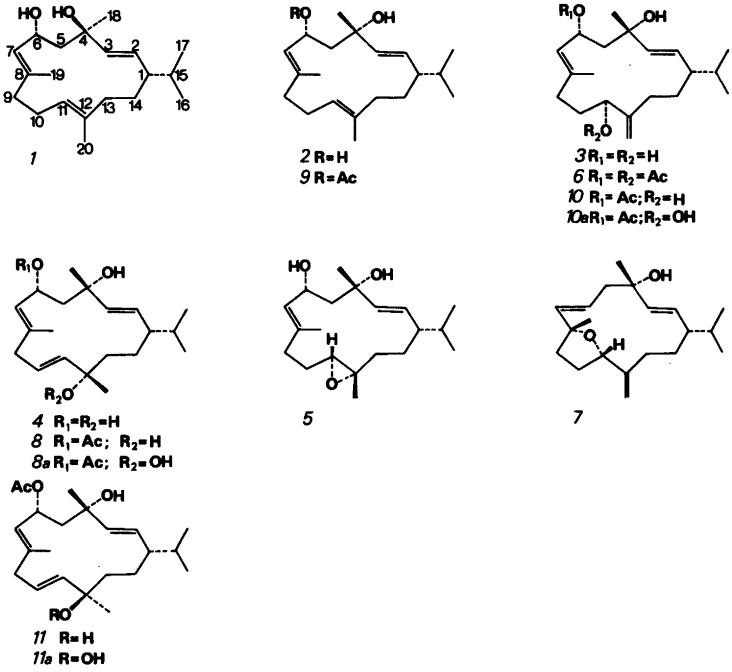
The spectral data indicated that the second tobacco isolate (*4*), $C_{20}H_{34}O_3$, is a cembratrienetriol and structurally closely related to triol *3*. However, in contrast to triol *3*, triol *4* incorporates one secondary hydroxyl group (a signal at δ 4.80 ddd, shifted to δ 5.74 in the ^1H NMR spectrum of monoacetate *8*) and two tertiary hydroxyl groups (^{13}C NMR signals at δ 74.0 (s) and 74.3 (s); OH-absorption in the IR spectrum of *8*). Of the double bonds, two are disubstituted and one trisubstituted.

Spin decoupling and spin simulation experiments carried out on both triol *4* and monoacetate *8* allocated the secondary hydroxyl group, the trisubstituted double bond and an *E*-disubstituted double bond ($J_{AB}=15.9$ Hz) to partial structure A (Fig. 2).

Since the remaining groups comprised one *E*-disubstituted double bond ($J_{AB}=15.5$ Hz), one isopropyl group, one sp^3 methine and two sp^3 methylene groups, triol *4* was provisionally identified as a (2*E*,10*E*)-2,7,10-cembratriene-4,6,12-triol.

With this result at hand, it could be inferred from the chemical shift value of the signal due to the C-19 methyl group in triol *4*, δ 18.0, that the geometry of the 7,8 double bond is *E*.

Additional structural information was obtained by chemical means. Thus, (1*S*,2*E*,4*S*,6ξ,7*E*,11*S*,12*S*)-



11,12-epoxy-2,7-cembradiene-4,6-diol (5)[6,9] was rearranged *via* an intermediate hydroxy selenide and an unstable selenoxide[10] to (1*S*,2*E*,4*S*,6ξ,7*E*,10*E*,-12*S*)-2,7,10-cembratriene-4,6,12-triol (*4*). The latter proved to be identical in all respects to the new tobacco diol.

A plausible biogenetic route to the new triols (*3*, *4*) would involve attack of oxygen on the 11,12 double bond in (1*S*,2*E*,4*S*,6ξ,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*2*). In harmony with this view, acetate *9*, which in contrast to diol *2* has a 7,8 double bond not susceptible to singlet oxygen reactions, proved to undergo facile ene reactions at the 11,12 double bond. Reduction of the reaction mixture using triethyl phosphite and separation by HPLC yielded two major and one minor product (*10*, *8* and *11*; ratio according to HPLC: 54:43:3).

The ^{1}H NMR spectrum demonstrated that the least polar, major product (*10*) retained the 2,3 and

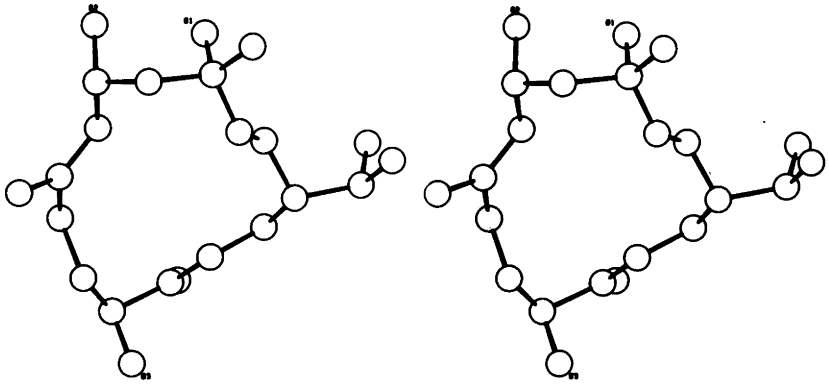
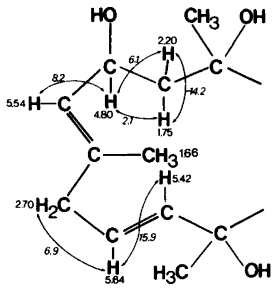


Fig. 1. Stereoscopic view of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*3*).



A

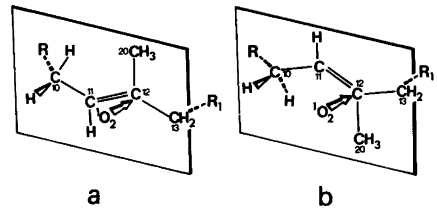
Fig. 2. Partial structure A. Chemical shift values (δ) are in Roman, coupling constants (Hz) are in italic type.

7,8 double bonds and the 6-acetoxyl group and contained a newly introduced secondary hydroxyl group (one-proton triplet at δ 4.03) and an exocyclic methylene group (one-proton signals at δ 4.92 and 5.07). Monoacetate *10* was converted by treatment with acid into (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*) and by acetylation into a diacetate (*6*), which proved to be identical to the diacetate derived from triol *3*. These results allowed the assignment of *10* as (1*S*,2*E*,4*S*,6*R*,7*E*,-11*S*)-6-acetoxy-2,7,12(20)-cembratriene-4,11-diol.

The most polar product (*8*), being indistinguishable from the acetate obtained from triol *4*, was hence identified as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol, whereas the minor product (*11*), whose ^{13}C NMR spectrum differed mainly with respect to the shielding of C-20, δ 29.4 as against δ 27.6, was formulated as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-6-acetoxy-2,7,10-cembratriene-4,12-diol.

As a consequence of the chemical correlations described above, diol *2*, which is a synthetic precursor of *10*, is now conclusively identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol. Likewise, it can be concluded that triol *4* as well as epoxide *5*, which has previously been described as a synthetic product[6,9] and is now reported as the third new tobacco isolate, have 6*R*-configurations.

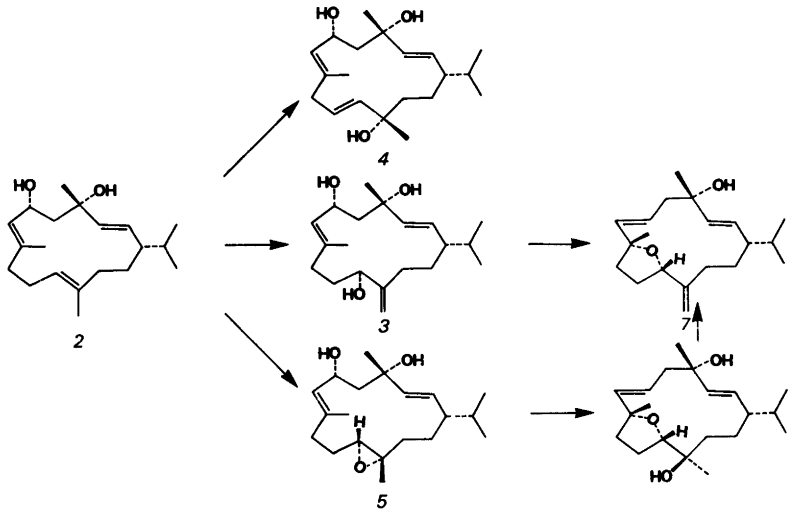
The outcome of the photo-oxygenation reaction deserves comment. Thus, in keeping with the *cis*-cyclic mechanism[11] the generation of both the major hydroperoxides *8a* and *10a* (43 % and 54 %) may be rationalized by ene reactions involving the 11,12 double bond in conformer *a* of acetate *9*. Compound *8a* arises by oxygen attachment to C-12 and migration of the *pro*-*R*-hydrogen at C-10 and compound *10a* by oxygen attachment to C-11 and migration of a hydrogen from C-20. The minor hydroperoxide *11a* (3 %) would be formed by attack of singlet oxygen at C-12 in conformer *b* and abstraction of the *pro*-*S*-hydrogen at C-10. Conformer **a** may then exist in preference to conformer **b** or react with a higher rate constant than the latter, a conclusion which accords with the fact that peracid oxidation of diol *2* yields predominantly the 11*S*,12*S*-epoxide *5*.[9]



a b

It is noteworthy that all three products arise by *syn* ene additions, *i.e.* singlet oxygen abstracts hydrogen from the 1,2-disubstituted side of the trisubstituted double bond.[12] This result indicates that 2*E*,7*E*,11*E*-cembratrienes having a fourteen-membered ring system react with singlet oxygen in the same fashion as has previously been found for acyclic and other cyclic systems, cyclohexenes being exceptions.[13]

The two new triols (*3*, *4*), being present in the cuticular wax of the leaf may well be generated in tobacco by sensitized photo-oxygenation of the 4*S*,6*R*-diol *2* (Scheme 1). Another possibility, which is currently exploited, is that the 4*S*,6*R*-diol *2* is converted to triols *3* and *4* by an enzyme-assisted reaction.



Scheme 1. Probable biogenesis of compounds *3*–*5* and *7*.

Triol *3*, in turn, may be an intermediate in the biogenesis of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*), whose alternative path of bioformation would involve a rearrangement of epoxide *5* and a subsequent dehydration.[9]

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 ^{1}H NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 14 were used.

Isolation. An extract (24 g) obtained by immersing green leaves of Greek *Nicotiana tabacum* (Basma Drama) in chloroform was distributed between hexane and methanol–water (80:20). The polar material obtained (16 g) was chromatographed over silica gel using a gradient of hexane–ethyl acetate as eluent to give three fractions, of which the least polar one (1 g) was a complex mixture and fraction 2 (8 g) was a mixture of the (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols (*1*, *2*). Fraction 3 (6 g) was separated further by chromatography over silica gel followed by HPLC using columns packed with μ-Porasil and μ-Bondapak/CN and gradients of hexane–ethyl acetate as eluents to yield 50 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,-11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*3*), 20 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-4,6,12-triol (*4*) and 105 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*5*).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-Cembratriene-4,6,11-triol (*3*) had m.p. 106–107 °C; $[\alpha]_D$ +48° (*c* 1.2, EtOH); (Found: $[M-18]^{\cdot+}$ 304.2374. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$) bands at 3605, 3440, 3090, 1650, 1390 and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.87 (d, *J* = 6.7 Hz)/0.89 (d, *J* = 6.6 Hz) (H-16/H-17), 1.28 (s, H-18), 1.67 (d, *J* = 1.2 Hz, H-19), 1.80 (dd, *J* = 2.7 and −14.4 Hz, H-5a), 2.22 (dd, *J* = 5.5 and −14.4 Hz, H-5b), 4.07 (dd, *J* = 4.5 and 8.5 Hz, H-11), 4.67 (ddd, *J* = 2.7, 5.5 and 9.1 Hz, H-6), 4.94 (m, $W_{1/2}$ = 3 Hz, H-20a), 5.07 (broad s, H-20b), 5.46 (d, *J* = 15.1 Hz, H-3), 5.55 (dd, *J* = 8.2 and 15.1 Hz, H-2) and 5.59 (broad d, *J* = 9.1 Hz, H-7); MS[*m*/*z* (%, composition)]: 304 (M−18, 3), 286 (5, $C_{20}H_{30}O$), 261 (4), 243 (8, $C_{14}H_{27}O_3$), 225 (3, $C_{17}H_{21}$), 205 (7, $C_{14}H_{21}O$, $C_{13}H_{17}O_2$ and $C_{15}H_{25}$), 177 (9, $C_{12}H_{17}O$ and $C_{13}H_{21}$), 159 (12, $C_{12}H_{15}$), 147 (23, $C_{11}H_{15}$ and $C_{10}H_{11}O$), 133 (27, $C_{10}H_{13}$ and C_9H_9O), 123 (30, C_9H_{15} and $C_8H_{11}O$), 109 (31), 95 (40, C_7H_{11} and C_6H_7O), 81 (54, C_5H_5O), 69 (36, C_5H_9 and C_4H_5O), 55 (39, C_4H_7 and C_3H_3O) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-Cembratriene-4,6,12-triol (*4*) had m.p. 140–143 °C; $[\alpha]_D$ +158° (*c* 0.3, $CHCl_3$); (Found: $[M-18]^{\cdot+}$ 304.2379. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR (CCl_4) bands at 3605, 3380, 1390 and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.80 (d, *J* = 6.7 Hz)/0.85 (d, *J* = 6.7 Hz) (H-16/H-17), 1.21 (s, H-20), 1.29 (s, H-18), 1.66 (broad s, H-19), 1.75 (dd, *J* = 2.1 and −14.2 Hz, H-5a), 2.20 (dd, *J* = 6.1 and −14.2 Hz, H-5b), 2.70 (dd, *J* = 0.8 and 6.9 Hz, H-9a and H-9b), 4.80 (ddd, *J* = 2.1, 6.1 and 8.2 Hz, H-6), 5.40 (d, *J* = 15.5 Hz, H-3), 5.42 (d, *J* = 15.9 Hz, H-11), 5.54 (d, *J* = 8.2 Hz, H-7), 5.54 (dd, *J* = 8.5 and 15.5 Hz, H-2) and 5.64 (dt, *J* = 6.9

and 15.9 Hz, H-10); MS [*m/z* (%, composition)]: 304 (M−18, 3), 286 (5, $C_{20}H_{30}O$), 268 (5, $C_{20}H_{28}$), 243 (6), 225 (5, $C_{17}H_{21}$), 203 (3, $C_{15}H_{23}$ and $C_{14}H_{19}O$), 185 (5, $C_{14}H_{17}$), 159 (10, $C_{12}H_{15}$), 145 (19, $C_{11}H_{13}$), 133 (21, $C_{10}H_{13}$), 119 (22, C_9H_{11}), 105 (27, C_8H_9), 93 (35, C_7H_9), 81 (38, C_5H_5O), 69 (27, C_5H_9 and C_4H_5O), 55 (28) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-Epoxy-2,7-cembradiene-4,6-diol (*5*) was identified by direct comparison with a synthetic sample (optical rotation, IR, ^{1}H NMR and MS).[9]

Acetylation of (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (3). Treatment of 8.2 mg of *3* with acetic anhydride in pyridine at room temperature for 4 h followed by work-up and HPLC using a column packed with μ-Porasil gave 6.5 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6,11-diacetoxy-2,7,12(20)-cembratrien-4-ol (*6*), which was an oil and had $[\alpha]_D+31°$ (*c* 0.21, $CHCl_3$); IR (CCl_4) bands at 3600, 3480, 3090, 1735, 1652 and 1245 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.86 (d, *J* = 6.5 Hz)/0.88 (d, *J* = 6.5 Hz) (H-16/H-17), 1.32 (s, H-18), 1.72 (d, *J* = 1.2 Hz, H-19), 2.03 (s, OCOC*H*$_3$), 2.05 (s, OCOC*H*$_3$), 4.97 (m, $W_{1/2}$ = 3 Hz, H-20a), 5.09 (broad s, H-20b), 5.14 (broad t, *J* = 6 Hz, H-11), 5.36 (broad d, *J* = 10 Hz, H-7) and 5.5−5.8 (overlapping signals, H-2, H-3 and H-6); MS [*m/z* (%)]: 328 (M−18−60, 3), 304 (1), 286 (6), 268 (8), 253 (3), 243 (6), 225 (14), 197 (5), 185 (10), 159 (11), 145 (19), 133 (21), 119 (19), 105 (37), 93 (36), 79 (23), 69 (17), 60 (22), 55 (19) and 43 (100).

Treatment of (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (3) with acid. To a solution of 12 mg of *3* in 2 ml of chloroform was added 0.1 ml of aqueous HCl (10 %). The reaction mixture was kept at room temperature for 30 min, washed with water, dried and evaporated. The residue was separated by HPLC using a column packed with μ-Porasil and ethyl acetate−hexane (20:80) as solvent to give 1.6 mg of a product, whose m.p., optical rotation, IR, ^{1}H NMR and mass spectra were indistinguishable from those of (1*S*,2*E*,4*S*,6*E*,-8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*).[6]

Acetylation of (1S,2E,4S,6R,7E,10E,12S)-2,7,10-cembratriene-4,6,12-triol (4). Acetylation of 8.4 mg of *4* using acetic anhydride in pyridine at room temperature for 1 h followed by chromatography over silica gel gave 5.8 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,-12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*8*), which had m.p. 105−107 °C, $[\alpha]_D+118°$ (*c* 0.56, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3450, 1720, 1670 and 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.80 (d, *J* = 6.7 Hz)/0.84 (d, *J* = 6.8 Hz) (H-16/H-17), 1.24 (s, H-20), 1.29 (s, H-18), 1.76 (broad s, H-19), 1.91 (dd, *J* = 3.4 and −13.6 Hz, H-5a), 2.05 (s, OCOC*H*$_3$), 2.07 (dd, *J* = 7.6 and −13.6 Hz, H-5b), 2.67 (dd, *J* = 8.1 and −18 Hz, H-9a), 2.76 (dd, *J* = 5.4 and −18 Hz, H-9b), 5.29 (d, *J* = 9.7 Hz, H-7), 5.41 (d, *J* = 15.5 Hz, H-3), 5.44 (d, *J* = 15.4 Hz, H-11), 5.55 (dd, *J* = 8.2 and 15.5 Hz, H-2), 5.67 (ddd, *J* = 5.4, 8.1 and 15.4 Hz, H-10) and 5.74 (ddd, *J* = 3.4, 7.6 and 9.7 Hz, H-6); MS [*m/z* (%)]: 286 (M−60−18, 3), 268 (7), 253 (2), 243 (3), 225 (7), 183 (8), 169 (8), 157 (8), 145 (20), 131 (19), 119 (18), 105 (29), 91 (32), 81 (25), 69 (18), 60 (12), 55 (24) and 43 (100).

Conversion of (1S,2E,4S,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diol (5) to (1S,2E,4S,-6R,7E,10E,12S)-2,7,10-cembratriene-4,6,12-triol (4). To a stirred suspension of 27 mg of diphenyl diselenide in 5 ml of dry ethanol was added, under nitrogen, 7 mg of $NaBH_4$. After addition of 45 mg of *5* the reaction mixture was refluxed for 26 h. The solution was cooled and 2.5 ml of tetrahydrofuran was added, followed by dropwise addition of 0.3 ml of hydrogen peroxide (30 %). The temperature was kept below 20 °C while cooling the mixture in an ice-bath. After 3 h the elimination was complete by TLC. The resulting slurry was diluted with water and extracted with ether. The organic phase was washed with aqueous sodium carbonate (10 %), brine and water, dried and concentrated. The residue, 65 mg, was chromatographed over silica gel to give 30.1 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triol, whose m.p., optical rotation, IR, ^{1}H and ^{13}C NMR and mass spectra were identical with those of the naturally occurring triol (*4*).

Acetylation of (1S,2E,4S,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (2). Acetylation using acetic anhydride in pyridine converted *2* into (1*S*,2*E*,4*S*,6*R*,-7*E*,11*E*)-6-acetoxy-2,7,11-cembratrien-4-ol (*9*), which was an oil and had $[\alpha]_D+139°$, (*c* 1.37, $CHCl_3$); IR ($CHCl_3$) bands at: 3600, 3460, 1725, 1670 and 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.79 (d, *J* = 6.7 Hz)/0.83 (d, *J* = 6.8 Hz) (H-16/H-17), 1.38 (s, H-18), 1.51 (broad s, H-20), 1.72 (d, *J* = 1.2 Hz, H-19), 1.97 (dd, *J* = 8.0 and −13.0 Hz, H-5a), 2.02 (dd, *J* = 3.2 and −13.0 Hz, H-5b), 2.03 (s, OCOC*H*$_3$), 5.03 (m, $W_{1/2}$ = 10 Hz, H-11), 5.24 (broad d, *J* = 10.2 Hz, H-7), 5.32 (dd, *J* = 8 and 16 Hz, H-2), 5.35 (d, *J* = 16 Hz, H-3) and 5.54 (ddd, *J* = 3.2, 8.0 and 10.2 Hz, H-6); MS [*m/z* (%)]: 288 (M−60, 2), 270 (27), 255 (10), 245 (4), 227 (36), 199 (6), 185 (11), 171 (17), 159 (32), 145 (38), 133 (40), 119 (43), 107 (50), 91 (52), 81 (74), 69 (40), 55 (46) and 43 (100).

Photo-oxygenation of (1S,2E,4S,6R,7E,11E)-6-acetoxy-2,7,11-cembratrien-4-ol (9). A solution of 110 mg of *9* and 10 mg of Rose Bengal in 25 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 80 min when TLC showed that all starting material had been consumed, 100 μl of triethyl phosphite was added, and the reaction mixture was kept at room tem-

perature for 45 min. The solvent was removed under reduced pressure. The residue was filtered through silica gel and subsequently separated by HPLC using a column packed with Spherisorb 5 Nitrile to afford 8.7 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*11*), 52.2 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6-acetoxy-2,7,12(20)-cembratriene-4,11-diol (*10*) and 36.1 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*8*).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-6-Acetoxy-2,7,10-cembratriene-4,12-diol (*11*) was an oil and had $[\alpha]_D$ +102° (*c* 0.23, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3440, 1725, 1670 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (d, J=6.8 Hz)/0.86 (d, J=6.7 Hz) (H-16/H-17), 1.26 (s, H-20), 1.30 (s, H-18), 1.78 (d, J=1.2 Hz, H-19), 1.94 (dd, J=3.6 and −13.4 Hz, H-5a), 2.04 (s, $OCOCH_3$), 2.05 (dd, J=8.8 and −13.4 Hz, H-5b), 2.68 (dd, J=6.6 and −16.2 Hz, H-9a), 2.79 (dd, J=6.1 and −16.2 Hz, H-9b), 5.27 (broad d, J=9.2 Hz, H-7) 5.45 (d, J=15.4 Hz, H-3), 5.50 (d, J=15.7 Hz, H-11), 5.57 (dd, J=8.0 and 15.4 Hz, H-2), 5.62 (ddd, J=6.1, 6.6 and 15.7 Hz, H-10) and 5.70 (ddd, J=3.6, 8.8 and 9.2 Hz, H-7); MS [*m/z* (%)]: 304 (M−60, 2), 286 (4), 268 (3), 243 (8), 225 (4), 203 (3), 185 (6), 159 (11), 145 (20), 133 (23), 119 (12), 105 (22), 93 (26), 81 (28), 74 (27), 59 (40) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6-Acetoxy-2,7,12(20)-cembratriene-4,11-diol (*10*) was an oil and had $[\alpha]_D$ +61° (*c* 0.57, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3430, 1720, 1665, 1645 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (d, J=6.6 Hz)/0.88 (d, J=6.5 Hz) (H-16/H-17), 1.32 (s, H-18), 1.73 (d, J=1.3 Hz, H-19), 1.99 (dd, J=3.3 and −13.8 Hz, H-5a), 2.04 (s, $OCOCH_3$), 2.09 (dd, J=7.5 and −13.8 Hz, H-5b), 4.03 (t, J=6.5 Hz, H-11), 4.92 (m, $W_{1/2}$=3 Hz, H-20a), 5.07 (broad s, H-20b), 5.35 (broad d, J=9.5 Hz, H-7), 5.46 (d, J=15.4 Hz, H-3), 5.59 (dd, J=8.3 and 15.4 Hz, H-2) and 5.65 (ddd, J=3.3, 7.5 and 9.5 Hz, H-6); MS [*m/z* (%)]; 304 (M−60, 2), 286 (10), 243 (10), 225 (7), 185 (9), 173 (7), 159 (11), 147 (16), 133 (23), 119 (22), 105 (32), 93 (32), 79 (42), 69 (26), 60 (38), 55 (32) and 43 (100).

The third product, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*8*) proved to be identical in all respects (m.p., optical rotation, IR, ^1H NMR and MS) to the acetate derived from the naturally occurring 4*S*,6*R*,12*S*-triol.

Treatment of (1S,2E,4S,6R,7E,11S)-6-acetoxy-2,7,11-cembratriene-4,11-diol (10) with acid. To a solution of 27 mg of *10* in 2 ml of chloroform was added 0.1 ml of aqueous HCl (10 %). The reaction mixture was kept at room temperature for 72 h, washed with aqueous Na_2CO_3 (10 %) and water, dried and evaporated. The residue (18 mg) was chromatographed over silica gel to give 3 mg of starting material (*10*) and 3 mg of a product, whose m.p., optical rotation, IR, ^1H NMR and mass spectra were indistinguishable from those of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*).[6]

Acetylation of (1S,2E,4S,6R,7E,11S)-6-acetoxy-2,7,12(20)-cembratriene-4,11-diol (10). Acetylation of 10 mg of *10* using acetic anhydride in pyridine gave, after work-up and HPLC over μ-Porasil, 6.2 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6,11-diacetoxy-2,7,12(20)-cembratrien-4-ol (*6*), whose optical rotation, IR, ^1H NMR and mass spectra were identical to those of the diacetate obtained from the naturally occurring 4*S*,6*R*,11*S*-triol.

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On the Correlation Between Solvent Scales and Solvent-induced ^{13}C NMR Chemical Shifts of a Planar Lithium Carbanion. A Multivariate Data Analysis Using a Principal Component–Multiple Regression-like Formalism

BERTIL ELIASSON,[a] DAN JOHNELS,[a] SVANTE WOLD[b] and ULF EDLUND[a,*]

[a] Department of Organic Chemistry, University of Umeå, S-901 87 Umeå, Sweden and [b] Research Group for Chemometrics, Department of Organic Chemistry, University of Umeå, S-901 87 Umeå, Sweden

The solvent influence on the ^{13}C NMR chemical shifts of indenyllithium has been measured. A principal component data analysis showed that the shift matrix is described to 80–90 % by a two-component model. Only 58 % of the systematic variation in the shift data could be explained by the generally accepted solvent scales as shown by a partial least squares analysis. Although the π^* scale seems to be the most appropriate, no single scale has such high relevance in this complex solute–solvent system that it could be of a practical predictive utility. However, for subsets of the solvent matrix, single solvent parameter correlations can give acceptable results.

It is well known that the physico-chemical properties of a solute are influenced by the solvent and that the theories for the nature of these interactions are quite insufficient. This lack of an accurate theory is one of the reasons for the large number of empirical solvent "polarity" scales which appear in chemical literature.[1]

In order to describe kinetic, thermodynamic and spectroscopic data, single solvent scales as well as multiparameter equations have been applied.[1b,d,2–7] Multiple regression using a linear combination of two or several empirical scales usually gives better correlations than models with single scales but demands more data points (*i.e.* data from a large number of solvents) if any conclusions are to be reached from the regression coefficients. Furthermore, if the scales are highly correlated to each other, the ratio between the regression coefficients becomes very unreliable.[8,9] A way to overcome the latter problem is to use uncorrelated variables in the multiple regression analysis. These variables can be extracted from the correlated ones (*i.e.* the empirical solvent scales) with the aid of factor analysis[10] or principal component analysis.[11,12] The uncorrelated vectors are then successively calculated from the data set. The first vector describes the largest amount of variation in the data, while the second accounts for the largest possible amount in the remaining data, and so on. Several applications of factor analysis of solvent effects have recently appeared in the literature.[2c,10,12c,13]

The present work concerns the solvent effects on the ion pair structure and the charge distribution of indenyllithium (*I*) as reflected by the ^{13}C NMR chemical shifts. We have chosen this conjugated planar system as a suitable model because of its rigidity and stability, and also due to the high sensitivity of the ^{13}C chemical shifts of this anion towards changes in solvent, temperature and cation.[14,15] By going from a solvent with low solvating ability to one with high solvating ability, the external solvation of the ion pair will increase and/or the ion pair structure will be altered from a contact ion pair (c.i.p.) to a solvent separated ion pair (s.s.i.p.) and/or free ions.[14] The existence of an ion pair equilibrium (1)[16,17] has been shown in many investigations.[18–22]

*To whom correspondence should be addressed.

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$$\underset{\text{c.i.p.}}{A^-C^+} \rightleftharpoons \underset{\text{s.s.i.p.}}{A^-||C^+} \rightleftharpoons \underset{\text{free ions}}{A^- + C^+} \quad (1)$$

Since the cation in *I* has its average position above the five-membered ring in the c.i.p. structure,[14a,15a] an increase in cation solvation causes a redistribution of negative charge to the six-membered ring. This change is a consequence of a decreased π-polarization of negative charge towards the five-membered ring when the cation becomes more solvated as indicated by the ^{13}C chemical shifts.[14,15a]

Although the theory for ^{13}C chemical shielding is quite intricate,[23] a simple correlation of 160 – 180 ppm/e$^-$ between changes in sp^2 carbon chemical shifts and π-electron density is often noticed.[24-27]

The dominating factor in the solvation of indenyllithium and similar systems, is the Coulombic forces between the cation and solvent dipoles.[19a,21] The theoretical basis for treating ion – solvent electrostatic interactions is the "sphere in continuum model",[28] developed by Born[29] and Bjerrum and Larsson.[30] In a simplified treatment of this model, the logarithm of the dissociation constant for the dissociation of the contact ion pairs into free ions, is linearly related to the inverse of the dielectric constant of the medium.[31]

However, this model has several defects. Since the medium is assumed to be structureless, coordination of ions and ion pairs with solvent molecules is neglected. This is a serious approximation, especially with small and highly polar solvent molecules. Aggregation effects and the size and the shape of the ions are other structural factors which are not accounted for. Furthermore, dielectric saturation of the solvent and charge density redistribution in ions and solvent molecules are ignored. In addition, hydrogen bonding with suitable solvent, π-complexing with aromatic solvents[32] *etc.*, may have importance in the solvation processes. Hence, the dielectric constant alone cannot be used to explain the observed induced changes in charge density and ion pair structure.[21,22]

In this work, we wish to present an empirical principal component (PC) model, which describes the ^{13}C chemical shifts of *I* as a function of the solvent. Our choice of solvents is determined by their inertness toward strong bases and by the solubility of the ion pair, but also by the existence of data in the empirical solvent matrix. The predominating factors causing ion pair solvation are discussed on the basis of the parameters in the PC model. We have also examined the correlation between the chemical shift data and the empirical solvent scales. This study was performed by the use of a partial least squares (PLS) data analysis, *i.e.* a method similar to PC analysis of the chemical shift data but including a multiple regression-like formalism. Finally, the PLS method was used to predict chemical shift differences of *I* in two additional solvents, not included in the initial data analyses.

EXPERIMENTAL

Solvent purification and sample preparation. Anisole was refluxed over Na and distilled. The remaining ethers were refluxed over an Na/K alloy and finally distilled. All ethers were stored over Na-wire. The remaining solvents were refluxed over CaH_2, distilled, and stored over molecular sieves, except for dimethylsulfoxide which only was treated with molecular sieves.

I was obtained by adding an equivalent amount of n-BuLi (90 % in c-hexane) to a cooled solution of indene in the actual solvent. However, the anion could not be prepared directly in dimethyl formamide (DMF) or acetonitrile (AcN) because of the high reactivity of these solvents towards n-BuLi. Therefore *I* was prepared in diethylether (DEE) followed by evaporation of the ether by a stream of argon and finally addition of DMF or AcN. All work was performed under argon atmosphere.

NMR measurements and signal assignments. All spectra were obtained at 62.89 MHz on a Bruker WM-250 NMR spectrometer. The chemical shifts were measured relatively internal c-hexane,[33] and adapted to the TMS scale using δ (c-hexane)= 27.7 ppm. (Table 1.) The probe temperature was 25 ± 1 °C.

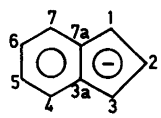
The assignment of the NMR signals of *I* in the solvents 3, 5, 6 and 9 has earlier been reported.[14a,34] In the remaining solvents, the signals of *I* were assigned by comparing the signal intensities and by the aid of standard decoupling techniques.

Before the PLS analysis was performed using all solvents, we found it necessary to undertake a concentration study of *I*. A comparison between the measured shift data and the calculated ones for anisole and dioxane might be erroneous since the ^{13}C shift values were obtained at much lower concentration of *I*. Three representative solvents were used in the investigation of the solvent dependence, TEA, THF, HMPA. The concentrations varied in the range of 0.04 – 1 M exept for TEA where solubility restrictions only allowed a maximum concentration of 0.4 M (Table 1). Only very small differences were observed by varying the concentration. This means that, if existent,[19b] stacking of

Table 1. ^{13}C NMR chemical shifts of indenyllithium (*I*) in various solvents.[a,b]

No.	Solvent[c]	Conc. (M)	C-1,3	C-2	C-3a,7a	C-4,7	C-5,6	$\Delta\delta_{av.}$
1	TEA	0.04	92.71	114.58	126.60	121.16	117.53	
		0.26	92.72	114.55	126.57	121.20	117.51	
		0.40	92.71	114.54	126.56	121.18	117.49	
2	i-Pr_2O	0.22	92.72	114.69	126.57	121.37	117.48	
			(0.00)	(0.14)	(0.00)	(0.17)	(−0.03)	0.05
3	Et_2O	0.27	92.13	115.42	128.14	120.66	116.13	
			(−0.59)	(0.87)	(1.57)	(−0.54)	(−1.38)	−0.11
4	THP[d]	0.23	91.69	115.38	128.78	120.30	115.24	
			(−1.03)	(0.83)	(2.21)	(−0.90)	(−2.27)	−0.35
5	DME	0.26	92.09	115.71	129.13	120.11	114.36	
			(−0.63)	(1.16)	(2.56)	(−1.09)	(−3.15)	−0.38
6	THF	0.04	91.66	115.55	129.72	119.76	114.25	
		0.25	91.68	115.59	129.76	119.66	114.06	
			(−1.04)	(1.04)	(3.19)	(−1.54)	(−3.45)	−0.52
		0.40	91.70	115.63	129.80	119.63	114.00	
		1.0	91.70	115.61	129.68	119.55	113.88	
7	AcN	0.20	93.60	118.5[e]	130.86	119.00	112.49	
			(0.88)	(4.0)	(4.29)	(−2.20)	(−5.02)	−0.01
8	Pyridine	0.26	94.83	119.43	132.16	119.87	112.82	
			(2.11)	(4.88)	(5.59)	(−1.33)	(−4.69)	0.92
9	DMSO	0.28	94.12	118.93	130.81	118.79	111.71	
			(1.40)	(4.38)	(4.24)	(−2.41)	(−5.80)	−0.08
10	DMF	0.20	93.60	118.48	131.40	118.48	111.19	
			(0.88)	(3.93)	(4.83)	(−2.72)	(−6.32)	−0.30
11	HMPA	0.05	93.29	118.02	131.37	118.15	110.19	
		0.20	93.24	117.98	131.33	118.15	110.19	
			(0.52)	(3.43)	(4.76)	(−3.05)	(−7.32)	−0.75
		1.0	93.27	118.04	131.31	118.15	110.27	
12	Anisole	0.07	93.09	115.65	127.60	121.3[e]	117.36	
			(0.37)	(1.10)	(1.03)	(0.1)	(−0.15)	0.4
13	Dioxane	0.05	92.01	115.59	130.44	119.86	114.52	
			(−0.71)	(1.04)	(3.87)	(−1.34)	(−2.99)	−0.14

[a] The chemical shifts were measured relatively internal c-hexane and adapted to the TMS scale using δ (c-hexane) = 27.7 ppm. [b] Values within parenthesis refer to differences in chemical shifts from TEA. [c] 1 = triethylamine, 2-diisopropyl ether, 3 = diethyl ether, 4 = tetrahydropyran, 5 = 1,2-dimethoxyethane, 6 = tetrahydrofuran, 7 = acetonitrile, 9-dimethylsulfoxide, 10 = *N*,*N*-dimethylformamide, 11 = hexamethylphosphoric triamide. [d] Measured relatively the methylene carbon in n-butane, with $\delta(-CH_2-) = 25.67$ ppm on the TMS scale. [e] Center of a solvent signal.



ion pairs is not reflected in the observed ^{13}C chemical shifts.

METHODS AND RESULTS

Solvent descriptors and ^{13}C chemical shift variables. Our choice of empirical solvent scales (Table 2) is mainly limited by the existence of at least eight data values per scale. In our data matrix, which is formed by eleven solvents and twelve solvent parameters, there are fourteen missing data values. We have tried to include solvent properties with expected relevance for the solvation process and thus tried to avoid parameters such as boiling points, molecular weights, viscosities, *etc.*

Although the dielectric constant, the dipole moment and the refractive index are bulk properties not representing the microscopic region, they have been included in the data analysis. Attempts to

Table 2. Empirical solvent scales.

No.	Symbol, name	Method, system	Solvents	Ref.
1	B	Difference in the IR stretching wave numbers of the free and hydrogen bonded OH-group in C_6H_5OH in CCl_4. Some LFER extensions are made, using data from similar measurements on CH_3OH and CH_3OD	All	35
2	pK_{HB}	^{19}F NMR of *p*-FC_6H_4OH in CCl_4 using *p*-$FC_6H_4OCH_3$ as an internal reference. The formation constant for the hydrogen-bonded complex *p*-FC_6H_4OH...Base is calculated. LFER extensions are applied, using calorimetric and IR data, and measurements on other alcohols.	No. 5 missing data	36
3	$\Delta\nu_D$	Difference in IR adsorption (wave number) of the OD-group in CH_3OD in pure solvent relative to that in benzene	No. 11 missing data	37
4	DN	Calorimetrically measured ΔH of the reaction $SbCl_5$ + Levis base with 1,2-dichloroethane as reference solvent	Nos. 2,4,12 missing data	38
5	$\Delta\nu_A$	Difference in the IR wave number of the C=O absorption in $C_6H_5COCH_3$, in the pure solvent relative to that in benzene	Nos. 8,10,11 missing data	37
6	AN	^{31}P NMR of $(C_2H_5)_3PO$ in pure solvents with hexane as reference solvent	Nos. 1,2,4,12 missing data	38
7	$E_T(30)$	UV/visible absorption spectroscopy. Exitation energy of a pyridinum-*N*-phenoxide betaine dye in pure solvents	No. 4 missing data	1b,c,39
8	π^*	UV/visible absorption spectroscopy. The wave numbers of seven indicators (*e.g.* 4-nitroanisole, *N,N*-diethyl-3-nitroaniline *etc.*) in pure solvents are forming a primary data set, which is extended using LFER and data from other indicators. Combinations of solvents and indicators with possible hydrogen bonding interacions are avoided	All	40
9	lg *P*	The logarithm of the octanol-water partition coefficient	Nos. 2,4,5 missing data	41
10	ε	Dielectric constant	All	standard handbooks
11	μ	Dipole moment	All	standard handbooks
12	n_D	Refractive index	All	standard handbooks

correlate these bulk solvent properties to solvation processes are frequently made.[1e]

The first four scales (Table 2) can be considered as representing solvent basicity, the next two scales as representing solvent acidity and the remaining ones as measures of "polarity".

The trend in the ^{13}C NMR chemical shifts by charging solvents, starting from TEA, is a decreased shielding in the five-membered ring and an increased shielding in the C4–C7 positions (Table 1). This trend is supposed to reflect changes in π-electron distribution caused by a varying cationic field. This proposal is supported by the observation that the average chemical shift ($\Sigma\Delta\delta/n$) is almost constant for most ethers, in spite of significant shift changes at individual positions.[25b] However, a deviating behaviour is noticed for some solvents, especially the anisotropic ones.

The ambition of this work is to derive a solvent scale which can be useful as a guide for the generation of delocalized anions. Hence, we want to exclude superimposed solvent–anion interactions. Therefore, in addition to a matrix formed by the relative chemical shift differentials referenced to internal cyclohexane, we have also analyzed a similar matrix where the $C_{5,6}$ chemical shift value was taken as an intramolecular reference (Table 3). Numerous studies in this field have confirmed that for aprotic *n*-electron donor solvents, the dominant role for the physical and chemical properties of organoalkali species can be ascribed to specific cationic solvation.[19,21] A more "polar" solvent would solvate the cation more effectively, producing an increase in the average interionic distance due to the weakening of the Coulombic attractions between anion and cation. Thus, an increased cation solvation will be the major cause for the generation of delocalized anion structures. The concentrations of *I* in the solvents 1–11 were 0.2–0.3 M, except for anisole and dioxane where, due to the limited solubility, the concentrations were 0.07 and 0.05 M, respectively. The low solubility made us exclude these two solvents in the first analysis, but they were used as test solvents for chemical shift predictions in the partial least squares analysis.

Data analysis. For a data matrix with variables i, *e.g.* chemical shifts, and objects *k*, *e.g.* solvents, the data y_{ik} can be described by the model

$$y_{ik} = \alpha_i + \sum_{a=1}^{A} \beta_{ia}\theta_{ak} + \varepsilon_{ik} \qquad (2)$$

where α_i is the variable mean, β_{ia} the loadings (corresponding to regression coefficients) and θ_{ak} are the component values corresponding to the solvent scales. The term ε_{ik} contains the remaining data variation *i.e.* model errors, and errors of measurements.

We have used the SIMCA program package for this principal component (PC) analysis. The rank of the matrix, A, is determined on a statistical basis using a cross–validation (CV) procedure.[42] A complete description of the data program package has earlier been reported.[12,43]

Initially a PC analysis was performed on the data matrix containing the ^{13}C chemical shift differentials referenced to cyclohexane, *i.e.* five variables measured in eleven solvents. A two-component model

Table 3. The ^{13}C NMR chemical shift differences between the $C_{5,6}$ signal and the four remaining signals of indenyllithium (I).

No.	Solvent	C-1,3–C-5,6	C-2–C-5,6	C-3a,7a–C-5,6	C-4,5–C-5,6
1	TEA	−24.79	−2.96	9.06	3.69
2	i-Pr_2O	−24.76	−2.79	9.09	3.89
3	Et_2O	−24.00	−0.71	12.01	4.53
4	THP	−23.55	0.14	13.54	5.06
5	DME	−22.27	1.35	14.77	5.75
6	THF	−22.38	1.53	15.70	5.60
7	AcN	−18.89	6.01	18.37	6.51
8	Pyridine	−17.99	6.61	19.34	7.05
9	DMSO	−17.59	7.22	19.10	7.08
10	DMF	−17.59	7.29	20.21	7.29
11	HMPA	−16.95	7.79	21.14	7.96
12	Anisole	−24.27	−1.71	10.24	3.94
13	Dioxane	−22.51	1.07	15.92	5.34

Table 4. Solvent θ values and F test values from principal component (PC) analysis of ^{13}C chemical shift data of indenyllithium (*I*).

No.	Solvent	Intermolecular chemical shift reference[a] θ_1	θ_2	F[c]	Intramolecular chemical shift reference[b] θ_1	θ_2	F^d
1	TEA	5.32	−0.70	1.0	9.80	−0.80	0.60
2	i-Pr_2O	5.28	−0.81	0.85	9.63	−0.82	0.45
3	Et_2O	3.19	0.00	0.34	6.08	0.22	0.94
4	THP	2.30	0.81	0.90	4.30	0.83	0.49
5	DME	1.27	0.83	0.33	2.08	0.62	1.0
6	THF	0.78	1.43	1.5	1.47	1.27	0.42
7	AcN	−2.73	−0.47	0.45	−4.69	−0.28	3.1
8	Pyridine	−3.58	−2.17	2.6	−6.15	−0.25	0.12
9	DMSO	−3.54	−0.58	1.4	−6.55	−0.85	0.48
10	DMF	−3.94	0.31	0.20	−7.33	−0.06	0.12
11	HMPA	−4.34	1.36	1.4	−8.64	0.13	3.3

[a] The chemical shifts are measured using c-hexane as reference compound. [b] The chemical shifts are referenced to the $C_{5,6}$ NMR signal of *I*. [c] *F*-test value, used in assigning the solvents to the class described by the PC model. The F values are compared to the critical value $F_{crit} = 3.0$ at the 95 % confidence level. (Degrees of freedom = 3.24). [d] $F_{crit} = 3.6$ (D.F. = 2.16).

($A = 2$) was found to be adequate by CV, accounting for 79 % of the original data standard deviation.

If the residual variance of one object (solvent) is compared with the total residual variance by means of an *F-test*, one gets a measure of how well the object is represented by the model (Table 4). It is found that pyridine is a "borderline" solvent. This is in accordance with the deviating behaviour noticed above for the anisotropic solvents. As the next step, the PC analysis was repeated using the four chemical shift variables earlier mentioned, *i.e.* having the $C_{5,6}$ carbon as an intramolecular reference. Again, a two-component model was obtained which described 94 % of the standard deviation of the four variables. This model has a reduced standard deviation compared with the model resulting from the first analysis. However, it should be noted that there is a good correlation between the θ scales of the models (Table 4).

Partial least squares analysis (PLS). A PC analysis of physicochemical data, followed by a multiple regression (MR) analysis of the latent variables θ_{ak} and biological activity data as dependent variables, has been shown to be a useful approach to multivariate data analysis problems.[44] One of the advantages of this analysis method lies in the fact that the problem using correlated variables and a limited object−variable ratio (at least 3−4 to avoid chance correlation[9]) can be eliminated.

In the PLS method, the PC analysis and the MR analysis are accomplished in one step. The method has all the advantages of an ordinary PC-MR routine, combined with a better predictive and classifying ability.[45]

The data set is divided into two blocks, one *y*-block containing the dependent variables, *e.g.* chemical shift differences, and one *x*-block containing the independent variables, *e.g.* solvent scales. The two blocks are described by one PC-like model each, (3) and (4), *cf.* eqn. (2).

$$y_{ik} = \alpha_i + \sum_{a=1}^{A} \gamma_{ia}\eta_{ak} + \varepsilon_{ik} \quad (3)$$

$$x_{jk} = \alpha_j + \sum_{a=1}^{A} \gamma_{ja}\xi_{ak} + \varepsilon_{jk} \quad (4)$$

The two blocks are related to each other by means of the latent variables η and ξ (5), where ρ is the least square regression coefficient.

$$\eta_{ak} = \rho_a\xi_{ak} + e_{ak} \quad (5)$$

This relationship is utilized in the PLS prediction model (6).

$$y_{pred.,ik} = \alpha_i + \sum_{a=1}^{A} \gamma_{ia}\rho_a\xi_{ak} \quad (6)$$

The relevance of each *x*-variable in describing the data variation of the matrix *Y* is expressed in its γ value.

Before the PLS analysis was performed, the missing values in the solvent matrix were calculated by

Table 5. Solvent component values and F-test values from partial least squares (PLS) analysis of the empirical solvent data and the chemical shift data of indenyllithium (*I*).

No.	Solvent	η^a	ξ^b	F^c
1	TEA	+9.79	+3.34	0.66
2	i-Pr_2O	+9.61	+2.59	2.3
3	Et_2O	+6.08	+2.81	0.20
4	THP	+4.31	+1.57	0.15
5	DME	+2.09	+1.03	0.13
6	THF	+1.50	+0.93	0.47
7	AcN	−4.70	−2.00	0.06
8	Pyridine	−6.15	−0.83	3.5
9	DMSO	−6.57	−4.47	4.5
10	DMF	−7.33	−2.79	0.03
11	HMPA	−8.63	−2.19	2.2

[a] Principal component of the chemical shift data. (*Cf.* θ_1 in Table 1). [b] Principal component of the empirical solvent data. [c] *F*-test value, used in assigning the solvents to the class described by the ξ model. The *F* values are compared to the critical value $F_{crit}=3.0$ (D.F. 3.27).

Table 6. γ values of the empirical solvent scales from the partial least squares analysis of chemical shift data of indenyllithium (*I*)[a].

	B	pK_{HB}	$\Delta\nu_D$	DN	$\Delta\nu_A$	AN
γ	−0.036	0.26	0.042	−0.089	0.34	0.33
	$E_T(30)$	π^*	lg*P*	ε	μ	n_D
γ	0.37	0.40	−0.31	0.34	0.38	0.25

[a] A value close to unity indicates a high relevance for the solvent scale in describing the ^{13}C chemical shift data.

Table 7. Partial least squares prediction shift data of indenyllithium (*I*) in anisole and dioxane.

No.	Solvent	η	ξ	$Y_{1,pred}$[a]	$Y_{2,pred}$	$Y_{3,pred}$	$Y_{4,pred}$
12	Anisole	+3.2	+1.3	−22.4	0.95	13.8	5.2
13	Dioxane	+3.5	+1.4	−22.6	0.65	13.5	5.1

[a] $y_{i,pred}$ represents the predicted chemical shift data (referenced to $C_{5,6}$) of *I*. (*Cf.* the measured values in Table 3.)

means of a PC model. The SIMCA program tolerates missing data in the matrix. Hence a PC model can be determined using an iterative procedure. A significant two-component model resulted from the SIMCA study of the solvent matrix. The first component dealt mostly with scales Nos. 5−11, while the second component was associated to Nos. 1−4. The refractive index was poorly described.

The PLS analysis of empirical solvent data in the *x*-matrix and chemical shifts relative to the $C_{5,6}$ signal in the *y*-matrix yielded a one-component model, explaining 58 % of the residual standard deviation in the *y*-matrix (Table 5). In this analysis the RSD in the *x*-data was reduced by 23 %. The γ values of the *x*-variables indicates that solvent scales Nos. 5−8, 10 and 11 are the most important when representing the *y*-block data, although of limited relevance (Table 6). In the second step, the *x*-variable data for anisole and dioxane were included followed by calculation of their η, ξ and *y*-values (Table 7). A comparison between the experimental shift data in the two solvents with those calculated (Tables 3, 7) shows an acceptable prediction.

Summary of results. A. The chemical shift matrix *y* is described to 80 or 94 % of the SD (5 and 4 variables, respectively) by a PC model.

B. The same chemical shift matrix *y* (4 variables) is described only to 58 % by a correlation model

with various solvent scales included as independent variables in a PLS model. Hence about 30 % of the systematic variation in y is not modelled by a relation with the solvent scale matrix x.

DISCUSSION

As mentioned earlier, it is well established that solvent coordination to the cation is of a major importance for the solvation and structure of the ion-pair. Moreover, it has been found from NMR studies, as well as from spectrophotometric studies, that the spectrum of the solvent-separated structures is mainly unaffected by a solvent change.[21] This means that solvent effects that are not affecting the anion – cation distance are of minor importance for the carbon shieldings, *i.e.* effects like solvent anisotropy, van der Waals and electric field contributions, acting on the anion.

It could possibly be argued that a ^{7}Li NMR study would be more appropriate than the present one to probe cation solvation. However, attempts to use ^{7}Li chemical shifts in this sense have been somewhat unsuccessful.[46] This can in fact be expected, since the very similar magnitude of the diamagnetic and paramagnetic screening constants for ^{7}Li allows for ring currents and other anisotropy effects to exercise a major influence on the chemical shifts.[46]

From above, one would intuitively expect some correlation between solvent basicity scales, *i.e.* Nos. 1 – 4, and the principal component(s) of the shift matrix. However, for the complete solvent matrix this is not observed, instead θ_1 which is the dominating "effect", has a moderate correlation to the "acicity" or "polarity" scales. Using the θ_1 values 1.7 and 8.0 for dioxane and anisole, respectively, the best fit to θ_1 is found for π^*, $r = 0.86$, $n = 13$. If anisole is excluded, the correlation is improved, $r = 0.96$, $n = 12$ (Fig. 1).

Future studies of other carbanions will show if the θ_1 scale has a wider generality. It can be mentioned that an identical trend, as the one indicated by our θ_1 values, has been found in an electron absorption study of 1,3-diphenylbut-1-enyllithium. The s.s.i.p. – c.i.p. ratio increased by going from from i-$Pr_2O \approx Et_2O$, dioxane, THP, THF $\approx$ DME, DMSO, HMPA.[22] The second component, θ_2, does not show any acceptable correlation to any of the empirical solvent scales, and can be considered as specific for the complex system at hand.

Another conclusion that can be reached from this study is that ion pair solvation cannot successfully be explained by a single solvent effect (θ's) nor a solvent scale derived in structurally different systems, at least not for such a variety of solvents. It has been suggested that the Brønsted pK_a values should be of utility when studying the solva-

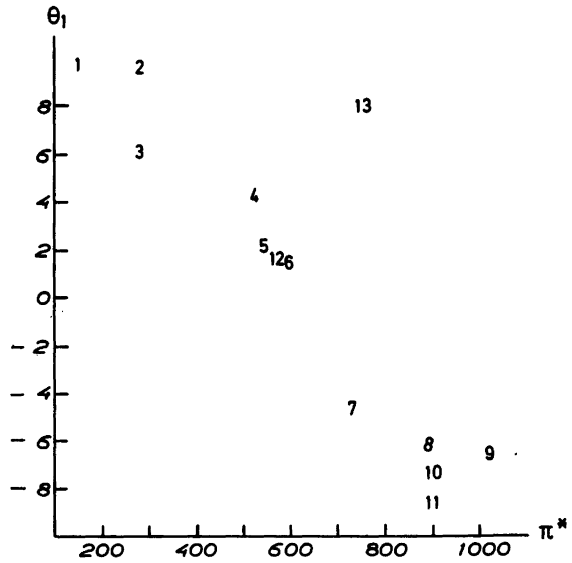


Fig. 1. Solvent θ_1 values from principal component analysis of ^{13}C NMR data of indenyllithium (*I*) are plotted against solvent π^* from solvatochromic shift data.

tion effects in various ethers.[18b] An acceptable correlation was indeed found in the ethereal subset between the θ_1 scale and a basicity scale, the pK_a scale of Arnett and Wu.[47] For the ethers 2,3,4,6,12,13 a correlation coefficient $r=0.77$ was achieved. This correlation was improved by excluding anisole, $r=0.93$.

Although *I* exists as a c.i.p. or tight ion pair in Et_2O the chemical shifts in TEA and i-Pr_2O are even more extreme. The most obvious explanation is that a different degree of "external" solvation causes a difference in the "effective" cation radius, *i.e.* a smaller positive sphere polarizes the π-cloud more strongly. Beyond cation solvation, other effects could influence the cation–anion distance such as anion solvation, π-cloud interactions and steric effects, especially when using other solvents than the saturated ethers.

To conclude, the present study confirms the complexity of the ion-pair system when it concerns the solvation of the ion pair(s). Reasonable correlations to singel solvent scales are obtained only on subsets of the original solvent matrix, which could indicate that, apart from cation solvation, solvent effects exercised on the anion also affect the anion–cation distance and thus the reflected π-polarization. These latter interactions are probably governed by other solvent characteristics than those valid for the cation solvation process and are more important for the non-etheral solvents.

Finally, we note that modern data analytic methods such as PC–CV and PLS give direct possibilities to find the systematic behaviour in multivariate data sets and to relate data sets to each other. This direct approach gives results which are easier to interpret than those obtained using indirectly derived solvent scales (and other empirical "substituent" scales) of dubious relevance for a given problem.

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Studies on Electrolytic Substitution Reactions. XXI.* Anodic Oxidation of *N,N*-Dimethyl-ω-hydroxyamides

ZOLTAN BLUM** and KLAS NYBERG***

Division of Organic Chemistry 3, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund 7, Sweden

Five *N,N*-dimethyl-ω-hydroxyamides were oxidized at a platinum anode in various solvents. No direct formation of the expected 1,3-oxazaheterocycles was observed but instead formation of *N*-methoxy-*N*-methyl-ω-hydroxyamides. The latter could in some cases (formation of 5-, 6- and 7-membered rings) easily be transformed to 1,3-oxaza-4-oxo heterocyclic systems by acid catalysis.

N-α-Alkoxylated amides are useful synthetic intermediates that are easily and inexpensively available *via* anodic alkoxylation of various types of amides.[1-5] The mechanism of anodic amide alkoxylation has so far been believed to be of the ECE type (eqns. (1) and (2)).

$$RCH_2N(R^1)CHO \xrightarrow{-e^-} [RCH_2N(R^1)CHO]^{\cdot +}$$
$$\xrightarrow{-H^+,\ -e^-} \overset{+}{R}CHN(R^1)CHO \qquad (1)$$

$$\overset{+}{R}CHN(R^1)CHO \xrightarrow{R^2OH} RCH(OR^2)N(R^1)CHO + H^+ \qquad (2)$$

With very few exceptions,[6,7] most studies have dealt with intermolecular alkoxylation, *i.e.* the alkoxy moiety has been derived from an external alcohol molecule. It thus seemed worthwhile to examine whether the intramolecular reaction, *i.e.* the formation of heterocycles, would proceed with the ease and smoothness earlier experienced with the intermolecular cases (Fig. 1).

We now report that five model amide derivatives (Fig. 1, n=0–4) *do not form cyclized products directly by anodic oxidation* in methanol but instead are *N*-α-methoxylated in the "normal" way. As expected these products can be cyclized in favorable cases (Fig. 1, n=0, 1, 2) *via* an acylimmonium ion by treatment with a catalytic amount of acid.[8] The intervention of such intermediates in the anodic process [according to eqns. (1) and (2)] thus seems rather unlikely, unless the intramolecular reaction is rendered impossible owing to the heterogeneous conditions.

*Part XX, see Eberson, L. and Webber, A. *Acta Chem. Scand. B 35* (1981) 53.

**Author to whom correspondence should be addressed.

***Present address: AB Bofors, Nobel Kemi, Box 800, S-690 20 Bofors, Sweden.

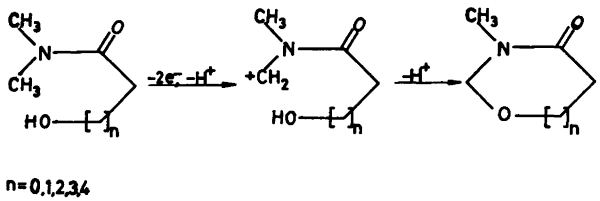

Fig. 1. Formation of heterocycles.

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RESULTS AND DISCUSSION

It would seem imperative that the formation of a cationic centre in a molecule possessing a fairly strong nucleophilic centre adequately located, ultimately would lead to ring formation (Fig. 1). Moreover the formation of a cyclized product from a properly designed starting material could indeed be taken as evidence for the presence of such a cationic centre somewhere in the reaction sequence. As the generally accepted mechanism [eqns. (1) and (2)] for anodic amide alkoxylation involves the formation and further reaction of an intermediate acylimmonium ion, the presence of an "internal" nucleophile should result in ring formation. Support for this assumption was found in the reports from Shono [6] and Ban [7] where formation of a 1,3-oxazolidine and various cyclic lactones, utilizing acyclic precursors, was achieved. As a model compound we chose 2-hydroxy-*N*,*N*-dimethylpropanamide capable of forming the six-membered ring *N*-methyl-4-oxotetrahydro-1,3-oxazine (Fig. 1, n=2). In order to circumvent competetive solvent interactions we initially tried to run the electrolysis in non-nucleophilic solvents. Surprisingly, anodic oxidation of the β-hydroxy compound in acetonitrile or dichloromethane solution failed to given even trace amounts of the desired cyclized product. GLC analysis showed complete consumption of starting material after ~2.2 F/mol and work-up demonstrated the formation of tars and resinous materials. This drawback led us to reconsider performing the electrolysis in methanol, hoping that the ring-forming reaction would be able to capitalize from the kinetic advantage of a monomolecular reaction. On anodic oxidation in methanol GLC analysis revealed the formation of a single product in high yield. Work-up and subsequent MS and ^{1}H NMR examination unambiguously demonstrated the formation of *N*-methoxymethylene-*N*-methyl-2-hydroxypropanamide. This remarkable finding was further emphasized by the demonstration of prompt cyclization of *N*-methoxymethylene-*N*-methyl-2-hydroxypropanamide to *N*-methyl-4-oxotetrahydro-1,3-oxazine in dichloromethane in the presence of either trifluoroacetic acid or Armberlyst 15 (eqns. (3) and (4), n=1).

$$CH_3OCH_2N(CH_3)CO(CH_2)_nCH_2OH \xrightarrow[-CH_3OH]{H^+} {}^+CH_2N(CH_3CO(CH_2)_nCH_2OH \quad (3)$$

$${}^+CH_2N(CH_3)CO(CH_2)_nCH_2OH \xrightarrow[-H^+]{} \begin{matrix} CH_2N(CH_3)CO \\ | \quad\quad | \\ O—CH_2—(CH_2)_n \end{matrix} \quad (4)$$

n=0, 1, 2

Evidently the cation formed by acid catalysis [eqn. (3)] was immediately trapped by the β-hydroxy group [eqn. (4)] suggesting either that no cation is formed during electrolysis or that if the cation is formed it is effectively blocked by solvent molecules. Another plausible explanation as to why the β-hydroxy group fails to engage in the cyclization step during electrolysis is that it for some reason becomes nonreactive due to the electrolysis conditions. A possible rationalization of the above-mentioned results would then have to involve anode surface adsorption phenomena. If one assumes that the amide is adsorbed at the electrode by the amide oxygen and the β-hydroxy oxygen, the nitrogen-alkyl moiety reaching out into the solution, the *N*-α cation formed would be an easy prey to surrounding solvent molecules (Fig. 2a). Further support of this interpretation is provided by known cases of direct anodic cyclization of methyl *N*-(2-

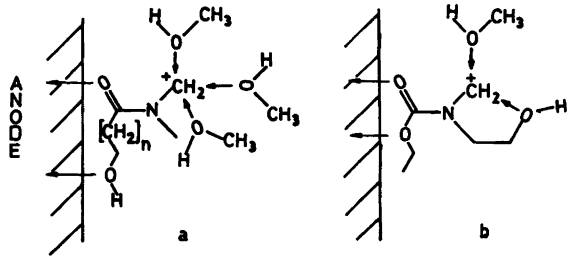

Fig. 2. Anode adsorption.

hydroxyethyl)-*N*-methylcarbamate[6] and 2-piperidone-5-acetic acid.[7] In both cases the internal nucleophile was located on the nitrogen side of the molecule. This would allow for successful competition for the cation formed (Fig. 2b).

The possibility of acid-catalyzed ring-opening/solvolysis caused by acidic species formed at the anode during electrolysis was recognized but seemed improbable. As no special precautions were taken to achieve dry reaction conditions the small concentration of water inevitably present would interfere in the solvolysis process and produce the demethylated analogue of the starting material.[9] Since secondary amides are known to be more oxidation resistant than tertiary ones,[10] the demethylated amide would accumulate and ultimately be detected at the end of the electrolysis. This was not the case. Moreover, the very high yields usually achieved in anodic alkoxylations contradict the involvement of solvolytic or/and hydrolytic processes.

In an effort to investigate whether the disinclination to cyclize was a structural feature of the chosen probe molecule we examined the behavior of the lower and some of the higher homologues. Thus, hydroxy-*N,N*-dimethylacetamide was electrolyzed according to the procedure adopted for 2-hydroxy-*N,N*-dimethylpropanamide. After 2.3 F/mol of substrate electrolysis was discontinued, and GLC analysis indicated the complete consumption of starting material and the formation of a single product. MS and NMR revealed its structure to be 2-hydroxy-*N*-methoxymethylene-*N*-methylacetamide. No trace of the cyclized *N*-methyl-4-oxo-1,3-oxazolidine could be detected in the reaction mixture. The homogeneous cyclization to the oxazolidine was not as easy an achievement as the formation of the above-mentioned oxazine. The oxazolidine, apparently being acid-sensitive, either failed to form or rapidly decomposed depending on the acid used. This obstacle was circumvented by the utilization of the Lewis acid boron trifluoride, promoting the formation of an oxazolidine $-BF_3$ complex in high yield [eqns. (3) and (4), n = 0].

4-Hydroxy-*N,N*-dimethylbutanamide was electrolyzed according to the procedure stated above. The cyclized compound, *N*-methyl-4-oxohexahydro-1,3-oxazepine, could not be detected in the electrolyte. The only new compound present was the readily formed 4-hydroxy-*N*-methoxymethylene-*N*-methylbutanamide. This compound readily cyclized to the oxazepine when treated with trifluoroacetic acid or Amberlyst 15 [eqns. (3) and (4), n = 2].

5-Hydroxy-*N,N*-dimethylpentanamide and 6-hydroxy-*N,N*-dimethylhexanamide were treated in the manner described above. The formation of *N*-methyl-4-oxoperhydro-1,3-oxazocine and *N*-methyl-4-oxoperhydro-1,3-oxazonine was not observed but merely *N*-α methoxylation giving 5-hydroxy-*N*-methoxymethylene-*N*-methylpentanamide and 6-hydroxy-*N*-methoxymethylene-*N*-methylhexanamide, respectively. Taking into account the known resistance towards forming rings larger than seven-membered, this was not surprising. Treatment of the methoxy compounds with various Brönsted and Lewis acids in various solvents and applying the high dilution approach failed to give even trace amounts of the perhydrooxazocine or perhydrooxazonine derivative.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Jeol MH 100 instrument, using $CDCl_3$ as solvent. GLC–MS analyses were obtained on a Finnegan 4021 spectrometer operating at 70 eV. GLC analyses were performed on an HP-5830A gas chromatograph fitted with an HP-18850A recorder/integrator. Columns used were 3 m × 3 mm 5 % OV 17 on Chromosorb W or 2 m × 3 mm 5 % NPGS on Chromosorb W. Methanol was of AnalR quality and was used as received. Tetrabutylammonium tetrafluoborate was prepared according to the method of Nyberg.[11] Trifluoroacetic acid, Amberlyst 15 and boron trifluoride etherate were used as received.

3-Hydroxy-*N,N*-dimethylpropanamide,[12] 4-hydroxy-*N,N*dimethylbutanamide[13] and 6-hydroxy-*N,N*-dimethylhexanamide[14] were prepared according to published procedures.

Hydroxy-N,N-*dimethylacetamide* was prepared by refluxing butyl glycolate (66.0 g, 0.5 mol) with 45.0 g (1 mol) of dimethylamine in 250 ml of toluene overnight. After evaporation of the solvent the residue was distilled giving a colorless liquid, b.p. 79–81 °C/3 mmHg, which solidified in the receiver, colorless crystals m.p. 33–34 °C, yield 43.6 g, 83 %. MS *m/e* (% rel int): 104 (M + 1, 7), 103 (M, 21), 72 (100). ^{1}H NMR: 2.74 (3 H, s), 3.00 (3 H, s), 3.70 (1 H, br s), 4.10 (2 H, s).

5-Hydroxy-N,N-*dimethylpentanamide*. The method of Powers *et al.*[14] was adopted. Thus, 50.0 g (0.5 mol) of valerolactone was added to a cooled (ice) Parr bottle. Gaseous dimethylamine, 100 g (2.2 mol) was condensed into the bottle, which was stoppered and was allowed to reach room temperature. After one week the bottle was cooled to 0 °C,

the stopper removed and the excess amine was evaporated by means of a flow of nitrogen. The residue was distilled giving a colorless liquid, b.p. 132–133 °C/1 mmHg. Yield 65.1 g, 90 %. MS *m/e* (% rel int): 145 (M, 3), 127 (11), 100 (15), 87 (21), 72 (53), 55 (42), 45 (100). ^{1}H NMR: 1.40–1.85 (4 H, m), 2.35 (2 H, t), 2.92 (3 H, s), 3.03 (3 H, s), 3.59 (2 H, t), 4.16 (1 H, br s).

Electrolyses were conducted in a 250 ml water-jacketed cell and stirred magnetically. Electrolytes were 1.0 M in substrate and 0.1 M in supporting electrolyte. Platinum was used as anode material and stainless steel as cathode. Current was passed by means of an Amel 552 potentiostat/galvanostat, operated in the galvanostatic mode and the amount of charge was monitored by an electronic integrator. The current density was maintained at 50 mA/cm^2.

Typical oxidation procedure: 3-Hydroxy-*N,N*-dimethylpropanamide (23.4 g, 0.2 mol) was dissolved in 200 ml of methanol. Bu_4NBF_4 (6.58 g, 0.02 mol) was added and the resulting solution was transferred to the electrolysis cell. A platinum foil anode and a a stainless steel rod cathode were immersed. Current was passed and the current density was adjusted to a constant 50 mA/cm^2. The consumption starting material was monitored by GLC. When the starting material was totally consumed equalling 2.4 F/mol of substrate, electrolysis was stopped. Methanol was evaporated and the residue transferred to a Claisen apparatus and distilled *in vacuo*. Yield 25.6 g, 87 %, b.p. 99–100 °C/1 mmHg of 3-hydroxy-*N*-methoxymethylene-*N*-methylpropanamide. MS *m/e* (% rel int): 148 (M+1, 2), 147 (M, 1), 132 (9), 79 (19), 60 (56), 45 (100). ^{1}H NMR: 2.29–2.42 (2 H, br t, $-COCH_2-$), 3.04 (3 H, 2 s, CH_3-N), 3.22, 3.29 (3 H, 2 s, CH_3-O), 3.42–3.78 (2 H, m, $-CH_2-OH$), 3.80 (1 H, br s, HO–), 4.71, 4.76 (2 H, 2 s, NCH_2O).

Hydroxy-N-methoxymethylene-N-methylacetamide. Yield 73 %, b.p. 92–94 °C/2.5 mmHg. MS *m/e* (% rel int): 134 (M+1, 2), 133 (M, 2), 131 (11), 118 (21), 100 (27), 45 (100). ^{1}H NMR: 2.92, 3.05 (3 H, 2 s, CH_3-N), 3.30 (3 H, s, CH_3-O), 3.80 (1 H, br s, HO–), 4.18, 4.27 (2 H, 2 s, $-COCH_2-$), 4.58, 4.83 (2 H, s, NCH_2O).

4-Hydroxy-N-methoxymethylene-N-methylbutanamide. Yield 78 %, b.p. 114–116 °C/0.3 mmHg, MS *m/e* (% rel int): 162 (M+1, 2), 161 (M, 1), 146 (7), 87 (21), 60 (78), 45 (100). ^{1}H NMR: 1.53–2.03 (2 H, m, $-CH_2-$), 2.39–2.66 (2 H, m, $-COCH_2-$), 2.99, 3.06 (3 H, 2 s, CH_3-N), 3.24, 3.30 (3 H, 2 s, CH_3-O), 3.39–3.75 (2 H, 2 t, $-CH_2OH$), 3.90 (1 H, br s, HO–), 4.72, 4.78 (2 H, 2 s, NCH_2O).

5-Hydroxy-N-methoxymethylene-N-methylpentaamide. Yield 69 %, b.p. 115–117 °C/0.1 mmHg. MS *m/e* (% rel int): 176 (M+1, 2), 160 (2), 144 (5), 101 (23), 83 (10), 74 (10), 60 (61), 55 (52), 45 (100). ^{1}H NMR: 1.39–1.93 (4 H, m, $-CH_2CH_2-$), 2.26–2.62 (2 H, m, $-COCH_2-$), 2.95, 2.98 (3 H, 2 s, CH_3N), 3.22, 3.28 (3 H, 2 s, CH_3-O), 3.49–3.91 (2 H, br t, $-CH_2-OH$), 3.70 (1 H, br s, HO–), 4.66, 4.75 (2 H, 2 s, NCH_2O).

6-Hydroxy-N-methoxymethylene-N-methylhexanamide. Yield 81 %, b.p. 137–139 °C/0.2 mmHg. MS *m/e* (% rel int): 190 (M+1, 2), 189 (M, 1), 174 (8), 115 (17), 69 (43), 60 (63), 55 (43), 45 (100). ^{1}H NMR: 1.39–2.05 (6 H, m, $-CH_2CH_2CH_2-$), 2.41–2.74 (2 H, m, $-COCH_2-$), 3.14, 3.17 (3 H, 2 s, CH_3-N), 3.41, 3.47 (3 H, 2 s, CH_3-O), 3.65–4.01 (3 H, m, $-CH_2-OH$), 4.68, 4.77 (2 H, 2 s, NCH_2O).

General procedure for the preparation/attempted preparation of cyclic derivatives of the methoxylated ω-hydroxyamides illustrated by the synthesis of *N-methyl-4-oxotetrahydro-1,3-oxazine:* (a) 3-Hydroxy-*N*-methoxymethylene-*N*-methylpropanamide (I) (25 g, 0.17 mol) was added to a solution of dichloromethane–trifluoroacetic acid, 200 ml (95:5, w/w). The resulting solution was stirred for 15 min and was then treated with saturated $NaHCO_3$ until neutral. The organic layer was separated and dried over $MgSO_4$. After removal of the solvent the residue was distilled through an efficient column giving 18.8 g of a colorless liquid, b.p. 67–68 °C/1 mmHg, yield 96 %. (b) 1.47 g (10 mmol) of (I) was added to a stirred suspension of 0.2 g Amberlyst 15 in 25 ml dichloromethane. After 15 min the solution was filtered and then evaporated. The crude oil was pure by NMR, yield 1.12 g, 98 %. MS *m/e* (% rel int): 115 (M, 47), 114 (50), 55 (100). ^{1}H NMR: 2.51–2.64 (2 H, t, $-COCH_2-$), 2.90 (3 H, s, CH_3-N), 3.93–4.05 (2 H, t, $-OCH_2-$), 4.73 (2 H, s, NCH_2O).

N-Methyl-4-oxohexahydro-1,3-oxazepine. Attempted distillation led to decomposition. The crude oil received after removal of the solvent was better than 95 % pure as demonstrated by GLC and NMR, yield 94 %. MS *m/e* (% rel int): 129 (M, 100), 114 (31), 98 (47), 87 (50), 55 (56). ^{1}H NMR: 1.78–2.09 (2 H, m, $-CH_2-$), 2.84–3.10 (2 H, t, $-COCH_2-$), 3.18 (3 H, s, CH_3-N), 4.08–4.22 (2 H, t, $-OCH_2-$), 5.09 (2 H, s, $-NCH_2O-$).

N-Methyl-4-oxo-1,3-oxazolidine. This compound could not be prepared by the above- mentioned general procedure but instead by treatment of hydroxy-*N*-methoxymethylene-*N*-methylacetamide (0.332 g, 0.0025 mol) with 0.355 g (0.0025 mol) of boron trifluoride etherate in 5 ml of dichloromethane. After stirring for 15 min at room temperature the solvent was removed and the semisolid residue was transferred to a sublimation apparatus. After treatment at 40–50 °C (care must be taken not to let the temperature exceed 50 °C whereupon excessive degradation sets in) and 0.05 mmHg colorless crystals (m.p. 132 °C dec.) could be collected. The yield was 0.296 g representing more

than 100 % of the theoretical and thus indicative of the formation of a BF_3 complex. This was confirmed by dissolution in water and titration of the resulting acidic solution with aqueous sodium hydroxide. Any attempt to isolate the oxazolidine in the pure state resulted in total decomposition of the organic moiety. On GLC–MS and ^{1}H NMR analysis the BF_3 was removed by vaporization and complexation with the solvent (DMSO-d_6), respectively, and thus behaved as the free oxazolidine. MS *m/e* (% rel int): 101 (M, 53), 100 (100). ^{1}H NMR (DMSO-d_6): 2.73 (3 H, s, CH_3-N), 4.10 (2 H, m, $-COCH_2-$),[15] 4.97 (2 H, br t, NCH_2O).[15]

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Kinetics and Mechanism of the Second Order Cyclization of the Cation Radicals Derived from 1,2-Diarylethanes

BJØRG AALSTAD,[a] ALVIN RONLÁN[b] and VERNON D. PARKER[a]

[a] Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and [b] Division of Organic Chemistry 2, Lund Institute of Technology, 220 07 Lund, Sweden

The cyclization of the cation radical derived from 1,2-bis(3,4-dimethoxyphenyl)ethane in dry acetonitrile or in acetonitrile containing trifluoroacetic acid was observed to follow rate law (i) which is

$$\text{Rate} = k_{app}[\text{Ar}-\text{CH}_2\text{CH}_2-\text{Ar}^{\cdot+}]^2 \quad \text{(i)}$$

consistent with either disproportionation (ii) followed by cyclization (iii) or cyclization (iv) followed by rate-determining electron transfer (v). A secondary deuterium kinetic isotope effect was detected when the ring protons were exchanged with deuterium. It was concluded that the most likely mechanism for the cyclization is mechanism (iv) – (v) with rate-determining electron transfer (v).

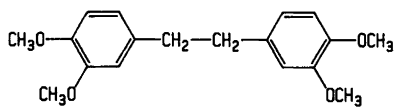
$$2\,\overset{\frown}{\text{Ar}}\,\text{Ar}^{\cdot+} \overset{k_{ii}}{\rightleftharpoons} \overset{\frown}{\text{Ar}^{\cdot+}}\text{Ar}^{\cdot+} + \overset{\frown}{\text{Ar}}\,\text{Ar} \quad \text{(ii)}$$

$$\overset{\frown}{\text{Ar}^{\cdot+}}\text{Ar}^{\cdot+} \longrightarrow \text{products} \quad \text{(iii)}$$

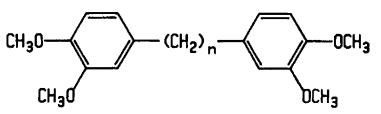
$$\overset{\frown}{\text{Ar}}\,\text{Ar}^{\cdot+} \rightleftarrows \overset{\frown}{\text{Ar}^{+}}\,\text{Ar}^{\cdot} \quad \text{(iv)}$$

$$\overset{\frown}{\text{Ar}^{+}}\,\text{Ar}^{\cdot} + \overset{\frown}{\text{Ar}}\,\text{Ar}^{\cdot+} \overset{k_v}{\rightarrow} \overset{\frown}{\text{Ar}^{+}}\,\text{Ar}^{+} + \overset{\frown}{\text{Ar}}\,\text{Ar} \quad \text{(v)}$$

The cyclization of the cation radical (3,4-dimethoxyphenyl)ethane (*1*) was one of the first examples reported[1] of the general reaction, oxidative intramolecular coupling of alkoxy-substituted biarylalkanes.[1-30] Most of the mechanistic work has been based upon product studies and cyclic voltammetric evidence. Symmetrically substituted compounds (*2*) were observed to undergo intramolecular cyclization while unsymmetrical ones (*3*) gave intermolecular dimers (*4*).[2] Potential step-sweep voltammetry experiments, supported by the product studies, indicated that the intramolecular cyclization of *2* involved the dication – diradical as an intermediate. It was suggested that the methylene groups separating the two aryl moeities have an insulating effect so that the potential difference,



1



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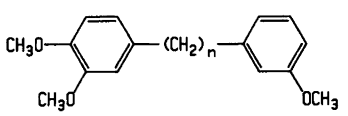

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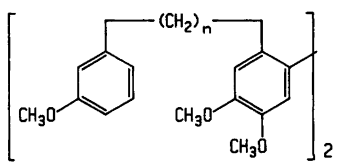

4

$$\overset{\frown}{\text{Ar}}\,\text{Ar} \overset{E_1}{\rightleftharpoons} \overset{\frown}{\text{Ar}}\,\text{Ar}^{\cdot+} + e^- \quad (1)$$

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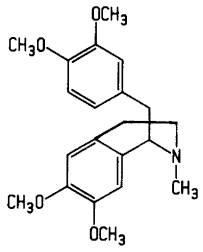
$$\overset{\frown}{\mathrm{Ar}}\ \overset{\frown}{\mathrm{Ar}}^{\cdot+} \overset{E_2}{\rightleftarrows} \overset{\frown}{\mathrm{Ar}}^{\cdot+}\overset{\frown}{\mathrm{Ar}}^{\cdot+} + e^- \quad (2)$$

$E_2 - E_1$, is small for symmetrically substituted compounds (*2*).[2] In the case of unsymmetrically substituted (*3*) the aryl moiety with two methoxy groups is oxidized about 0.5 V more easily than the anisyl group. The cation radicals of (*3*) were observed to undergo intermolecular coupling to give (*4*).

More recently, linear sweep voltammetry (LSV) and convolution potential sweep voltammetry (CPSV) have been applied to the study of the mechanism of the cyclization of two compounds related to (*1*), (*5*) and (*6*).[30] On the basis of the voltammetric results, different mechanisms were



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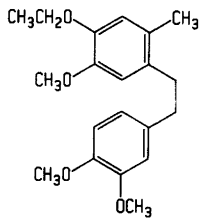


6

postulated for the oxidative cyclization of (*5*) and (*6*). In the acidic media employed (*5*) is protonated on nitrogen ($5-H^+$). The mechanism in this case was suggested to consist of reactions (3)–(6) which would give rise to rate law (7). Rate law (7) leads

$$(5-H^+) \rightleftarrows (5-H^+)^{\cdot+} + e^- \quad (3)$$

$$(5-H^+)^{\cdot+} \overset{K_4}{\rightleftarrows} (C-H^+)^{\cdot+} \text{ (C indicates cycle)} \quad (4)$$

$$(C-H^+)^{\cdot+} \overset{K_5}{\rightleftarrows} C^{\cdot+} + H^+ \quad (5)$$

$$C^{\cdot+} + (5-H^+)^{\cdot+} \xrightarrow{k_6} \text{products} \quad (6)$$

$$\text{Rate} = k_6 K_4 K_5 [(5-H^+)^{\cdot+}]^2/[H^+] \quad (7)$$

to the prediction that $dE^p/d\log v = (\ln 10)\, RT/3F$ and $dE^p/d\log C_A = 0$ during LSV mechanism analysis. The independence of the peak potential on the substrate concentration can be derived from equation (8) where a, b and i are the reaction orders in

$$dE^p/d\log C_A = (a+b+i-1)/(b+1)\,(\ln 10)RT/F \quad (8)$$

substrate, cation radical and protons, respectively.[31] An apparent inconsistency in the analysis is that the voltammetric experiments were carried out in strongly acidic media and $|H^+|$ is expected to be constant under these conditions. This inconsistency was commented on in a footnote and the implication was made that the region near the electrode is not buffered since protons are produced in the reaction. It is, of course, possible that the acidity in the double layer is enhanced but it must be kept in mind that the reaction in question is second order in cation radical and even if k_6 is a diffusion controlled second order rate constant, the reaction layer extends far beyond the immediate vicinity of the electrode. In our opinion, this is adequate reason for rejecting rate law (7) as accounting for the observed LSV results.

A simple disproportionation mechanism (9)–(10) with rate limiting cyclization was postulated to account for the oxidative cyclization of *6*. This

$$2\,6^{\cdot+} \overset{K_9}{\rightleftarrows} 6^{2+} + 6 \quad (9)$$

$$6^{2+} \xrightarrow{k_{10}} \text{products} \quad (10)$$

mechanism gives rise to rate law (11) and the same

$$\text{Rate} = k_{10} K_9 [6^{\cdot+}]^2/[6] \quad (11)$$

LSV predictions as for (7).

The reason that different mechanisms were postulated for the two reactions, in spite of very similar LSV behaviour, is that the CPSV analyses differed. Two factors should be considered in the evaluation of the latter data. The first is that the systems do not give ideal response and the voltammograms do not have the theoretical shapes necessary for the analysis. This is evident from Fig. 3 of Ref. 30. The second is that the calculations[32,33] upon which the CPSV predictions were made were derived for much simpler systems where the overall wave consists of the transfer of two electrons. During the oxidative cyclization of *5* and *6* and *2* in general, the overall reaction at the LSV wave involves the transfer of four electrons resulting in the dication of the cyclized product.[2,34] This added complication would surely have some effect on the wave shape. Thus, we feel that there is adequate reason to doubt the validity of the conclusions based upon the CPSV analyses in this case.

A further point which should be considered is

that it is not possible to distinguish between cyclization reaction sequences (12)–(13) and (14)–(16) by reaction orders, which is essentially the information

$$2\,\overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}}^{\cdot+} \rightleftarrows \overset{\frown}{\mathrm{Ar}}^{\cdot+}\overset{\frown}{\mathrm{Ar}}^{\cdot+} + \overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}} \qquad (12)$$

$$\overset{\frown}{\mathrm{Ar}}^{\cdot+}\overset{\frown}{\mathrm{Ar}}^{\cdot+} \longrightarrow \overset{\frown}{\mathrm{Ar}}\overset{\pm}{-}\mathrm{Ar}^{+} \qquad (13)$$

$$\overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}}^{\cdot+} \rightleftarrows \overset{\frown}{\mathrm{Ar}}\overset{\pm}{-}\mathrm{Ar}^{\cdot} \qquad (14)$$

$$\overset{\frown}{\mathrm{Ar}}\overset{\pm}{-}\mathrm{Ar}^{\cdot} + \overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}}^{\cdot+} \rightleftarrows \overset{\frown}{\mathrm{Ar}}\overset{\pm}{-}\mathrm{Ar}^{+} + \overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}} \qquad (15)$$

$$\overset{\frown}{\mathrm{Ar}}\overset{\pm}{-}\mathrm{Ar}^{+} \longrightarrow \text{products} \qquad (16)$$

available from the LSV wave,[31] since both give rate law (17).

$$\text{Rate} = k_{\text{app}}[\overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}}^{\cdot+}]^2/[\overset{\frown}{\mathrm{Ar}}\;\overset{\frown}{\mathrm{Ar}}] \qquad (17)$$

A possible way to distinguish between the two reaction sequences is to test for a secondary deuterium kinetic isotope effect when the ring protons are replaced by deuterium. Such an effect has recently been demonstrated in a related case, the dimerization of 4-methoxybiphenyl cation radical.[35] The secondary deuterium kinetic isotope effect arises when the carbon atoms attached to the isotopes undergo a change in hybridization,[36] sp^2 to sp^3 in this case. The disproportionation scheme (12)–(13) in the case where electron transfer (12) is rate-determining is not expected to show the kinetic isotope effect while the other mechanism (14)–(16) could when either (14) or (15) are rate-determining.

In this paper we report the results of an LSV kinetic study of the oxidative cyclization of *1*.

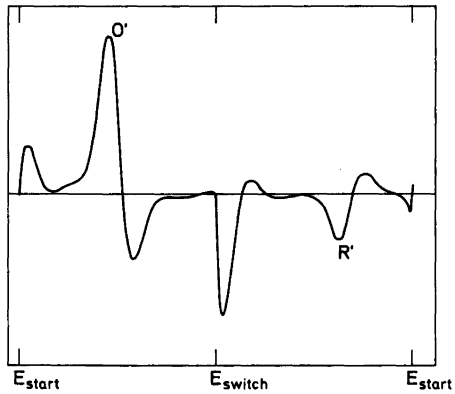


Fig. 1. Derivative cyclic voltammogram for the oxidation of (3,4-dimethoxyphenyl)ethane in acetonitrile–trifluoroacetic acid (19/1) containing Bu_4NBF_4 (0.1 M) at 243 K and 80 V/s. $E_{switch} - E_{start} = 900$ mV.

RESULTS AND DISCUSSION

Cyclic voltammetric oxidation of 1. The derivative cyclic voltammogram observed during the oxidation of *1* in acetonitrile–trifluoroacetic acid (AN/TFA, 19/1) containing Bu_4NBF_4 (0.1 M) at a voltage sweep rate (v) of 80 V/s at a platinum electrode is illustrated in Fig. 1. Similar voltammetric behaviour was observed when the solvent was AN. The potential difference between O' and R' was observed to be 265 mV at −30 °C. Normalized potential sweep voltammetry (NPSV),[37] which gives a direct comparison of the experimental LSV wave with theoretical data, gave a slope of 1.009 ± 0.004 when experimental data measured under the conditions given above were

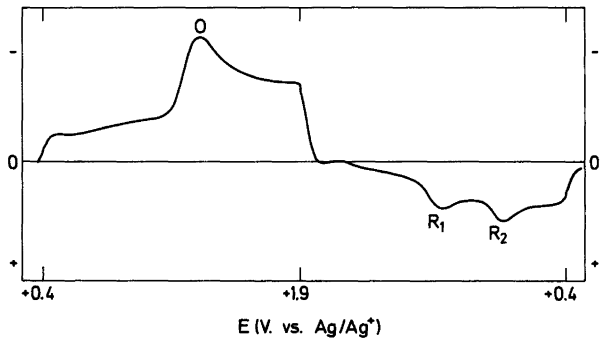
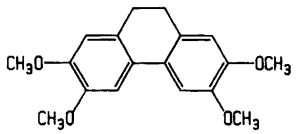


Fig. 2. Cyclic voltammogram for the oxidation of *1* in AN/TFA (19/1) containing Bu_4NBF_4 (0.1 M) at 100 V/s and −30 °C at a platinum electrode.

correlated as the Y axis *vs.* theoretical data for Nernstian charge transfer as the X axis. This analysis rules out the possibility that O′−R′ is due to a quasi-reversible oxidation of *1*. The NPSV slope for a quasi-reversible process with a cyclic voltammetric peak separation of 275 mV is much greater than unity.[38] An indication of the nature of the process giving rise to R′ can be obtained from the cyclic voltammogram measured at 100 V/s over an extended potential range illustrated in Fig. 2. On the reverse scan two reduction peaks, R_1 and R_2, are evident. The first, R_1, corresponds to R′ in Fig. 1. A comparison with voltammograms already published[2,34] showed that R_1 and R_2 are due to the reduction of the dication and cation radical, respectively, of the cyclized product (7).



7

LSV kinetic study. Preliminary experiments carried out in AN which had been passed through neutral alumina indicated that $dE^p/d\log v$ was of the order of 30 mV/decade. Under similar conditions it has been found that the yield of cyclized products is low.[30] When the water concentration is kept to a very low level by carrying out the voltammetry experiments in AN in the presence of alumina[39] the reaction appears to approach second order kinetics in cation radical. Data measured under these conditions are summarized in Table 1. Each peak potential listed is the mean of five measurements and the numbers in parentheses are the standard deviations. The precision, even at the exceedingly low concentrations (0.05 mM), is noteworthy. The last column gives $dE^p/d\log v$ obtained by linear regression analysis of the peak potential data. The last row of data gives the corresponding values of $dE^p/d\log C_A$. The data can be analyzed using the LSV equations (8) and (18). For a reaction second order in $1^{\cdot+}$, application of (18)[31] results in

$$dE^p/d\log v = 1/(b+1)(\ln 10)RT/F \qquad (18)$$

$dE^p/d\log v$ equal to 19.3 mV/decade and assuming that a and i are 0, eqn. (8) predicts $dE^p/d\log C_A$ to be −19.3 mV/decade. Solving (8) and (18) simultaneously for b then results in a value of 1.65. This result, along with the fact that the reaction tends to first order in $1^{\cdot+}$ when water is less rigorously excluded, suggests that the kinetics correspond to competing reaction mechanisms, the major one (~83%) being second order and the minor component (~17%) first order in $1^{\cdot+}$.

The results in the previous paragraph suggested that acetonitrile containing TFA to reduce the water activity[40] would be a more suitable medium to study the cyclization kinetics in. Results of measurements in AN/TFA(19/1) are summarized in Tables 2 and 3 carried out at 18 and 0 °C, respectively. Some drift in the reference electrode was observed in this solvent system. The most convenient method to determine the LSV slopes under these conditions was found to be to switch back and forth between sweep rates of 100 and 1000 mV/s. The sequence of measurement used was v

Table 1. Derivative linear sweep voltammetry analysis of the kinetics of the cyclization of $1^{\cdot+}$ in acetonitrile.[a]

	E^p at v/mV s^{-1}				
C_A/mM[b]	100	200	400	1000	$dE^p/d\log v$[c]
0.05	227.9(0.3)	234.3(0.4)	241.0(0.5)	250.3(0.5)	22.4
0.10	224.4(0.1)	231.5(0.2)	238.4(0.3)	249.0(0.3)	24.5
0.20	221.5(0.1)	227.0(0.1)	233.8(0.1)	244.0(0.3)	22.6
0.40	216.6(0.0)	221.5(0.1)	228.2(0.2)	237.7(0.2)	21.3
0.80	–	215.7(0.1)	222.4(0.3)	231.9(0.2)	23.2
$dE^p/d\log C_A$[c]	−12.2	−15.7	−15.7	−16.0	

[a] Measurements at 18 °C at a platinum electrode in solvent containing Bu_4NBF_4 (0.1 M) in the presence of neutral alumina. The peak potentials are expressed in mV *vs.* a potentiostat bias setting of +1.000 V relative to an Ag/Ag^+ reference electrode. [b] Substrate concentration. [c] Expressed in mV/decade.

Table 2. Derivative linear sweep voltammetry analysis of the kinetics of the cyclization of $1^{\cdot+}$ in AN/TFA at 18 °C.[a]

	$dE^p/d \log v$[b]				
C_A/mM	1	2	3	4	Ave.
0.05	20.2	22.0	20.7	18.9	20.5(1.3)
0.10	22.9	20.2	20.9	23.6	21.9(1.6)
0.20	20.8	22.3	21.0	19.5	20.9(1.1)
0.40	21.8	19.6	20.6	22.8	21.2(1.4)
0.80	18.1	19.1	17.6	16.6	17.9(1.0)
$dE^p/d \log C_A$[c]	−21.6	−23.6	−20.9	−23.6	−22.4(1.4)

[a] Solvent ratio (19/1) containing Bu_4NBF_4 (0.1 M). Measurements at a platinum electrode. [b] The difference in potentials measured at 100 and 1000 mV s^{-1} expressed in mV/decade. [c] Expressed in mV/decade.

Table 3. Derivative linear sweep voltammetry analysis of the kinetics of the cyclization of $1^{\cdot+}$ in AN/TFA at 0 °C.[a]

	$dE^p/d \log v$[b]				
C_A/mM	1	2	3	4	Ave.
0.05	18.5	16.6	17.8	19.7	18.2(1.3)
0.10	16.2	16.6	17.2	16.8	16.7(0.4)
0.20	16.2	18.9	14.9	17.6	16.9(1.7)
0.40	14.3	15.2	15.1	14.2	14.7(0.5)
$dE^p/d \log C_A$[c]	−18.1	−17.8	−19.8	−17.2	−18.2(1.1)

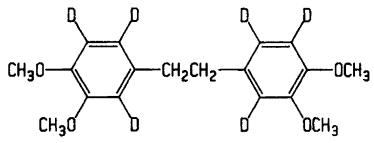
[a] Solvent ratio (19/1) containing Bu_4NBF_4 (0.1 M). Measurements at a platinum electrode. [b] The difference in potentials measured at 100 and 1000 mV s^{-1} expressed in mV/decade. [c] Expressed in mV/decade.

equal to 100 then 1000 then 100 then 1000. The numbers in column 1 of both Table 2 and Table 3 are the differences in E^p between the first 100 and the first 1000 mV/s measurement. Column 2 is for the first 1000 and the second 100, column 3 for the second 100 and the second 1000, column 4 is the difference in E^p for the first 100 and the second 1000 mV/s meaurement. Each E^p used in the analysis was the mean of 5 measurements. The somewhat larger than usual standard deviations listed in the average column reflects the slow reference potential drift during the measurements. At 0 °C the numerical values of $dE^p/d \log v$ and $dE^p/d \log C_A$ are +18.0 and −18.0 mV/decade when b is 2 and a and i are 0 as is evident from eqns. (8) and (18). The observed values were +16.6(1.4) and −18.2(1.1), respectively, both within experimental error of the theoretical values (Table 3). The slopes obtained at 18 °C were +20.5(1.5) and −22.4(1.4) which are slightly greater that the theoretical values, +19.3 and −19.3, for $dE^p/d \log v$ and $dE^p/d \log C_A$, respectively. The larger deviation in the latter value is probably a consequence of the reference potential drift mentioned earlier.

Analysis for a possible secondary deuterium kinetic isotope effect. The LSV kinetic results presented above indicate that in AN/TFA (19/1) the cyclization of $1^{\cdot+}$ follows rate law (19). This is the situation

$$\text{Rate} = k_{app}[1^{\cdot+}]^2 \qquad (19)$$

where it may be possible to differentiate between cyclization mechanism (12)−(13) and (14)−(16) by the secondary deuterium kinetic isotope effect as was described earlier. For this analysis, compound *8* was prepared by exchanging the ring hydrogens of *1* with deuterium in trifluoroacetic acid (d) containing trifluoromethanesulfonic acid.



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At any given value of v, deuterium kinetic isotope effects (k_H/k_D) can be calculated from the difference in E^p observed for the H and D isomers using eqn. (20).[41] Thus, for k_H/k_D of 0.7 and 0.9, negative shifts

$$\Delta E^p = \log(k_H/k_D)\, dE^p/d\log v \qquad (20)$$

in E^p of 3.1 and 0.9 mV are expected for a reaction order in cation radical of 2 at 298 K.

Ordinarily, the measurements of a 1 mV peak potential difference in two different solutions does not represent any special problem with the precision that we are able to attain.[42] However, the reference potential drift that we observe in the AN/TFA solutions make this type of measurement rather uncertain. In order to attempt to detect the effect, pairs of solutions containing *1* and *8* in concentrations identical within experimental error were made up. The accuracy of the concentrations is important since the peak potenatils shift linearly with $\log C_A$. After removing the electrodes from one solution and placing them in another, several minutes are usually required for the potential to settle down to a constant value under ordinary conditions. In this particular case, the initial rapid change in potential with time settled down in a few minutes to a slowly drifting value. The procedure used was firts to make a set of 15 measurements on a solution of either *1* or *8*, the interval between measurements being constant at about 30 s. After the first 5 measurements, the change in successive values was 0.2 mV or less. After the completion of the first set of 15 measurements, measurements were begun on the solution of the other form. The alternating measurements were continued until 10 sets had been gathered for each solution. The procedure was carried out on three different pairs of solutions. In all three cases, the oxidation of *1* was observed at a potential positive of the potential for the oxidation of *8*, with peak potential differences ranging from 0.9 to 3.2 mV. In view of the potential drift problem encountered in the measurements in solvent containing TFA, the same procedure was carried out on two pairs of solutions in acetonitrile with neutral alumina in the cell. Once again $E^p_H - E^p_D$ was positive, 1.0 and 1.1 mV, and this time there was no potential drift problem. The data along with the values of k_H/k_D calculated using eqn. (20) are summarized in Table 4. Secondary deuterium kinetic isotope effects ranging from 0.69 to 0.90 were obtained.

The mechanism of the cyclization reaction. In either AN with low water content or in AN/TFA, the LSV kinetic studies indicate that the major reaction pathway gives rise to rate law (19). This rate law is equally consistent with disproportionation mechanism (12)–(13) or that involving cyclization of the cation radical (14)–(16). In either case electron transfer, either (12) or (15) must be the slow step. The mechanism must take into account the secondary deuterium kinetic isotope effect observed during the comparison of the reactions of $1^{\cdot+}$ with those of $8^{\cdot+}$. Reaction (12) which involves only the transfer of electrons between cation radicals does not involve any hybridization changes of the carbons which are subsequently bonded in the cyclized product. Thus, the observation of k_H/k_D ranging from 0.7 to 0.9 makes the disproportions mechanism highly unlikely. On the other hand, reaction (14) does involve a change in hybridization of the two carbons through which the two rings are joined in the product. The change in hybridization from sp^2 to

Table 4. The secondary deuterium kinetic isotope effect for the cyclization of $1^{\cdot+}$ and $8^{\cdot+}$.

Solution	Solvent	$E^p_H - E^p_D$/mV[a]	k_H/k_D
1	AN/TFA	3.2(0.6)	0.64–0.74
2	AN/TFA	1.3(0.9)	0.77–0.95
3	AN/TFA	0.9(1.0)	0.80–1.01
4	AN[b]	1.0(0.5	0.84–0.94
5	AN[b]	1.1(0.6)	0.82–0.94

[a] The potential difference measured at 100 mV/s in solutions containing either *1* or *8* with a substrate concentration of 0.5 mM. [b] Measurements in the presence of alumina.

sp^3 in forward reaction (14) is expected to be accompanied by a kinetic isotope effect in the range observed.[35,36] Thus, the kinetic data suggest that the mechanism of cyclization of $1^{\cdot+}$ involves initial cyclization of the cation radical (14) followed by electron transfer reaction (15). It must be pointed out that there is a high degree of uncertainty in the value* of k_H/k_D due to the difficulties in measurement of the electrode potential difference to a high degree of precision. However, in the numerous experiments on which the data in Table 4 are based, no negative values of $E_H^p - E_D^p$ were observed, *i.e.* k_H/k_D in all cases was found to be less than unity.

The two mechanisms (12)–(13) and (14)–(16) are a special case of the two mechanisms of electrodimerization, radical ion dimerization or radical ion-substrate coupling that are currently under discussion.[35,43,44] It is perhaps not surprising that mechanism (14)–(16) is a favorable reaction pathway in view of the fact that the charged and uncharged rings are held in close proximity for the intramolecular coupling reaction. In order for the ion radical dimerization analog, dication diradical cyclization in this case, to occur second order electron transfer (12) must first take place. No data are available for the equilibrium constant for reaction (12) but it must surely be less than unity and probably 10^{-2} or smaller. Thus, the radical – substrate analog (14) is more favorable during the cyclization reactions than in the intermolecular reactions. Previous work has shown that the intermolecular radical ion – substrate reaction is a favorable reaction pathway.[35,43,44]

The question arises as to why the cyclization of $1^{\cdot+}$ follows rate law (19) while that for the related cation radical of *6* is consistent with rate law (17). A significant structural difference between *1* and *6* is that one of the rings in *6* does not have an unsubstituted position *para* to an alkoxy group. This then requires that the intramolecular coupling take place at a substituted ring position. This factor could change the balance between mechanisms (12)–(13) and (14)–(16) in favor of the disproportionation. The same argument can be put forth for the cyclization of $5^{\cdot+}$. However, in this case the apparent kinetics are consistent with rate law (19), providing the arguments against rate law (7) are accepted. There are no data vailable to distinguish between rate-determining (12) or (15) in this case. The added complication of protonation equilibria of the side-chain nitrogen makes this system very much more complicated.

EXPERIMENTAL

The practical aspects of making LSV kinetic measurements have been described in detail.[42] The solvent and electrolyte handling procedures were the same as those described in related work.[35,45]

Deuteration of *1* was carried out by dissolving in trifluoroacetic acid (d) containing trifluoromethyanesulfonic acid and allowing to stand at room temperature as described in a previous preparation.[35]

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*The uncertainty is in the magnitude of the effect, not the existance of an isotope effect.

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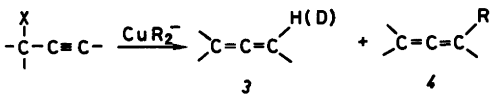
Allenes and Acetylenes. XXIV. Synthesis of α-Allenic Amines by Organocuprate Reactions of Acetylenic Aminoethers

CHRISTER SAHLBERG and ALF CLAESSON

Department of Organic Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden

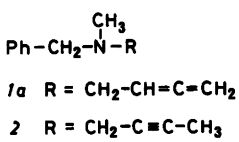
Tertiary α-allenic amines *1a–d* are formed in good yields in reactions of acetylenic aminoethers with an organocuprate reagent, derived from butylmagnesium bromide and 5–20 % of CuI, in tetrahydrofuran (THF) or THF–diethyl ether 3:1. The overall reduction reaction proceeds through an organometallic intermediate which is protonated on work-up. Primary and tertiary α-allenic amines *7a–d* are formed in moderate to good yields in 1,3-substitution reactions (S_N2') of acetylenic aminoethers with organocuprates in diethyl ether. The organocuprate reagents are derived from methylmagnesium or benzylmagnesium halide and 10 % of CuI or CuBr. The choice of solvent in these allene-forming reactions is of crucial importance in steering the reactions in one of the two directions.

Mitochondrial monoamine oxidase (MAO) is an enzyme which plays an important role in the oxidative deamination of transmitter amines.[1] MAO is inhibited by several types of compounds,[2] some of which can be used clinically, mainly in the treatment of depression.[3] Acetylenic (propargylic) amines are among the most studied inhibitors. They are mechanism-based irreversible inhibitors of MAO and their mode of action involves enzyme-mediated formation of an unknown reactive intermediate which reacts in the active site to form covalent bonds with the flavin unit of MAO. The allenic amine *1a*, a known inhibitor of MAO,[4] was shown by Krantz and Lipkowitz to give an unidentified adduct with MAO.[5] The unstable adduct is not identical with that obtained from the isomeric acetylene *2* which gives a flavocyanine.[6] The structure of the adduct is currently being elucidated through collaborative efforts by the research groups of Professor Krantz, Dr. Salach and our group.[7] For these studies a method for preparing isotope-labeled α-allenic amines, particularly amine *1a*, was needed. Since the work, on our part, involves studies of structure-activity relationships of allenic amines as MAO inhibitors, it was highly desirable to use synthetic methods which permit structure variation. The synthetic methods[8] known at the time of commencement of this work were not judged suitable. Since then some synthetic methods for allenic amines have been reported.[9] The above considerations have led us to develop new synthetic methods for α-allenic amines. A method for primary α-allenic amines has been published.[10] The present paper



(1)

describes in detail[11] a synthetic method for tertiary α-allenic amines which is based on the known ability of organocuprates to give "reduced" allenes *3* besides alkylated allenes *4* in reactions with propargylic acetates[12] and other propargylic derivatives[13] (eqn. 1). The method has been applied in synthesis of allenic prostaglandins.[14] Speculations have been made about the mechanism of this reduction reaction[12b,13a] and studies on the same type of reduction of allylic ethers have been reported.[15]



1a R = CH_2–CH=C=CH_2

2 R = CH_2–C≡C–CH_3

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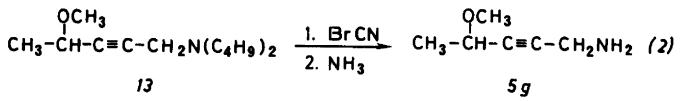


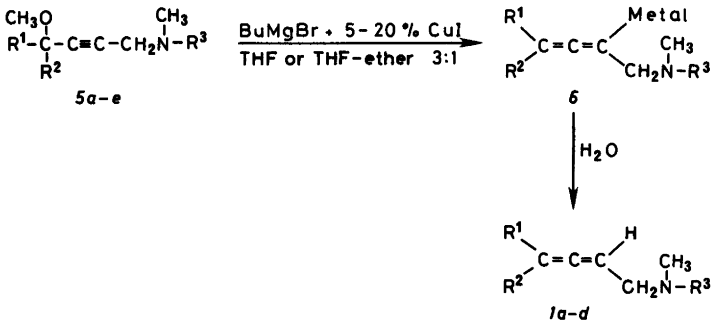
13 5g

A synthesis of allenic primary and tertiary amines by, formally, S_N2' reactions of the same aminoethers with organocuprates is also reported in the present paper (Scheme 2).

RESULTS

The starting aminoethers (*5a–f*) were prepared in high yields from a secondary amine, paraformaldehyde and a propargylic methyl ether by the Mannich reaction. Compound *5g* was synthesized as outlined in eqn. 2.

The aminoethers *5a–e* were allowed to react with a butylcuprate, derived from butylmagnesium bromide and CuI in tetrahydrofuran (THF) or THF–diethyl ether (ether) 3:1. The allenic amines (*1a–d*) were obtained in good yields (Table 1, Scheme 1). In entry 1, allene *1a* was accompanied by an equal amount of the isomeric amine *2*. Compound *1a* was isolated by preparative GLC. In entries 2, 3 and 4, allenes *1b–d* were formed in good isolated yields, and only small amounts, <5 %, of isomeric acetylenes were detected. The existence of the organometallic intermediate *6* was confirmed by hydrolyzing the reaction mixture in entry *8* with D_2O. The allene *1b*-d_1 thus obtained had a deuterium incorporation of >99 %, as determined by mass spectrometry and NMR. Several unsuccessful attempts were made to achieve reduction of the aminoether *5e* under the above conditions. Preliminary attempts (not shown in Table 1) to prepare primary and secondary α-allenic amines by the method described in Scheme 1 led to mixtures of products and further attempts were not made.

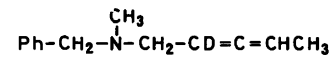


Scheme 1.

Table 1. Reactions of *5* with BuMgBr+CuI in THF–ether 3:1 (Scheme 1).

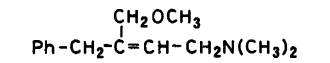
Entry	Methyl ether *5*	R^1	R^2	R^3	CuI (%)[a]	Reaction time (h)	Allenic amine *1*	Isolated yield (%)
1	*5a*	H	H	CH_2Ph	20	3	*1a*	40[b]
2	*5b*	CH_3	H	CH_2Ph	20	3.5	*1b*	62
3	*5c*	CH_3	CH_3	CH_2Ph	20	4	*1c*	70
4[c]	*5d*	C_3H_7	H	CH_3	10	2	*1d*	63
5	*5e*	$C(CH_3)_3$	H	CH_2Ph	20			0
6	*5a*	H	H	CH_2Ph	10	2.5	*1a*	40[b]
7	*5b*	CH_3	H	CH_2Ph	5	3	*1b*	80[b]
8[d]	*5b*	CH_3	H	CH_2Ph	20	2.5	*1b*–d_1	80[b]

[a] Relative to BuMgBr. [b] GLC-yield. [c] Solvent: THF. [d] Hydrolyzed with D_2O.



1b-d₁

If, instead of THF, ether is used as the solvent in similar organocuprate reactions, alkylated allenes are obtained. Thus, amines *7a,b,f* and *g* were obtained in variable yields through reactions of the aminoethers *5a,b,f* and *g* with a methyl or benzyl cuprate in ether (Table 2, Scheme 2). This reaction



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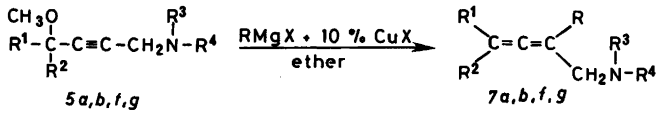
was found to be 100 % regioselective, since in no case were acetylenic amines obtained. From the reaction of compound *5f* with benzylmagnesium chloride and 10 % of CuBr, the addition product *8* was isolated in a yield of about 20 %.

DISCUSSION

The results in Table 1 indicate that the reduction depicted in Scheme 1 is quite general and that it can be used as a synthetic method for many tertiary α-allenic amines. However, certain bulky substituents (R^1 or R^2) on the propargylic carbon in the aminoether *5*, as in *5e*, obviously present hindrance when *5e* is attacked by the cuprate (entry 5, Table 1). No other reasons but steric ones are available to explain this unexpected result. Similar failures to reduce a propargylic aminoether containing an amide function in the R^1 substituent ($R^2 = H$) have been reported.[16]

In the two types of reactions described above the acetylenic aminoethers are either reduced or undergo substitution, mainly depending on the solvent. Thus, THF gives reduction and ether gives substitution. Butylmagnesium bromide plus 10 % of a Cu(I) halide, however, gives about 35 % of reduction products on reaction with the aminoether *5b* when the solvent is ether.[17] Neither we[18] nor other authors,[19] however, have noted any reduction under similar conditions when the acetylenic ether does not contain an amino group. The ability of the solvent to steer the reaction in either direction can be explained by considering the reaction mechanism(s) which, however, is not yet fully elucidated. It is generally assumed that cuprate reagents, like other d^{10} complexes, often react with electrophiles by oxidative addition, *i.e.* a copper(III) species is a transient intermediate which then gives the products by reductive elimination.[20] An alternative explanation pertaining to substrates having π-electrons is loose complex formation followed by addition (unsaturated ketones) or addition–elimination (*e.g.* the present propargylic compounds).[21]

As mentioned above, reduction of propargylic compounds has been encountered earlier. Other authors[12b,13a] have attempted to explain the formation of these products by postulating a stable trialkylcopper(III) species which is hydrolyzed during work-up. Since no such alkyl complex has ever been isolated,[22] the postulation is indeed remarkable and, if true, has far-reaching consequences. A more likely explanation is a reduction–oxidation process



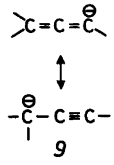
5a,b,f,g *7a,b,f,g*

Scheme 2.

Table 2. Reactions of *5* with RMgX + 10 % CuX in ether (Scheme 2).

Entry	Methyl ether *5*	R^1	R^2	R^3	R^4	RX	CuX	Reaction time (h)	Allenic amine *7*	Isolated yield (%)
1	*5a*	H	H	CH_2Ph	CH_3	CH_3I	CuI	3	*7a*	60
2	*5b*	H	CH_3	CH_2Ph	CH_3	CH_3I	CuI	21	*7b*	70
3	*5f*	H	H	CH_3	CH_3	$PhCH_2Cl$	CuBr	15	*7f*	20
4	*5g*	H	CH_3	H	H	CH_3I	CuBr	20	*7g*	42

in which the cuprate is oxidized to alkanes by the acetylenes, which are thus reduced. In some unknown way, THF obviously promotes this reduction reaction more than ether does. We have also found that propargylic methyl ethers are reduced to a somewhat greater extent than are propargylic acetates.[17] This is in accordance with the more pronounced differences found in the corresponding reactions of certain allylic ethers and acetates.[15] The results in both cases might be explained by relative slowness of a reductive elimination of a copper-(III) intermediate caused, at least in part, by a copper-bound methoxide ligand. It is conceivable that such an intermediate complex might simply be reduced by another copper(I) species or rearrange internally before elimination.[15] In both cases an ambident propynyl–propadienyl anion *9*, *cf.* *6*, would be the result. The possible role of the solvent could be to alter the structure and/or dynamics of the copper(III) intermediate (*e.g.* σ



9

versus π-complex). These speculative mechanistic considerations are developed more fully elsewhere for the allylic case.[15]

The protonation of the ambident anion *9* was surprisingly regioselective when R^1 and/or R^2 equalled the alkyl. Only $\leq 5\%$ acetylenic amines were formed, which were readily removed. However, when $R^1 = R^2 = H$ the allenic and acetylenic amines *1a* and *2*, respectively, were formed in equal amounts. The same regioselectivity was not displayed by a corresponding anion which lacked the amino function.[17] Here, the acetylene was the main product. The role of the amino nitrogen is not readily apparent, but some kind of chelating effect in the anion *6* might be invoked.

Formation of tertiary α-allenic amines by reactions of Grignard reagents with acetylenic aminoethers of the present type is known.[23] However, the yields increase when catalytic amounts of CuBr are used, as we have done (Table 2, Scheme 2). Similar improvements of the yields have been observed in the synthesis of allenic hydrocarbons from acetylenic ethers.[19] The alkylation reaction in Scheme 2 was highly regioselective when R = Me, CH_2Ph. Moreover, no reduced products were detected. However, reactions of *5b* with ethyl- or butylcuprate also gave "reduced" allenes besides alkylated ones.[17]

The mechanism of the reaction in Scheme 2 might be either an oxidative addition–reductive elimination process[20] or a *cis* addition of alkylcopper over the acetylenic bond followed by 1,2-elimination of copper methoxide.[24] Similar additions of organocopper reagents to acetylenes are known.[25] The last-mentioned mechanism might be valid in some cases since the reaction of *5f* with a benzylcuprate (entry 3, Table 2) produced equal amounts of the addition product *8* and the allene *7f*.

The alkylation reaction in Scheme 2, in contrast to the reduction reaction, is applicable to the synthesis of primary amines (*cf.* entry 4, Table 2). The yields are not, however, as high as for the synthesis of tertiary amines.

EXPERIMENTAL

General. IR spectra were recorded on a Perkin-Elmer Infracord 157G spectrophotometer, and mass spectra (at 70 eV) on an LKB 9000 instrument. These spectra were routinely recorded and are in full accordance with the proposed structures. Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were carried out by the Microanalytical Laboratory, Royal Agricultural College, Uppsala. GLC analyses were performed on a Varian 1700 chromatograph equipped with a 2.7 m glass column packed with 8 % Carbowax 20 M + 2 % KOH on Chromosorb W (60–80 mesh) or, for preparative use, a 3.0 m steel column packed with 20 % Carbowax 20 M on Chromosorb W (60–80 mesh). THF was distilled from $LiAlH_4$. All reactions with organocopper reagents were carried out under nitrogen.

Preparation of starting materials

3-Methoxy-1-hexyne (10). This was prepared by standard methods, using 3-hexyn-3-ol, NaH and CH_3I in ether–dimethyl formamide (DMF) 1:1. Yield: 41 %, b.p. 48 °C/32 mmHg. NMR ($CDCl_3$) 0.95 (t,3H), 1.4–1.9 (m,4H), 2.46 (d, $^4J \sim 2$Hz, 1H), 3.44 (s, 3H), 3.85–4.1 (m, 1H).

4,4-Dimethyl-3-methoxy-1-pentyne (11). This was prepared by standard methods, using 4,4-dimethyl-1-pentyn-3-ol, prepared according to a general procedure,[26] NaH and CH_3I in ether–DMF 1:1. Yield: 50 %, b.p. 52 °C/75 mmHg. NMR ($CDCl_3$) 0.96 (s, 9H), 2.34 (d, $^4J \sim 2$Hz, 1H), 3.37 (s, 3H), 3.48 (d, $^4J \sim 2$Hz, 1H).

1-Bromo-4-methoxy-2-pentyne (12). To a stirred solution of cyanogen bromide (32.0 g, 0.3 mol) in ether (400 ml) at 25 °C was added *N,N*-dibutyl-4-methoxy-2-pentynylamine (*13*) (68.0 g, 0.3 mol) over a 30-min period. The mixture was stirred for an additional 12 h and then washed with 1 M HCl (3 × 50 ml) and water (2 × 50 ml). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and distilled at reduced pressure to give 39.2 g (74 %) of *12*, b.p. 70–75 °C/10 mmHg. IR (film) 2200 cm^{-1}. NMR ($CDCl_3$) 1.35 (d, $^3J \sim 7$Hz, 2H), 3.37 (s, 3H), 3.94 (d, $^5J \sim 2$Hz, 2H), 3.9–4.3 (m, 1H).

4-Methoxy-2-pentynylamine (5g). 8.5 g (0.048 mol) of *12* was added to about 50 ml of liquid NH_3 in a tube which was sealed for 3 h. The excess of NH_3 was then allowed to evaporate and ether (200 ml) was added. The ether phase was washed with H_2O (10 ml) and dried over Na_2CO_3. The ether was distilled off and the residue was distilled *in vacuo* to give 5.4 g (60 %) of *5 g*, b.p. 62–63 °C/12 mmHg. NMR ($CDCl_3$) 1.32 (s, 2H), 1.38 (d, $^3J \sim 7$Hz, 3H), 3.33 (s, 3H), 3.42 (d. $^5J \sim 7$Hz, 2H), 3.8–4.2 (m, 1H).

General procedure for preparation of acetylenic aminoethers (Mannich reactions). A mixture of an appropriate secondary amine (1.1 equiv.), paraformaldehyde (1.2 equiv.), an appropriate acetylenic methyl ether (1.0 equiv.) and CuCl (0.01 equiv.) in dioxane was refluxed for 2 h. The solvent was removed *in vacuo* and the reaction mixture was poured into ice-water. The mixture was acidified and extracted twice with ether. The aqueous layer was made basic and extracted three times with chloroform. Drying over K_2CO_3, evaporation of the solvent and distillation *in vacuo* gave the appropriate aminoether.

N-*(4-Methoxy-2-butynyl)*-N-*methylbenzylamine (5a).* The standard procedure was followed, using 3-methoxy-1-propyne[26] and *N*-methylbenzylamine. Yield: 89 %, b.p. 90–92 °C/0.2 mmHg. NMR ($CDCl_3$) 2.33 (s, 3H), 3.35 (t, 2H), 3.40 (s, 3H), 3.58 (s, 2H), 4.15 (t, 3H), 7.35 (s, 5H).

N-*(4-Methoxy-2-pentynyl)*-N-*methylbenzylamine (5b).* The standard procedure using 3-methoxy-1-butyne[27] and *N*-methylbenzylamine gave *5b* in a yield of 71 %, b.p. 106–108 °C/0.6 mmHg. NMR ($CDCl_3$) 1.43 (d, $^3J \sim 7$Hz, 3H), 2.32 (s, 3H), 3.33 (d, 2H), 3.41 (s, 3H), 3.55 (s, 2H), 3.95–4.3 (m, 1H), 7.35 (s, 5H).

N-*(4*-Methoxy-*4*-methyl-*2*-pentynyl)-N-*methylbenzylamine (5c).* The standard procedure was followed, using 3-methoxy-3-methyl-1-butyne[28] and *N*-methylbenzylamine. Yield: 76 %, b.p. 87 °C/0.1 mmHg. NMR ($CDCl_3$) 1.50 (s, 6H), 2.32 (s, 3H) 3.34 (s, 2H), 3.41 (s, 3H), 3.56 (s, 2H), 7.35 (s, 5H).

N,N-*Dimethyl-4-methoxy-2-heptynylamine (5d).* The mixture was heated in a sealed tube; otherwise the standard procedure was followed, using *10*, dimethylamine hydrochloride and one equivalent of K_2CO_3. Yield: 53 %, b.p. 84–86 °C/12 mmHg. NMR ($CDCl_3$) 0.95 (t, 3H), 1.35–1.8 (m, 4H), 2.30 (s, 6H), 3.29 (d, 2H), 3.39 (s, 3H), 3.8–4.2 (m, 1H).

N-*(5,5-Dimethyl-4-methoxy-2-hexynyl)*-N-*methylbenzylamine (5e).* The standard procedure using *11* and *N*-methylbenzylamine gave *5e* in a yield of 70 %. B.p. 98–99 °C/0.06 mmHg. NMR ($CDCl_3$) 1.00 (s, 9H), 2.35 (s, 3H), 3.3–3.5 (m, 5H), 3.5–3.7 (m, 3H), 7.35 (s, 5H).

N,N-*Dibutyl-4-methoxy-2-pentylamine (13).* The standard procedure was followed, using 3-methoxy-1-butyne[27] and dibutylamine. Yield: 82 %, b.p. 84–87 °C/0.4 mmHg. NMR ($CDCl_3$) 0.90 (t, 6H), 1.15–1.75 (m, 11H), 2.45 (t, 4H), 3.35–3.50 (m, 5H), 3.9–4.3 (m, 1H).

N,N-*Dimethyl-4-methoxy-2-butynylamine (5f)* was prepared according to Ref. 29.

General procedure for organocuprate reactions of acetylenic aminoethers. A Grignard reagent, derived from Mg (0.065 mol) and an organic halide (0.065 mol) in an appropriate solvent, was cooled to −30 °C. CuI or CuBr (0.0038–0.013 mol) was then added over a 5-min period. A solution of an aminoether *5a–g* in THF or ether was then added dropwise in 5-min. Thereafter the reaction mixture was allowed to slowly reach room temperature. The mixture was quenched with water (10 ml) when the reaction was complete (2–21 h), as determined by GLC. Diluted NH_3 solution (0.01 M, 40 ml) was added to the mixture and it was extracted with ether (3 × 50 ml). The organic phase was washed with additional NH_3 solution and water, dried over Na_2SO_4–K_2CO_3 and concentrated *in vacuo*. Distillation at reduced pressure gave the allenic amines *1a–d*, *7a,b,f* and *g*. They were characterized by NMR, IR and elemental analyses of their oxalates or hydrochlorides.

N-*2,3-Butadienyl*-N-*methylbenzylamine (1a).*[4] Purified by preparative GLC. IR (film) 1955 cm^{-1}. NMR ($CDCl_3$) 2.24 (s, 3H), 3.0–3.2 (m, 2H), 3.53 (s, 2H), 4.8–4.55 (m, 2H), 5.0–5.35 (m, 1H), 7.32 (s, 5H).

N-*2,3-Pentadienyl*-N-*methylbenzylamine (1b).* B.p. 94–96 °C/1.0 mmHg. IR (film) 1960 cm^{-1}. ($CDCl_3$) 1.66 (dd, 3H), 2.25 (s, 3H), 3.04 (dd, 2H), 3.53 (s, 2H), 4.95–5.3 (m, 2H), 7.31 (s, 5H). The oxalate of *2b* had a m.p. of 135–136 °C after recrystallization from ethanol–ether. Anal. $C_{15}H_{19}NO_4$: C,H,N.

N-*(4-Methyl-2,3-pentadienyl)*-N-*methylbenzylamine (1c).* B.p. 78–79 °C/0.25 mmHg. IR (film) 1960 cm^{-1}. NMR ($CDCl_3$) 1.69 (d, $^5J \sim 3$ Hz, 6H), 2.24 (s, 3H), 3.03 (d, $^3J \sim 7$Hz, 2H), 3.55 (s, 2H), 4.5–5.3 (m, 1H), 7.32 (s, 5H). Oxalate of *2c*: m.p. 150–151 °C (recrystallized from ethanol–ether). Anal. $C_{16}H_{21}NO_4$: C,H,N.

N,N-*Dimethyl-2,3-heptadienylamine (*1d*)*. B.p. 98–101 °C/65 mmHg. IR (film) 1960 cm^{-1}. NMR ($CDCl_3$) 0.95 (t, 3H), 1.2–2.2 (m, 4H), 2.30 (s, 6H), 2.98 (dd, 2H) 4.9–5.25 (m, 2H). Oxalate of *2d*: m.p. 122–123 °C (recrystallized from ethanol–ether). Anal. $C_{11}H_{19}NO_4$: C,H,N.

N-*(2-Methyl-2,3-butadienyl)*-N-*methylbenzylamine (*7a*)*. B.p. 64–65 °C/0.6 mmHg. IR (film) 1960 cm^{-1}. NMR ($CDCl_3$) 1.64 (t, $^5J \sim 3$Hz,3H), 2.16 (s, 3H), 2.91 (t, $^5J \sim 2$Hz, 2H), 3.49 (s, 2H), 4.5–4.75 (m, 2H), 7.30 (s, 5H). *7a* was converted to a hydrochloride which, after recrystallization from ethyl acetate, had an m.p. of 119–121 °C. Anal. $C_{13}H_{18}ClN$: C,H,N.

N-*(2-Methyl-2,3-pentadienyl)*-N-*methylbenzylamine (*7b*)*. B.p. 76–77 °C/0.2 mmHg. IR (film) 1960 cm^{-1}. NMR ($CDCl_3$), 1.61 (d, $^3J \sim 7$Hz, 3H), 1.73 (d, $^5J \sim 3$Hz, 3H), 2.18 (s, 3H), 2.90 (d, $^5J \sim 2$Hz, 2H), 3.50 (s, 2H), 4.8–5.2 (m, 1H), 7.32 (s, 5H). Oxalate of *7b*: m.p. 124–125 °C (recrystallized from ethanol–ether). Anal. Calc. for $C_{16}H_{21}NO_4$: C 66.0; H 7.3; N 4.8. Found: C 65.4; H 7.4; N 4.8.

N,N-*Dimethyl-2-benzyl-2,3-butadienylamine (*7f*)*. B.p. 56–60 °C/0.15 mmHg. IR (film) 1960 cm^{-1}. NMR ($CDCl_3$) 2.21 (s, 6H), 2.76 (t, 2H), 3.36 (t, 3H), 4.45–4.8 (m, 2H), 7.28 (s, 5H). Hydrochloride of *7f*: m.p. 125–127 °C (recrystallized from ethanol–ether). Anal. $C_{13}H_{18}ClN$: C,H,N.

*2-Methyl-2,3-pentadienylamine (*7g*)*. B.p. 76–78 °C/100 mmHg. IR (film) 1960 cm^{-1}. NMR ($CDCl_3$) 1.52 (s, 2H), 1.62 (d, $^3J \sim 7$Hz, 3H), 1.66 (d, $^5J \sim 4$Hz, 3H), 3.05 (d, $^5J \sim 3$Hz, 2H), 4.8–5.35 (m, 1H). Hydrochloride of *7g*: m.p. 148–149 °C (recrystallized from ethanol–ether). Anal. $C_6H_{12}ClN$: C,H,N.

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The Kinetics and Mechanism of the Cyclization of the Anion Radicals of 1,3-Dibenzoyl-1,3-diphenylpropane and 1,3-Dibenzoylpropane

BJØRG AALSTAD and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The kinetics of the cyclization of the anion radical of 1,3-dibenzoyl-1,3-diphenylpropane in acetonitrile were investigated by linear sweep voltammetry, normalized potential sweep voltammetry and derivative cyclic voltammetry. The results of all of the measurements, including determination of the activation energy, are consistent with a mechanism consisting of reversible (i) followed by essentially irreversible electron transfer (ii) giving rise to rate law (iii). The major product of the

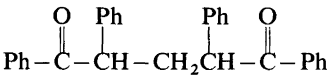
$$A^{\cdot -} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} C^{\cdot -} \text{ (C is the cyclic product)} \qquad \text{(i)}$$

$$C^{\cdot -} + A^{\cdot -} \xrightarrow{k_{ii}} C^{2-} + A \qquad \text{(ii)}$$

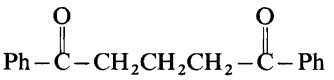
$$\text{Rate} = k_i k_{ii} |A^{\cdot -}|^2 / (k_{-i} + k_{ii} |A^{\cdot -}|) \qquad \text{(iii)}$$

cyclization was observed to be an isomer of 1,2,3,5-tetraphenyl-1,2-cyclopentanediol. ^{1}H NMR analysis of the product indicated a *trans* configuration of the diol with adjacent phenyl groups being either *cis-cis* or *trans-trans*. The latter possibility was deemed most likely on steric grounds. Apparent first order kinetics were observed during the cyclization of the anion radical of 1,3-dibenzoylpropane which is consistent with the same mechanism with the cyclization step (i) being rate-determining or with the disproportionation mechanism as had previously been proposed.

As a part of our continuing program on the study of the dimerization[1-9] and cyclization[10-20] reactions of ion radicals, we have carried out studies on the reactions of the anion radicals of 1,3-dibenzoyl-1,3-diphenylpropane (*1*) and 1,3-dibenzoylpropane (*2*).



1



2

The electrocyclization of *2* had previously been investigated by Savéant and co-workers[21,22] by linear sweep voltammetry (LSV) and convolution potential sweep voltammetry (CPSV). On the basis of their studies they proposed a disproportionation mechanism, eqns. (1) to (3) with either the cyclization or the protonation step being rate-determining. No evidence for the oxidation of the anion radical

$$2\,A^{\cdot -} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A^{2-} + A \qquad (1)$$

$$A^{2-} \xrightarrow{k_2} C^{2-} \qquad (2)$$

$$C^{2-} + ROH \xrightarrow{k_3} \text{products} \qquad (3)$$

($A^{\cdot -}$) could be detected using voltage sweep rates as high as 1,000 V/s.[21] We elected to investigate the reaction in more detail and in order to do this chose *1* as the substrate with the thought that the phenyl substituents in the 1,3-positions might crowd the transition state for the cyclization and thus moderate the rate of the reaction.

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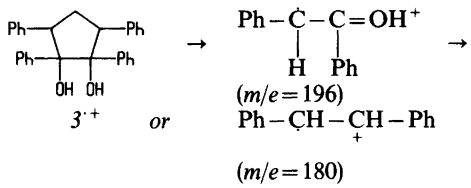
EXPERIMENTAL

Preparative scale electrolysis. A solution of *1* (1.01 g) in acetonitrile (100 ml) containing Me_4NBF_4 (0.07 M) was reduced at a platinum gauze electrode at a constant current of 50 mA (~1 mA/cm^2) over a period of 2.7 h (2 F/mol) in a divided cell under an atmosphere of nitrogen. After diluting with water and allowing to stand overnight, the crystals which had formed were gathered and recrystallized from methanol. More material was obtained by extraction of the solution with dichloromethane to give an isolated yield of about 50 %. No attempts were made to optimize the yield. The structure of the product was assigned on the basis of IR (no carbonyl absorption), mass and ^{1}H NMR (100 MHz) spectral data.

Kinetic studies. The instrumentation and data retrieval systems were the same as those recently described.[1] The working electrodes were constructed from platinum wire (0.4 mm) imbedded in glass and polished to a planar surface before electrolytically depositing mercury on the surface. The cells and reference electrodes were the same as previously described.[1] The temperature was controlled either by immersing the cell in an ice-water bath or with a Haake cryostat.

RESULTS

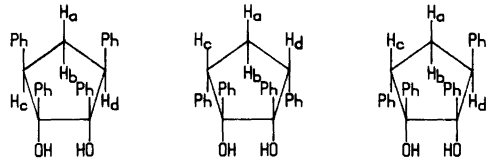
Structure of the isolated product. The mass spectral data are consistent with eqn. (4). The fragmentation pattern of the isolated product is



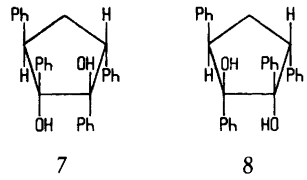
$$Ph-CH_2-\overset{Ph}{\underset{|}{C}}=OH^{\cdot+} \rightarrow PhC=O^+ \quad (m/e=105) \quad + C_7H_7^+ \quad (m/e=91) \qquad (4)$$

compared with that of the corresponding *cis*-diol prepared by reduction of *1* with amalgamated aluminum[29] in Table 7. All of the major ionic species are common to the two compounds. The double cleavage giving rise to the ions of mass 196 and 180 are of different proportions but of major importance in both spectra.

The ^{1}H NMR spectrum in $CDCl_3$ consisted of signals at δ 7.3–7.0 (20 H, m), 4.79 [2 H, t(J=9 Hz)], 2.85 [2 H, t(J=9 Hz)] and 1.84 (2 H, s). The singlet at 1.87 disappeared upon exposure to D_2O indicating exchange of hydroxyl H. There are three possible isomers with a *cis* arrangement of the hydroxyl groups. In structures *4* and *5* protons H_a and H_b are not equivalent as required by the



triplet at δ 2.85. Structure *6* is satisfactory with respect to H_a and H_b but in this case H_c and H_d are not equivalent as required by the triplet at δ 4.79. Thus, *trans* (with respect to the hydroxyl groups) isomers *7* and *8* remain as possibilities. This is also



consistent with the fact that the spectrum of the product of amalgamated aluminum reduction of *1* was quite complex and did not contain the triplet at δ 4.79. That latter is at somewhat higher field than expected for benzylic protons and the reason for this is not clear. Protons H_a and H_b in the *cis* isomers give rise to signals close to δ 4.0. Molecular models suggest that there are considerable steric interactions between the *cis*-phenyl substituents in *7*. The transition state leading to cyclization of the intermediate giving rise to *8* would be of lower energy which suggests that *8* is the more likely structure of the isolated product.

LSV mechanism analysis. The data summarized in Table 1 can be analyzed according to eqns. (5) and (6)[23] in order to determine the reaction orders

$$dE^p/d \log v = [1/(b+1)]RT/nF \qquad (5)$$

$$dE^p/d \log C_A = (a+b-1)/(b+1)RT/nF \qquad (6)$$

Table 1. Linear voltammetry study of the cyclization reaction.[a]

C_A/mM	$dE^p/d \log v$[b]
2.00	21.1
1.00	22.4
1.00	21.8
1.00	21.1
0.500	20.2
$dE^p/d \log C_A$[c]	−16.4

[a] In acetonitrile containing Bu_4NBF_4 (0.1 M) at 0 °C. [b] In mV/decade from correlation of data with v ranging from 100 to 1000 mV/s. [c] In mV/decade from correlation of data measured at 1000 mV/s with C_A ranging from 0.50 to 2.00 mM.

in substrate (*a*) and anion radical (*b*). At 273.15 K, (ln 10)RT/F is equal to 54.17 mV. The mean value of $dE^p/d \log v$ in Table 1 is 21.3±0.8 mV/decade. This implies a reaction order of 1.55 in the anion radical. The value required for a second order reaction is 18.1 mV/decade at this temperature. The fractional reaction order implies a complex rate law or competing mechanisms. If the reaction order a is 0, application of (6) with b equal to 1.55 results in a prediction of −14.9 mV/decade for $dE^p/d \log C_A$, somewhat lower than the observed value, −16.4. In order to be consistent, $dE^p/d \log v$ must differ from 18.1 by the same number as $dE^p/d \log C_A$ differs from −18.1. The values most consistent with the data are 20.6 and −15.6 mV/decade for the two slopes. These values are within experimental error of the observed values. Application of eqn. (5) using 20.6 mV/decade for $dE^p/\log v$ results in a reaction order of 1.64 for the anion radical.

Normalized potential sweep voltammetry (NPSV) analysis. A direct comparison of experimental data with theoretical electrode potentials can be made by normalizing the current along the voltammetric wave by dividing by the peak current and expressing the potential relative to that where the current is half the peak value.[24] The theoretical data selected for the analysis corresponded to processes with rate law (7). NPSV data are summarized in Table 2. For a perfect data fit for

$$\text{Rate} = k_{app} C_B^2 \qquad (7)$$

mechanism assignment, the NPSV slope is equal to unity. The data show that the slope is close to 1.0,

Table 2. Normalized potential sweep voltammetry study of the cyclization reaction.[a]

v/mV s^{-1}	C_A/mM	NPSV slope[b]
973	0.53	1.060±0.012
96.1	0.53	0.979±0.005
973	1.01	1.046±0.004
96.1	1.01	0.954±0.004

[a] Measured in acetonitrile containing Bu_4NBF_4 (0.1 M) at 0 °C. [b] Analysis using theoretical data for the rate law, $\text{Rate} = k_{app} C_A^{2\cdot-}$. The error limits are the standard deviations in 10 analyses.

is somewhat dependent upon the sweep rate and is independent of the substrate concentration. The sweep rate dependence and the deviation from unity of the slope suggest that the rate law deviates from the simple second order case (7). That the deviations are not large suggests that the mechanism of the electrode process gives rise to a rate law similar to (7) but perhaps somewhat more complex. Thus, the NPSV analysis is consistent with the LSV data.

The disproportionation equilibrium constant. The observation of kinetics with a reaction order in anion radical greater than 1 suggests that disproportionation might be involved as was concluded for a related cyclization reaction.[21,22] Since both the anion radical and the dianion are very short-lived a direct measure of the potential difference for the first and second reduction stage needed to calculate the equilibrium constant for reaction (1) cannot be obtained by cyclic voltammetry. Phase selective second harmonic *a.c.* voltammetry[25] gives a reliable estimate of electrode potentials even in the case of very reactive systems.[26] The data summarized in Table 3 illustrate the determination of K_{disp}. The

Table 3. Phase selective second harmonic *a.c.* voltammetric determination of the disproportionation equilibrium constant.[a]

Frequency/Hz	$\Delta E°$/mV	K_{disp}
100	259.2±0.2	3.7×10^{-5}
300	261.9±0.1	3.4×10^{-5}
1,000	266.3±0.2	2.8×10^{-5}

[a] Measured in acetonitrile containing Bu_4NBF_4 (0.1 M). *D.c.* voltage sweep rate equal to 50 mV/s. The error limits are the standard deviations in six determinations.

Table 4. Derivative cyclic voltammetry reaction order analysis.[a]

C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A$
1.00	170.2 ± 14.4	170.2
0.50	84.3 ± 7.7	168.6
0.50	82.1 ± 6.9	168.3

[a] Measured during the reduction of *1* in acetonitrile containing Bu_4NBF_4 (0.1 M) at 0 °C. The error limits are the standard deviations in 10 measurements.

fact that the potential difference is frequency dependent indicates that the value at the highest frequency is most reliable and that this will give a maximum estimate of K_{disp}, probably quite close to the real value.

Derivative cyclic voltammetric kinetic analysis. Direct electrode kinetic techniques such as cyclic voltammetry can also be used to obtain reaction orders.[27] In this case, it is not possible to separate *a* and *b* and the reaction order expressing the contributions of both A and B, $R_{A/B}$, is given by eqn. (8) where $v_{\frac{1}{2}}$ is the voltage sweep rate necessary for the ratio of the peak heights on the first derivative

$$R_{A/B} = 1 + z(v_{\frac{1}{2}}/C_A^z = \text{constant}) \quad (8)$$

of the reverse and forward scans of a cyclic voltammogram, R_I', to be constant at 0.500 and *z* is the power to which C_A must be raised in order that $v_{\frac{1}{2}}/C_A^z$ be constant. Data for the reduction of *1* in acetonitrile are summarized in Table 4. The data indicate that *z* in this case is unity which results in $R_{A/B} = 2$. This reaction order is consistent with rate law (7) but does not rule out rate law (9). However, we are unable to find a reasonable

$$\text{Rate} = k_{app} C_A C_B \quad (9)$$

mechanism for the cyclization reaction giving rise to (8). The LSV and NPSV analyses both indicate that *a* is zero and *b* closer to 2 than 1. The data in Table 4 are over a limited concentration range. The reason for this is that the high rate of reaction places an upper limit on the substrate concentration and poor reproducibility in the response, which gets severe at low concentrations, setting a lower limit at about 0.5 mV for meaningful measurements. That the system does not behave ideally is obvious from the standard deviations in $v_{\frac{1}{2}}$ of the order of 10 %. We normally find deviations of the order of ±1 % for well behaved systems. A possible reason for the problems with reproducibility is suggested by the fact that it was necessary to renew the mercury surface frequently. This is normally not necessary and indicates that some species formed during the electrode process attack the surface.

Activation parameters for the cyclization. Data from two activation energy determinations are summarized in Table 5. Due to the problems mentioned in the previous section, several attempts to measure the activation parameters failed. The data reported in Table 5 were obtained by first making the measurements at 0 °C then at the other temperatures and finally the 0 °C measurement was repeated. If the two 0 °C measurements differed by more than 10 %, the data was considered unreliable. Two similar runs were discarded because of deviations as great as 50 %. The rate constants reported in the third column were cal-

Table 5. Rate constants and activation parameters for the cyclization reaction in acetonitrile.[a]

T/K	$v_{\frac{1}{2}}$/V s^{-1}	$10^{-6}\,k$/M^{-1} s^{-1}	
273.2	174.9	1.52	$E_a = 2.84$ kcal/mol
285.5	243.8	2.02	$k_{298} = 2.39 \times 10^6$ M^{-1} s^{-1}
294.8	274.2	2.20	$\Delta S_{298} = -29.3$ cal/K mol
273.2	176.9	1.53	$r = -0.986$
259.6	103.3	0.94	$E_a = 2.69$ kcal/mol
273.2	153.9	1.34	$k_{298} = 1.91 \times 10^6$ M^{-1} s^{-1}
285.1	185.2	1.54	$\Delta S_{298} = -29.8$ cal/K mol
296.2	226.9	1.82	$r = -0.989$
273.2	146.2	1.27	

[a] Measured during the reduction of *1* in solvent containing Bu_4NBF_4 (0.1 M) at a mercury electrode. The activation parameters on the right were calculated for the two separate runs.

culated from $v_{\frac{1}{2}}$ assuming rate law (7). The feature of most interest in the data is that the E_a values are much lower than expected for a bimolecular reaction with a rate constant of 2×10^6 M^{-1} s^{-1} at 298 K. In fact, the activation energy appears to be lower than expected for a diffusion-controlled reaction which is a clear indication of a complex mechanism.[28] The entropy of activation, −29 cal/K mol, is also somewhat larger than would be expected for a simple second order electron transfer reaction as in forward step (1).

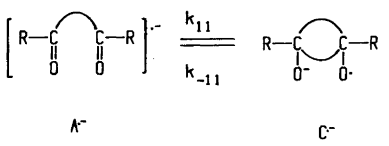
Derivative cyclic voltammetric kinetic study of the cyclization of 1,3-dibenzoylpropane anion radical. Since the previous work on the reduction of *2* indicated that the mechanism of the cyclization was a disproportionation as in eqns. (1) to (3) with rate law (10) which differs significantly from that we find during the reduction of *1*, we reinvestigated

$$\text{Rate} = k_{app} C_B^2 / C_A \qquad (10)$$

the kinetics at a lower temperature where the reaction rate was low enough to be measured by cyclic voltammetry. Data measured at −26 °C are summarized in Table 6. As in the case of *1* the response was less reproducible than usual with variations of the order of ±10 % in $v_{\frac{1}{2}}$. Within the limits of experimental error, $v_{\frac{1}{2}}$ was observed to be independent of the substrate concentration indicating apparent first order kinetics. The latter is consistent with previous work[21,22] and with rate law (10). However, the data do not rule out mechanism (11)−(12) with rate determining cyclization (11).*

DISCUSSION

The observation that kinetic data fit either rate law (7) or (10) can be taken as evidence for the disproportionation mechanism (1) to (3) for the cyclization reactions of *1* and *2*. However, the evidence is not unambiguous. An alternative mechanism involving the cyclization of the anion radical in reversible reaction (11) followed by electron transfer reaction (12) and product-forming reaction (3) also gives rise to the same rate laws



A⁻ C·⁻ (11)

$$A^{\cdot -} + C^{\cdot -} \xrightarrow{k_{12}} C^{2-} + A \qquad (12)$$

depending upon whether (12) or (3) is rate-determining. However, if (11) cannot be considered to be in equilibrium the rate law is more complex and the reaction order in $A^{\cdot -}$ would be expected to be less than 2 as is evident from (13).

$$\text{Rate} = k_{12} k_{11} |A^{\cdot -}|^2 / (k_{-11} + k_{12} |A^{\cdot -}|). \qquad (13)$$

The fact that the apparent activation energy for the cyclization of $1^{\cdot -}$ is less than 3 kcal/mol under conditions where the observed second order rate constant is of the order of 10^6 M^{-1} s^{-1} is a very clear indication that the reaction does not involve rate-determining disproportionation reaction (1). The value of E_a observed is even too low for a diffusion-controlled reaction. On the other hand, mechanism (11)−(12) readily accounts for the low E_a. It is probable that equilibrium (11) would be shifted to the right by lowering the temperature. Thus, this inverse temperature effect on (11) would

* This mechanism was ruled out under the conditions of previous studies.[21,22]

Table 6. Kinetic data for the cyclization of the anion radical of 1,3-dibenzoylpropane in acetonitrile.[a]

C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	k_{app}/s^{-1}
0.50	353±53	3.7×10^3
1.00	395± 8	4.2×10^3
2.00	367±23	3.9×10^3

[a] Measured in solvent containing Bu_4NBF_4 (0.1 M) at a mercury electrode at −26 °C.

Table 7. Mass spectral data from the electrolytic and amalgamated aluminum reduction products.[a]

	Relative abundance	
m/e	Electrolytic	Amalgamated aluminum
406	18.5	17.6
196	100	95.8
180	42.1	100
105	97.8	97.1
91	20.0	21.2
77	45.5	38.6

[a] Reduction products of *1*.

counteract that on (12) and result in an apparently anomolous E_a.

Rate law (13) is consistent with the LSV results as well. Under the conditions of the LSV study, the reaction order in $A^{\cdot -}$ is clearly less than 2. It was also pointed out that the NPSV results suggest a rate law more complex than (7). Other than the low E_a obtained by derivative cyclic voltammetry, the latter technique did not reveal the complex rate law. Complex reactions sometimes do appear more simple as has been amply demonstrated in related work.[28] Thus, the fact that the reaction appears to be second order under some conditions cannot be considered as evidence against mechanism (11)–(12). This mechanism can give reaction orders in $A^{\cdot -}$ ranging from 1 to 2, depending upon the relative magnitudes of the terms in the denominator of (13).

The disproportionation equilibrium constant calculated from the second harmonic *a.c.* measurements, $<2.8\times10^{-5}$, can be used to estimate the maximum possible second order rate constant for forward reaction (1). If the back reaction (1) is assumed to be diffusion-controlled, $k_{-1}\sim10^{10}$ M^{-1} s^{-1}, the maximum value of k_1 consistent with the data is $<2.8\times10^{5}$ M^{-1} s^{-1}. The value of k_{298} obtained from the Arrhenius correlation, Table 5, is about an order of magnitude greater. While this is not compelling evidence because of the approximations made in the estimate it does add further support to the arguments based on the other kinetic data.

The reactions of $2^{\cdot -}$, as can be seen from the data in Table 6, are so rapid that an activation energy determination was not attempted. Thus, we have no evidence in this case that the reaction does not follow a simple disproportionation. On the other hand, mechanism (11)–(12) cannot be ruled out. In fact, it seems highly unlikely that the structural change in going from $1^{\cdot -}$ to $2^{\cdot -}$ would cause a change in the mechanism of cyclization. Equilibrium (11) would be expected to be even more favorable for $2^{\cdot -}$ and would be more rapid because of the unfavorable steric interactions for the reactions of $1^{\cdot -}$. We therefore conclude that (11)–(12) represents the most likely mechanism for both anion radicals.

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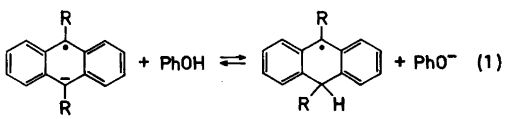
Structural Effects on the Mechanism of the Protonation of Anion Radicals of Aromatic Compounds. The Protonation of 1,1-Diphenylethylene Anion Radical by Methanol

OLAV LERFLATEN and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The initial proton addition to the anion radical of 1,1-diphenylethylene generates $Ph_2C^{\cdot}-CH_3$. By virtue of the fact that the proton becomes attached to an aliphatic carbon the reaction is irreversible. The kinetics of the reaction are clearly first order in anion radical and the overall reaction is of the ECE_h type. These results are in contrast to those anion radical protonations during which protons become attached to benzylic carbons reversibly giving rise to complex kinetic behaviour. The dependence of the linear sweep voltammetry peak potential on the methanol concentration during the reduction of 1,1-diphenylethylene in *N,N*-dimethylformamide indicated an apparent reaction order in methanol less than 1. The results could be explained by assuming either a dimerization equilibrium and that only monomeric methanol serves as the proton donor or association of the anion radicals with methanol resulting in a shift in E^{rev}. The latter was deemed most likely.

Recent studies on the protonation of anthracene and substituted anthracene anion radicals by phenol (1) indicate that the reactions exhibit complex



(1)

kinetic behaviour.[1,2] It was observed that the reaction orders in anion radical and phenol vary between the limits of 1 and 2 and that the rate of the reaction is inhibited by phenoxide ion. These results indicate that reaction (1) is reversible and further steps along the reaction pathway contribute to controlling the rates of the reactions.

The structural features common to the initial proton adducts of the various anthracene anion radicals, as well as those of other condensed aromatic compounds, is that the proton becomes attached to a benzylic position. The objective of the present study was to show that this structural feature is responsible for the reversibility of reaction (1) and when this feature is removed by suitable structural modification then the reactions become irreversible and exhibit simple second order kinetics. The obvious structural modification is one in which the initial proton attachment occurs at an aliphatic position not conjugated with an aromatic ring. For this purpose we selected the 1,1-diphenylethylene (DPE) anion radical which was expected to react with a proton donor as in reaction (2).

$$(Ph_2C=CH_2)^{\cdot -} + ROH \longrightarrow Ph_2C^{\cdot}-CH_3 + RO^- \quad (2)$$

RESULTS AND DISCUSSION

LSV sweep rate dependence. The dependence of the peak potential (E^p) on the voltage sweep rate (v) during linear sweep voltammetry (LSV) analysis of purely kinetic waves is given by (3).[3] The response

$$dE^p/d\log v = [\ln 10/(b+1)]RT/F \quad (3)$$

is dependent upon the reaction order in the primary intermediate of the charge transfer reaction (4) and the temperature. The data in Table 1 are a summary

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$$A \pm e \rightleftharpoons B \quad (4)$$

of LSV experiments on solutions of DPE in DMF in the presence of methanol (25 mM). Each value of $dE^p/d \log v$ listed resulted from measurements at 100, 200, 400 and 1000 mV s^{-1} with 5 replicates at each sweep rate with standard deviations being of the order of ± 0.1 mV. The last column gives the theoretical value for $b=1$ corresponding to rate determining protonation reaction (5) with methanol in excess. The mean values at the four different

$$DPE^{\cdot -} + MeOH \xrightarrow{k_5} DPE^{\cdot} - H + MeO^- \quad (5)$$

substrate concentrations were all reasonably close to the theoretical values. Since a reaction order of two in anion radical results in a prediction (3) of 19.7 mV/decade at 298 K, the results are only compatible with $b=1$.

LSV substrate concentration dependence. The dependence of E^p on the substrate concentration (C_A) is given by (6) where a is the reaction order

$$d \log E^p/d \log C_A = [(a+b+i-1)/(b+1)] \ln 10\,(RT/F) \quad (6)$$

in A and i that of products formed which further participate in the process.[3] For the case where $a=0$, $b=1$, and $i=0$ which is the predicted result for rate determining reaction (5), $dE^p/d \log C_A$ is zero. The data summarized in Table 2 are values of E^p at four C_A and v measured at 23 °C. Once again each E^p is the mean of 5 replicate measurements with standard deviations of the order of ± 0.1 mV. The values listed in the last column of Table 2 give the mean values of E^p averaged over all concentrations. The standard deviations found are of the order of 0.6 mV which is a clear indication that both a and i are zero and $dE^p/d \log C_A$ is zero as well.

LSV methanol concentration dependence. For a pseudo first order reaction of intermediate B (eqn. 1) with a reactant in excess (X), the dependence of the peak potential on C_X is given by eqn. (7) where x

$$dE^p/d \log C_X = (x \ln 10)/(b+1)\, RT/F \quad (7)$$

is the reaction order in X.[3] For rate determining reaction (5), x (X = MeOH) is expected to be 1 and $dE^p/d \log C_X$ to equal (1/2) RT/F. The data summarized in Table 3 show that this relationship is not followed. At all four sweep rates, $dE^p/d \log C_X$, which result from measurements at four different concentrations, are markedly dependent upon temperature. The theoretical slopes for $x=1$ are those

Table 1. LSV sweep rate dependence during the reduction of DPE in DMF containing methanol.[a]

	$dE^p/d \log v$ (mV/decade) at C_{DPE}					
T/°C	0.3 mM	0.5 mM	0.7 mM	1.0 mM	Mean (s.d.)[b]	Theory[c]
−7	27.5	27.9	28.8	30.1	28.6(1.2)	26.4
11	27.0	27.8	28.1	29.2	28.0(0.9)	28.2
23	28.0	28.6	28.7	29.5	28.7(0.6)	29.4
39	29.5	29.7	30.2	29.1	29.6(0.5)	31.0

[a] Measurements at a mercury electrode in solvent containing Bu_4NBF_4 (0.1 M) and methanol (25 mM). [b] The mean and standard deviation at all substrate concentrations. [c] The theoretical value for a reaction first order in anion radical.

Table 2. The variation of the peak potential with changes in the substrate concentration.[a]

	$-E^p$ (mV)[b] at C_{DPE}(mM)				
v/mV s^{-1}	0.3	0.5	0.7	1.0	Mean (s.d.)
100	689.8	689.7	690.2	690.7	690.1(0.5)
200	697.2	697.2	697.7	678.5	697.7(0.6)
400	705.7	706.2	706.4	707.3	706.4(0.7)
1000	717.6	718.2	718.7	(720.1)[c]	718.2(0.6)

[a] For conditions see Table 1, T=23 °C. [b] The LSV peak potential *vs.* a potentiostat bias potential of −2.100 V *vs.* Ag/Ag^+. [c] Not included in the averaging.

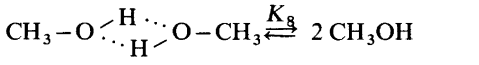
Table 3. The dependence of the peak potential on the methanol concentration during the reduction of DPE in DMF.[a]

v/V s^{-1}	T/°C	$dE^p/d \log C_{MeOH}$[b]
100	−7	−20.3
100	11	−24.8
100	23	−27.6
200	−7	−19.8
200	11	−24.7
200	23	−27.5
400	−7	−18.9
400	11	−24.3
400	23	−27.8
1000	−7	−18.6
1000	11	−23.7
1000	23	−26.7

[a] Conditions as in Table 1, $C_{DPE} = 1.0$ mM. [b] Methanol concentrations of 25, 50 and 100 mM.

given in the last column of Table 1. The deviations from the theoretical values are temperature dependent and are greatest at the lower temperature. The data indicate that the apparent reaction order in methanol is less than unity and is variable.

A possible dimer-monomer equilibrium. The data in Table 3 suggested that methanol may possibly be involved in a concentration dependent equilibrium which reduces the effective concentration of the proton donor. An obvious possibility is the dimerization equilibrium (8) which suggests that



$$CH_3-O\overset{H\cdots}{\underset{\cdots H}{\diagup}}O-CH_3 \overset{K_8}{\rightleftharpoons} 2\,CH_3OH \qquad (8)$$

the hydrogen bonded cyclic dimer could be a poorer proton donor than monomeric methanol. The data in Table 4 are based on the experimental data in Table 3. At each temperature the apparent reaction order in methanol was obtained by applying eqn. (7) to estimate the effective methanol concentration. Assuming equilibrium (8), the total methanol concentration, $|MeOH|_{tot}$, is given by (9) and the effective concentration, $|MeOH|_{eff}$, is related to the equilibrium constant K_8 by (10). Equilibrium

$$|MeOH|_{tot} = |MeOH|_{eff} + 2|(MeOH)_2| \qquad (9)$$

$$|MeOH|_{eff} = ((K_8{}^2 + 8\,K_8|MeOH|_{tot})^{\frac{1}{2}} - K_8)/4 \qquad (10)$$

constants were estimated at each temperature and $|MeOH|_{eff}$ obtained from (10) were then used to calculate $dE^p/d \log C_{MeOH}$. The mean values, standard deviations, and theoretical values are summarized in Table 4. As a further test to determine whether or not eqn. (10) gives consistent values of K_8, the data were treated according to eqn. (11). If the K_8 values are consistent, a linear relationship

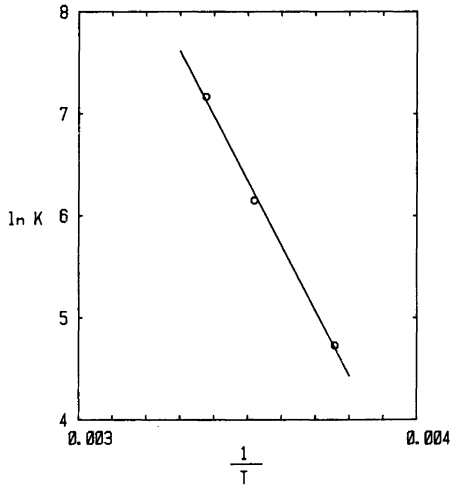


Fig. 1. The temperature dependence of the equilibrium constants calculated assuming a dimerization equilibrium of methanol in DMF.

Table 4. Correction of the peak potential dependence on the methanol concentration assuming a methanol dimerization equilibrium.[a]

T/K	296	284	266
K_8/M	1.300	0.470	0.113
$(-dE^p/d \log C_X)_{corrected}$	29.3(0.5)	28.2(0.6)	26.4(1.1)
$(-dE^p/d \log C_X)_{theory}$	29.4	28.2	26.4

[a] Data from Table 3 treated as described in the text for a reaction first order in anion radical and methanol.

Table 5. Correction of the peak potential dependence on the methanol concentration assuming association of the anion radical and methanol.[a]

T/K	296	284	266
K_{13}/M^{-1}	0.60	1.30	2.90
$\Delta E^{rev}(C_X = 25$ mM)/mV	0.38	0.78	1.60
$\Delta E^{rev}(C_X = 50$ mM)/mV	0.75	1.54	3.10
$\Delta E^{rev}(C_X = 100$ mM)/mV	1.49	2.99	5.84
$(-dE^p/d \log C_X)_{corrected}$ [b]	29.3(0.4)	28.0(0.5)	26.4(0.8)
$(-dE^p/d \log C_X)_{theory}$ [c]	29.4	28.2	26.4

[a] Data from Table 3 corrected as described in the text assuming eqn. (13). [b] In mV/decade corrected for the appropriate change in E^{rev}. [c] In mV/decade assuming rate determining reaction (14).

$$\ln K_8 = -\Delta H_8/RT + c \quad (11)$$

is expected. The data plotted in Fig. 1 resulted in $\Delta H_8 = 12.7$ kcal/mol with a correlation coefficient of 0.999.

Possible anion radical–methanol association equilibrium. Another plausible explanation for the low and variable values of $dE^p/d \log C_X$ is that the anion radicals are reversibly associated with methanol and the overall electrode process is described by eqns. (12)–(14). The reversible potential for

$$DPE + e^- \rightleftarrows DPE^{\cdot -} \quad (12)$$

$$DPE^{\cdot -} + MeOH \overset{K_{13}}{\rightleftarrows} DPE^{\cdot -}/MeOH \quad (13)$$

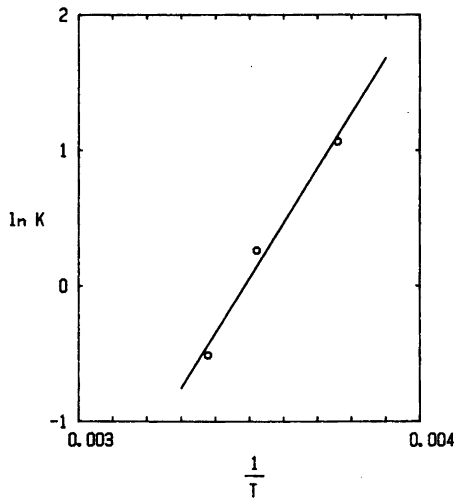


Fig. 2. The temperature dependence of the equilibrium constants calculated assuming association of diphenylethylene anion radical with methanol.

$$DPE^{\cdot -}/MeOH \xrightarrow{k_{14}} DPE^{\cdot} - H + MeO^- \quad (14)$$

reactions (12)–(13) is described by eqn. (15) and the difference in E^{rev} measured in the presence and absence of methanol (ΔE^{rev}) by (16).

$$E^{rev} = E^\circ + RT/F \ln (K|MeOH| + 1) \quad (15)$$

$$\Delta E^{rev} = RT/F \ln (K|MeOH| + 1) \quad (16)$$

In order to test the applicability of eqn. (16) to explain the LSV data, the value of ΔE^{rev}, at each methanol concentration and temperature, was estimated from the observed values of $dE^p/d \log C_X$ and (ln 10) $RT/2F$, the expected value for mechanism (12)–(14). The ΔE^{rev} values were then used in (16) to estimate K_{13}. The results are summarized in Table 5. Correlation of ln K_{13} *vs.* 1/T (Fig. 2) resulted in ΔH_{13} of -8.1 kcal/mol with a correlation coefficient of 0.98.

CONCLUSIONS

The point of crusial importance in terms of the overall mechanism of the protonation of $DPE^{\cdot -}$ by methanol is which of the alternatives for the dependence of the LSV peak potential on the methanol concentration is correct. Unfortunately, it was not possible to measure the reversible potential, to the degree of precision required, by phase sensitive second harmonic *a.c.* voltammetry because of interference by the kinetics of the follow-up reaction. If it had been possible to measure E^{rev} in the absence and presence of methanol, the question would have been resolved. However, the very small ΔE^{rev} values required by the second alternative, that of association of $DPE^{\cdot -}$ with methanol, as shown in

Table 5 are less than the error in the reversible potential measurements.

Methanol dimer has recently been shown to be significant in non-polar solvents.[6] A theoretical study[7] predicts that the most stable form of the dimer in isolation is a *trans* near-linear form in which only one of the hydrogens is involved in hydrogen bonding. It does not seem likely that such a dimer would be ineffective as a proton donor. However, it is possible that in DMF the dimer is cyclic as in eqn. (8) and this form would be expected to donate protons less effectively than monomeric methanol. We have been unable to find literature data for the dimerization of methanol in DMF. On the other hand infrared spectral evidence has been reported which indicates that methanol is monomeric in acetonitrile at concentrations as great as 4 M.[8] The methanol–nitrile interactions are apparently greater than those between solute molecules. DMF would be expected to be a better hydrogen bonding solvent than acetonitrile which makes methanol dimerization appear highly unlikely.

We are then left with eqns. (12)–(14) as the most likely initial steps in the mechanism. These are then followed by solution electron transfer (17) and rapid protonation of the resulting carbanion (18). This

$$DPE^{\cdot} - H + DPE^{\cdot -} \rightleftharpoons DPE^{-} - H + DPE \quad (17)$$

$$DPE^{-} - H + MeOH \longrightarrow DPE(H_2) + MeO^{-} \quad (18)$$

mechanism is similar to that earlier suggested to be the general reaction pathway for the protonation of aromatic hydrocarbon anion radicals.[5] But even in this relatively simple case, a pre-equilibrium is highly probable and the rate law for the reaction is (19). Rate data are not related to a simple microscopic step but the LSV analysis provides values for

$$\text{Rate} = k_{14}K_{13}|DPE^{\cdot -}||MeOH| \quad (19)$$

K_{13}. Under the conditions of our measurements the apparent first order rate constant for the reaction was greater than about 10^4 and out of range of measurement by cyclic voltammetry at voltage sweep rates less than about 1000 V/s.

EXPERIMENTAL

Reagent grade 1,1-diphenylethylene was reagent grade and used as received. The procedure for solvent and electrolyte purification as well as data handling was similar to that described for related work from this laboratory.[9] The practical details of the LSV analysis have recently been summarized.[10]

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Rate Constants and Activation Parameters for the Cyclization of the Tetraphenylethylene Dication in Acidic Dichloromethane

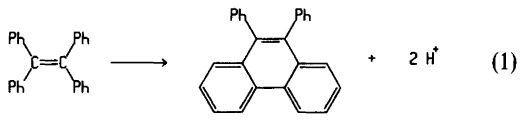
BJØRG AALSTAD,[a] ALVIN RONLÁN[b] and VERNON D. PARKER[a]

[a] Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and [b] Division of Organic Chemistry 2, Lund Institute of Technology, 220 07 Lund, Sweden

The kinetics and activation parameters for the cyclization of the tetraphenylethylene dication in dichloromethane – trifluoroacetic acid – trifluoroacetic acid anhydride (8.5/1.0/0.5) containing Bu_4NBF_4 (0.1 M) were determined by derivative linear sweep voltammetry. The rate constant at 298 K was found to be equal to $3.2 \times 10^3\ s^{-1}$, E_a was observed to be 11.4 kcal/mol and the entropy of activation at 298 K was −6 cal/K mol. The relatively large activation energy is believed to arise from charge – charge repulsion in the transition state. The negative entropy of activation arises because of solvent reorganization around the more localized charges in the transition state.

Cyclization reactions are frequently encountered during the oxidation and reduction of difunctional organic molecules. The intramolecular cyclization of 1,2-diarylethanes during oxidation has been studied in detail.[1–10] These systems are of special interest due to their relationship to naturally occurring alkaloids.

A related cyclization, that of tetraphenylethylene (TPE) to 9,10-diphenylphenanthrene (DPP) during anodic oxidation in acetonitrile (eqn. 1) was reported some years ago.[11] The cation radical ($TPE^{\cdot+}$) was



(1)

TPE DPP

found to be sufficiently stable so that solution could be prepared and the kinetics of the reaction were studied.[12] The reaction was observed to follow rate law (2) and a disproportionation mechanism, eqns. (3)–(4), was proposed to account for the data.

$$\text{Rate} = k_{obs}|TPE^{\cdot+}|^2/|TPE| \quad (2)$$

$$2\ TPE^{\cdot+} \underset{}{\overset{K_3}{\rightleftharpoons}} TPE^{2+} + TPE \quad (3)$$

$$TPE^{2+} \xrightarrow{k_4} DPP + 2\ H^+ \quad (4)$$

A rotating disk electrode study of the oxidation of the cation radical to the dication supported the mechanism assignment.

Disproportionation mechanisms have been proposed for two related cyclization reactions, those involving 1,2-diarylethane cation radicals[9] and 1,3-dibenzoylpropane anion radicals.[13,14] More recently, it has been suggested that the observation of rate laws of the type represented by (2) is not unambiguous evidence for the disproportionation mechanism.[15] The reaction investigated was the cyclization of 1,3-dibenzoyl-1,3-diphenylpropane anion radicals. Under some conditions the reaction was observed to follow simple second order kinetics (5) which could indicate the disproportionation

$$\text{Rate} = k_{app}|A^{\cdot-}|^2 \quad (5)$$

mechanism with rate-determining electron transfer analogous to (3). However, the apparent activation energy was observed to be only 2.7 kcal/mol which rules out a simple bimolecular reaction. Under linear sweep voltammetry (LSV) conditions, the rate law (6) was more complex and suggested mechanism (7)–(8). In this mechanism, $Cy^{\cdot-}$ is the

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$$\text{Rate} = k_7 k_8 |A^{\cdot -}|^2/(k_{-7} + k_8 |A^{\cdot -}|) \quad (6)$$

$$A^{\cdot -} \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} Cy^{\cdot -} \quad (7)$$

$$Cy^{\cdot -} + A^{\cdot -} \xrightarrow{k_8} Cy^{2-} + A \quad (8)$$

product of cyclization bridging the two carbonyl carbons. The pertinent point is that the apparent rate laws for the disproportionation mechanism (3)–(4) and ion radical cyclization (7)–(8) are not sufficient evidence to establish the mechanism. For example if (8) is considered reversible and is followed by irreversible (9), the anion radical cyclization mechanism gives a rate law identical in form to (2).

$$Cy^{2-} \xrightarrow{k_9} \text{products} \quad (9)$$

There are two other types of evidence which can be used to distinguish between the two mechanisms. The first is the apparent activation energy. In the case cited above, under purely second order conditions the apparent activation energy was two low to be due to rate determining electron transfer. Thus, the magnitude of the apparent activation energy can serve as a guide in the analysis of the mechanism. The second arises from the deuterium kinetic isotope effect when the carbons at the sites of ring closure are substituted with deuterium as illustrated in (10). Values of k_H/k_D in the range 0.7–0.9 have been observed during the dimerization of

$$|Ar{-}D \quad D{-}Ar|^{\cdot -} \rightarrow |\underset{D}{Ar}\underset{D}{-}Ar|^{\cdot -} \quad (10)$$

arene cation radicals.[16] The secondary deuterium kinetic isotope effect arises when the substituted carbons undergo a change in hybridization,[17] in this case from sp^2 to sp^3. Under purely second order conditions, the disproportionation mechanism involves rate-determining electron transfer which should not be dependent upon the isotopic substitution. On the other hand, two carbons change hybridization in the ion radical cyclization mechanism and appropriate deuterium substitution would be expected to be accompanied by a deuterium kinetic isotope effect of the usual magnitude.

In this paper we address the first mechanism criterion, that of the activation energy. The predominant contribution to the activation energy for the disproportionation mechanism of ion radical cyclization is expected to come from the cyclization of the doubly charged ion as in (4) for an example. Prior to this study, activation parameters were not available for any model systems undergoing the cyclization reaction. The purpose of the work presented here was to establish the activation parameters for reaction (4) to made the data available for comparison with other systems of unknown mechanism.

RESULTS AND DISCUSSION

The kinetic method. All kinetic measurements were carried out in dichloromethane – trifluoroacetic acid – trifluoroacetic acid anhydride (8.5:1.0:0.5). The first derivative of the linear sweep voltammogram for the oxidation of TPE at −33 °C is illustrated in the figure. The first peak (p_1) is due to the oxidation to $TPE^{\cdot +}$ which is stable under the reaction conditions. The second peak (p_2) is the one describing the chemistry of interest and consists of the oxidation to TPE^{2+} which reacts to form DPP which is oxidized to $DPP^{\cdot +}$. The voltammetry for these two redox systems have been reported earlier.[12] The exact nature of the process occurring at p_3 is unknown but it corresponds to an overall two electron oxidation. At low sweep rates, the relative

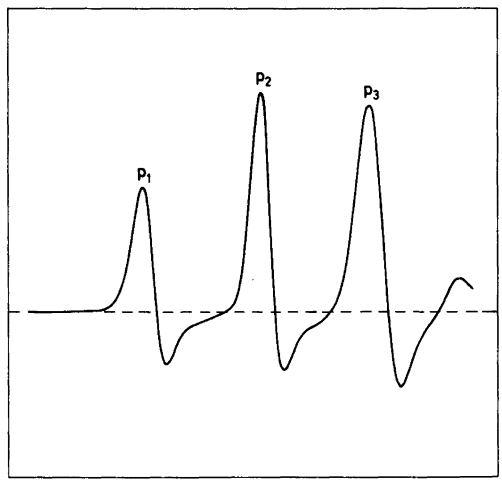

Fig. 1. Derivative linear sweep voltammogram for the oxidation of tetraphenylethylene in dichloromethane – trifluoroacetic acid – trifluoroacetic acid anhydride (8.5:1.0:0.5) containing Bu_4NBF_4 (0.1 M) at −33 °C. Voltage sweep rate = 10 V s^{-1}.

peak heights are 1:2:2 for p_1:p_2:p_3. Because p_2 involves both $TPE^{\cdot+}$ and DPP, this peak and the reduction counterpart cannot be used for derivative cyclic voltammetry kinetics. However, p_1 involves only the substrate TPE and p_3 only the product $DPP^{\cdot+}$ and the ratio p_3:p_1 or p_3:p_2, can be used to determine the rate constant for the cyclization of TPE^{2+}.

Theoretical data were obtained by digital simulation[18] using the measured potentials for the processes occurring at p_1, p_2 and p_3. The calculations assumed, based on experimental observations, that the process occurring at p_3 give a purely kinetic wave. This is necessary in the analysis so that the peak height is a measure of the amount of TPE^{2+} consumed at p_2. Calculations were carried out over a range of rate constants, normalized for changes in sweep rate. Since these calculations are specific to this system, they will not be reported here. Any other system with different electrode potentials for the three waves would require a complete set of calculations for a kinetic analysis.

Since $TPE^{\cdot+}$ is stable under the reaction conditions, a convenient method of analysis was observed to be a potential program consisting of a step to a value mid-way between p_1 and p_2 and then a potential sweep encompassing p_2 and p_3 after a hold time long enough for TPE to be depleted in the reaction layer. Carrying out experiments in this manner made the contribution from back reaction (3) negligible. The ratio (p_3/p_2) obtained experimentally could then be used in conjunction with theoretical data to obtain k_4.

Kinetics of the cyclization of TPE^{2+}. Kinetic experiments were carried out at −31.1 °C with the substrate concentration varied from 0.50 to 2.00 mM. At each substrate concentration, p_3:p_2 was held at either 0.500 or 0.700 by making appropriate adjustments in the voltage sweep rate. Any change taking place in the apparent rate constant would then be reflected in changes in the sweep rate.[19] The correspondence of the two rate constants derived at each concentration is a good indication of the fit of the experimental data to the theoretical model. The very close correspondence in all three pairs indicate an excellent fit. Although the rate constants measured at |TPE| equal 0.50 mM are little higher than at the other two concentrations, the standard deviation in the rate constants was observed to be less than ±10 %. The latter indicates that the kinetics are first order and describe the cyclization of the dication. This is the principal advantage of using the potential step to deplete the reaction layer of TPE. If this were not done the kinetic scheme would be more complicated because of the necessity to contend with reverse reaction (3).

Activation parameters of the cyclization of TPE^{2+}. The same procedure for measuring the rate constants for the cyclization of the dication was used for experiments carried out over a 32 K temperature range. Once again the rate constants derived from the two different p_3:p_2 ratios were in excellent agreement. Arrhenius correlation of the two data sets resulted in activation energies of 11.6 and 11.2 kcal/mol with corresponding activation entropies of −5.4 and −7.0 cal/K mol. In both cases the correlation coefficient was −0.998. The probable error in E_a was estimated by taking the errors in $v_{0.5}$ into account for data at the two extreme temperatures. This resulted in values of 11.4 and 12.2 kcal/mol and the conclusion that the error in E_a is of the order of ±0.5 kcal/mol. This is consistent with the fact that the value estimated from $v_{0.7}$ data differed from that using $v_{0.5}$ by 0.4 kcal/mol. An error of 0.5 kcal/mol in E_a corresponds to an error of ±1.8 cal/K mol in $\Delta S^{\neq}$. This again is reflected in the two values given in Table 2.

Conclusions. The activation energy for the cyclization of the tetraphenylethylene dication is of the same order of magnitude as was observed previously for the dimerization of 4-methoxybiphenyl cation radicals.[16] This is most likely due to loss of conjugation and to the localizing of the positive charge. The negative activation entropy could arise from the restriction of rotations in the transition state during

Table 1. Rate constants for the cyclization of tetraphenylethylene dication in acidic dichloromethane.[a]

C_A/mM[b]	p_3:p_2[c]	v/V s^{-1}	k/s^{-1}
0.50	0.500	19.3(1.9)	40.5
0.50	0.700	11.1(0.7)	39.8
1.00	0.500	16.2(1.0)	34.0
1.00	0.700	9.9(0.7)	35.5
2.00	0.500	16.2(1.6)	34.0
2.00	0.700	9.7(0.9)	34.8
			36.4(2.9)

[a] Measurements in dichloromethane – trifluoroacetic acid – trifluoroacetic acid anhydride (8.5:1.0:0.5) at −32.1 °C. [b] Tetraphenylethylene concentration. [c] The ratio of derivative peak 3 to peak 2 heights during linear sweep voltammetry as described in the text.

Table 2. The effect of temperature on the rate of cyclization of tetraphenylethylene dication.[a]

T/K	$v_{0.5}/V\ s^{-1}$ [b]	k/s^{-1}	$v_{0.7}/V\ s^{-1}$ [b]	k/s^{-1}
273.2	326(6)	604	178.2(2.5	563
263.1	117.5(7.0)	226	65.6(2.5)	215
251.6	45.1(4.7)	90.8	27.5(2.4)	94.8
241.2	16.1(1.5)	33.8	9.6(1.0)	34.4
E_a (kcal/mol)	11.6		11.2	
k_{298} (s^{-1})	3.4×10^3		3.0×10^3	
$\Delta S^{\neq}_{298}$ (cal/K mol)	−5.4		−7.0	
r [c]	−0.998		−0.998	

[a] Conditions as in Table 1. [b] The voltage sweep rates necessary for $p_3:p_2$ to equal 0.500 and 0.700. [c] Correlation coefficient from the Arrhenius correlation.

cyclization or to increased ordering of the solvent. In any case ΔS_{298} was not observed to be very large, only −6 cal/K mol as compared to −30 cal/K mol in the related cyclization of the anion radical of 1,3-dibenzoyl-1,3-diphenylpropane.[15] This comparison suggests that the restriction of rotations in the transition state in the latter reaction is much more severe. This is not unexpected since the carbonyl groups of the anion radical are separated by three methylenes and rotations are relatively free until bond formation begins.

The data reported here, along with that from other recent studies[15,16] provide a basis for the analysis of the mechanism of dimerization and cyclization reactions utilizing activation parameters. Mechanism assignments which include such studies as well as the determination of reaction orders are on much safer grounds than those based on experimental rate laws alone.

EXPERIMENTAL

The instrumentation and data retrieval system was the same as used in other recent publications.[15,16,20] TFA was reagent grade and used without further purification. Reagent grade dichloromethane containing the supporting electrolyte (Bu_4NBF_4) was passed through a column of neutral alumina before use.

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Short Communications

HPLC of Biopolymers on Columns of Agarose *

STELLAN HJERTÉN

Institute of Biochemistry, Biomedical Center, University of Uppsala, Box 576, S-751 23 Uppsala, Sweden

In 1961 agarose was introduced as a supporting medium for electrophoresis[1] and immuno-electrophoresis[2] and one year later for chromatography.[3] Very soon – and particularly when agarose became commercially available – it became routinely employed in most biochemical laboratories for these and similar purposes. The broad applicability of agarose can be ascribed to its low adsorption of most biopolymers, high rigidity and high porosity. For instance, a 12% (w/v) agarose gel has a more open structure than dextran and polyacrylamide gels of this concentration – and yet it is more rigid. This explains why agarose gels – and particularly the cross-linked ones[4,5] – can be used for HPLC with its requirement of hard and small gel beads for high resolution also at relatively high flow rates.[6,7] An example is shown in Fig. 1. The beads were prepared as described in Ref. 8. Even at the high flow rate used (0.6 ml/min) the resolution was satisfactory although it was better at lower flow rates, as expected.

There is an extensive literature on methods for the derivatization of agarose gels for their use in conventional affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, *etc.* All these derivatization methods can be applied to the high-concentration, crosslinked agarose beads. The way is therefore open to transform the above chromatographic methods [and others, including molecular-sieve chromatography (Figs. 1 and 2)] to the HPLC mode with agarose as a matrix.[6,7] Since agarose is comparatively inexpensive one can expect that not

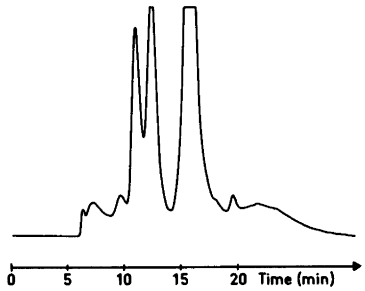

Fig. 1. High performance molecular-sieve chromatography of model proteins on 12% cross-linked agarose. Sample: a mixture of thyroglobulin (mol. wt. 669,000), catalase (232,000), aldolase (158,000), albumin (67,000), ovalbumin (43,000), chymotrypsinogen A (25,000), and ribonuclease A (14,000). Bed dimensions: 6 (i.d.) × 300 mm. Bead diameter: 1–10 μm. Sample volume: 6 μl. The amount of each protein: 3–25 μg. Buffer: 0.1 M sodium phosphate, pH 7.4. Pressure: 28 atm. Flow rate: 0.6 ml/min.

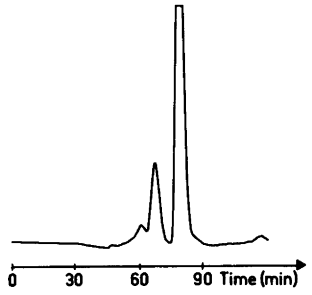

Fig. 2. Separation of monomer, dimer and trimer of human albumin by high performance molecular-sieve chromatography on 12% cross-linked agarose. Bed dimensions: 6 (i.d.) × 340 mm. Bead diameter: 2–10 μm. Sample volume: 10 μl. The amount of protein: 40 μg. Buffer: 0.2 M sodium phosphate, pH 6.8. Flow rate: about 0.1 ml/min. This diagram illustrates that a simple peristaltic pump (which was used in the experiment) can be employed for elution when there is no definite requirement of very short running times. A similar result was obtained on a column only 130 mm long (i.d. = 6 mm) when the flow rate was decreased to 0.02 ml/min (the run took about 3 h) and somewhat smaller non-cross-linked beads were used.

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only analytical but also preparative HPLC of biopolymers on agarose beds will be widely used in the future. An attractive feature of agarose columns is that they have a relatively low flow resistance, which means that they need not be operated by high pressure pumps but by cheaper peristaltic pumps, provided that moderate flow rates can be accepted (see Fig. 2). Another advantage is that the column tubes can be made from transparent glass or Plexiglass instead of stainless steel.

One can expect a higher resolution if the experiments shown in Figs. 1 and 2 are performed with gel beads of a more uniform size.

This communication is not a final report, but a presentation of work in progress.

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Separation of Glutathione *S*-Transferases from Rat Liver Cytosol by Chromatofocusing*

HELGI JENSSON, PER ÅLIN and BENGT MANNERVIK

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Glutathione *S*-transferases (EC 2.5.1.18) are a group of proteins, which play an important role in detoxification of electrophilic compounds.[1] They are present in all mammals investigated and high specific activities have been found in the liver of rodents.[2] Five forms of glutathione *S*-transferase, with isoelectric points in the range of pH 7 – 10, have been purified to homogeneity from rat liver cytosol. Conventional preparative isoelectric focusing has not given satisfactory separation of these forms. In the present study glutathione *S*-transferases from rat liver cytosol were separated and purified by use of affinity chromatography on *S*-hexylglutathione bound to epoxy-activated Sepharose 6B and by chromatofocusing. The chromatofocusing revealed 7 peaks of activity towards the substrate CDNB.**

Experimental. Rat liver cytosol was obtained as earlier described.[3] The microsome-free supernatant fraction (78 ml) was chromatographed on an *S*-hexylglutathione Sepharose affinity column, prepared as earlier described.[4] The affinity matrix (2 × 12 cm) was equilibrated with 10 mM Tris-HCl (pH 7.8). After application of the sample, the column was washed with the same buffer fortified with 0.2 M NaCl until protein was not further eluted. The GSH *S*-transferases were eluted with 5 mM *S*-hexylglutathione dissolved in 10 mM Tris-HCl (pH 7.8) containing 0.2 M NaCl. The pooled fractions from the affinity chromatography (74 ml) were desalted on a Sephadex G-25 column (4 × 30 cm) equilibrated with 5 mM Tris-HCl, pH 8.0. The desalted solution (130 ml) was concentrated to 4.5 ml by ultrafiltration and then applied on a column of chromatofocusing gel PBE 118 (1 × 30 cm) equilibrated with 25 mM triethylamine-HCl (pH 11) according to the instructions of the manufacturer

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**Abbreviations used: CDNB: 1-Chloro-2,4-dinitrobenzene; SDS: Sodium dodecylsulfate.

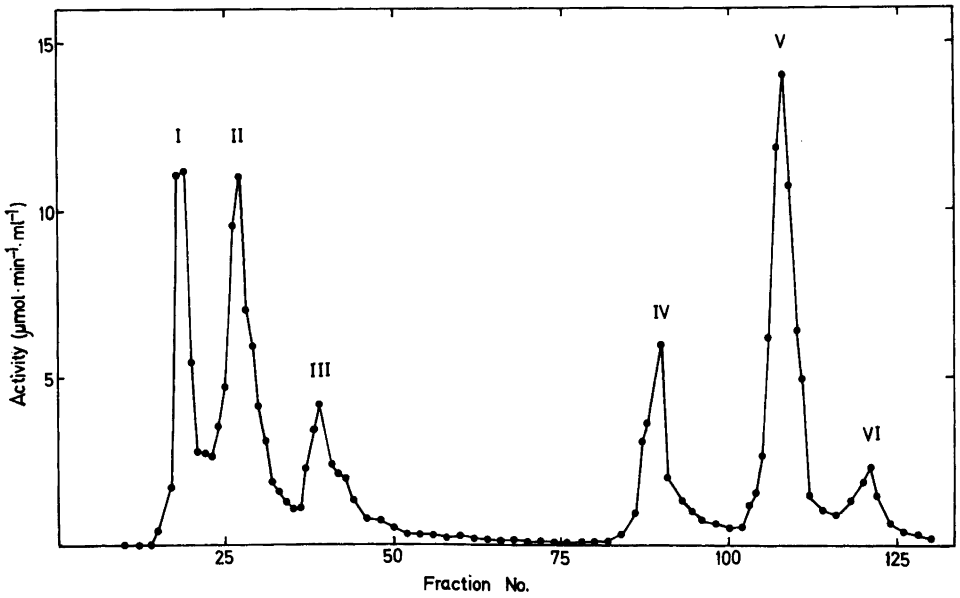

Fig. 1. Separation of glutathione *S*-transferases from rat liver cytosol by chromatofocusing. A sample partially purified by affinity chromatography was chromatographed on a column (1 × 30 cm) of chromatofocusing gel PBE 118. The enzymes were eluted with a pH gradient, pH 11 – 8, and enzymatic activity (●) was measured with CDNB as electrophilic substrate.

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Table 1. Separation of glutathione *S*-transferases.

Fraction	Volume (ml)	Total act.[a] (μmol/min)	Specific act. (μmol/mg min)	Yield (%)
Supernatant	78.0	859.6	1.15	100
S-Hexylglutathione Sepharose 6B	74.0	–	–[b]	–
Sephadex G-25	130.0	548.2	15.6	63.7
Chromatofocusing PBE 118, pH 10.5–8				
Peak I	5.0	44.5	30.9	
Peak II	9.0	72.6	25.1	
Peak III	9.0	27.6	17.7	
Peak IV	13.0	51.2	38.6	36.1
Peak V	13.0	92.2	30.0	
Peak VI	8.0	15.1	43.0	
Peak VII	5.0	6.8	3.0	

[a] Activity measured with CDNB as the electrophilic substrate. [b] *S*-Hexyl glutathione inhibits activity and interferes with protein measurements.

(Pharmacia Fine Chemicals, Uppsala). The enzymes were eluted with 380 ml of Pharmalyte, pH 8–10.5, diluted 1:80 and adjusted to pH 8 with HCl. Fractions of 2 ml were collected. Glutathione *S*-transferase activity was measured with CDNB as the electrophilic substrate as earlier described.[5] Protein concentrations were determined by the method of Lowry *et al.*[6] SDS slab gel electrophoresis was performed essentially as described by Laemmli.[7] Antibodies against glutathione *S*-transferases A and B were raised in rabbits using standard techniques of immunization. The antigens were mixed with Freund's complete adjuvant.

Results and discussion. Chromatofocusing resulted in seven clearly separated peaks of activity towards the substrate CDNB. Six of them were eluted with Pharmalyte buffer as shown in Fig. 1; an additional peak, peak VII, was eluted with 1 M NaCl. Table 1 summarizes the separation of the transferases. When tested by the double diffusion method of Ouchterlony against antibodies to glutathione *S*-transferases B and A, peaks I–III reacted with antibodies to transferase B and IV–VI with antibodies to transferase A, but not *vice versa*. Peak VII gave no precipitate with any of the two antisera. The transferases in peaks I, II, IV, V and VI were homogeneous as judged from SDS slab gel electrophoresis, but peak III contained one major band and one minor band. Peak VII was contaminated with a colored protein (hemoglobin?). The molecular weights of the subunits were estimated as 25 000 and 22 500 for the transferases in peaks I–III and to 23 500 for the transferases in peaks IV–VI. In view of these results the peaks I and II probably contained two different forms of glutathione *S*-transferase B and peaks IV and V correspond to transferases A and C, respectively. The identities of the three remaining peaks are unclear, although the data presented here suggest that peak III is related to transferase B and peak VI to transferases A and C. Peak VII seems not to be related to any of these transferases. Further characterization is required to definitely establish the identity of the peaks. The present paper describes a rapid and efficient procedure to prepare the various glutathione *S*-transferases in pure state. The novel procedure may facilitate further characterization of the molecular properties of and clarification of the relationship between the different forms.

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Tetrathiooxalate. Electrochemical Preparation and X-Ray Structure Determination of a Tetrathiooxalate

HENNING LUND,[a] EBERHARD HOYER[b] and RITA GRØNBÆK HAZELL[a]

[a]Department of Chemistry, University of Aarhus, 8000 Aarhus C, Denmark and [b]Department of Chemistry, Karl-Marx University, 7010 Leipzig, DDR

Some years ago it was shown [1,2] that earlier claims of the preparation of tetrathiooxalic acid [3,4] by reduction of carbon disulfide (*1*) could not be substantiated. Later a number of groups have investigated the reduction of carbon disulfide [5-14] electrochemically, by means of sodium amalgam, sodium in liquid ammonia or in hexamethylphosphoric triamide, potassium in DMF, or sodium naphthalenide in DMF. Similarly, carbon diselenide has been reduced.[15]

The reduction of *1* in aprotic medium followed by methylation yields [1,2] 4,5-bis(methylthio)-1,3-dithiole-2-thione (*2*) together with dimethyl trithiocarbonate (*3*), tetrakis(methylthio) ethylene and some minor products. It has been shown [14] by high-pressure liquid chromatography (HPLC) that tetrathiooxalate is formed during the electrochemical reduction in DMF, but the compound reacts slowly on standing in the mixture to trithiocarbonate dianion and other products.

Dimethyl tetrathiooxalate has been prepared [16,17] from *2* through photochemical decarbonylation of 4,5-bis(methylthio)-1,3-dithiol-2-one and some of its chemical reactions have been described.[18]

The formation of *2* has been suggested [1] to proceed through a primary formation of tetrathiooxalate dianion (*4*) by dimerization of $1^{\cdot -}$; an alternative mechanism has been proposed [6] in which *2* is formed by reaction between carbon sulfide and trithiocarbonate dianion.

The former reaction route has been shown [14] to be a major route to *2*, so there should be a chance of scavenging *4* in some way before it reacts further. An alkylating or acylating agent would produce compounds which would be expected to be further reducible under the employed conditions and thus not produce derivatives of *4*. A more promising possibility would be to work under conditions where *4* would form a slightly soluble salt; the tetrathiooxalate dianion would be expected to be difficultly reducible.

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It has been reported [10,12] that a yellow-brown precipitate has been obtained on electrochemical reduction of *1* in acetonitrile with tetraethylammonium bromide or perchlorate as supporting electrolyte; the precipitate has been suggested [12] from its elementary analysis, IR spectrum and chemical properties to be a bis(tetraethylammonium)hexathioperoxydicarbonate.

Electrochemical reduction of *1* in acetonitrile saturated with potassium iodide gave a brownish potassium salt in modest yield which was dissolved in water forming a red solution and precipitated with tetraphenylphosphonium chloride. Recrystallized from acetonitrile–diethyl ether it formed orange crystals (*4A*) which were analyzed as $C_{50}H_{44}O_2P_2S_4(C_{48}H_{40}P_2, C_2S_4, 2\,H_2O)$. The crystals were not suitable for X-ray structure analysis, but recrystallization from water produced needle-formed, orange crystals (*4B*) which according to the elementary analysis contained 6 mol of water of crystallization; the crystals were large enough for an X-ray examination. The elementary analysis suggested a tetrathiooxalate salt, but in order to establish the structure of *4B* an X-ray crystallographic analysis was made.

The crystals of *4B* are tetragonal, *P4*, with $a = 13.026(3)$; $c = 13.786(4)$ Å; $V = 2339$ Å^3; $D_x = 1.330$ Mg/m^3; μCuK$\alpha = 2.86$ mm^{-1}. The structure consists of an I-centred arrangement of the tetraphenylphosphonium ions in a cell with the *c*-axis halved. The anions and water molecules are placed in holes in this lattice as shown in Fig. 1. The tetrathiooxalate ion is placed across a twofold axis but is far from planar, the torsion angle being 79.5° (1.0), in contrast to the oxalates (*5*) which are usually planar [19] or nearly so,[20] but analogous to the torsion angle in potassium dithiooxalate (76.5°).[21]

Table 1. Selected distances and angles. The mark ′ denotes symmetry relation $2-x,1-y,z$.

Distance	Å	Angle	Degrees
C–S1	1.713(9)	S1–C–S2	128.6(6)
C–S2	1.691(10)	S1–C–C′	114.7(8)
C–C′	1.461(19)	S2–C–C′	116.6(8)
S2–S2	3.068	C–P1–C	107.5 × 2
S1–S2′	3.530		110.5 × 4
S1–O3	3.339	C–P2–C	106.7 × 2
S2–O4	3.347		110.9 × 4
O1–O3	2.961	C–P3–C	107.7
O2–O4	2.905		110.8 × 4
P1–C	1.794		105.9
P2–C	1.796		
P3–C	1.793	Torsion angle	
	1.802	S1–C–C′–S2′	79.5(1.0)

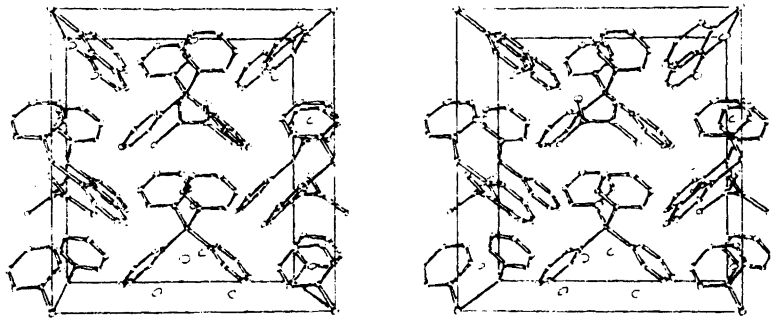

Fig. 1. Stereoscopic view of the structure of *4B*. The *a*-axis is horizontal, *c* vertical in the projection. The unconnected atoms are water molecules.

The large torsion angle in *4B* prevents close contacts between the sulfur atoms, but may also be enforced by the lattice. Indeed, the four water molecules in the similar hole *c*/2 away are arranged in almost the same way as the four sulfur atoms. The most important dimensions are given in Table 1. Unfortunately the accuracy is limited, and it is not possible to decide whether the two C–S bonds are equal, but they do show considerable double bond character. The C–C bond (1.46 Å) is apparently shorter than the bond in oxalate (1.56 – 1.58 Å);[19,20] the C–C length in potassium dithiooxalate is 1.516.[21]

The π-electron systems in the two dithiocarboxyl groups of *4* are situated nearly as in allene and the length of the central C–C bond can be understood in this context. The difference between *4* and *5* with respect to torsion angle and C–C distance is probably connected with the much higher electronegativity of oxygen compared to that of sulfur.

Experimental. Preparation of ditetraphenylphosphonium tetrathiooxalate. Carbon disulfide (2 ml) was reduced at a mercury cathode at 0 °C in acetonitrile saturated with potassium iodide (~0.2 M) at −1.0 V *vs.* Ag/AgI (0.2 MI^-). During the reduction was formed a brown precipitate which was washed with ether and dissolved in water. On addition of tetraphenylphosphonium chloride (2 g in 20 ml H_2O) a precipitate (0.7 – 1.5 g) was obtained; the yield varied for unknown reasons, in some cases a low water content in the acetonitrile (~0.1 %) during electrolysis seemed preferable to (nominally) anhydrous acetonitrile. The precipitate was filtered, washed with cold acetonitrile, and recrystallized from acetonitrile – diethyl ether (*4A*) or water (*4B*). The crystals from A darkened at 130 °C, melted about 160 °C, solidified about 190 °C. Found: C 69.21; H 5.05; S 14.60. Calc. for $C_{50}H_{40}P_2S_4$, 2 H_2O: C 69.26; H 5.11; S 14.79. IR spectrum (KBr, cm^{-1}): 3500 – 3300(w), 3040(w), 1580(w), 1478(m), 1437(s), 1105(s), 983(s), 968(sh), 925(w), 750(m), 720(s),

Table 2. Final coordinates and mean square vibration amplitudes for the tetrathiooxalate ion, the water molecules and the phosphorus atoms. The coordinates of the phenyl carbon atoms and the hydrogen atoms can be obtained from the author Rita Hazell. The expression for the temperature factor is exp$[-2\pi^2(h^2a^{*2}u_{11}+...2klb^*c^*u_{23})]$.

	x	*y*	*z*	u_{11}	u_{22}	u_{33}	u_{12}	u_{13}	u_{23}
S1	0.9711(2)	0.3590(2)	0.4467(2)	0.070(2)	0.033(2)	0.039(2)	−0.007(2)	0.010(1)	0.005(1)
S2	1.1326(2)	0.4410(2)	0.3045(2)	0.039(2)	0.070(2)	0.039(2)	0.011(2)	0.008(1)	−0.005(2)
C0	1.0263(8)	0.4505(7)	0.3742(8)	0.068(7)	0.036(6)	0.040(5)	0.008(5)	−0.016(6)	−0.004(5)
O1	0.5422(11)	0.1386(11)	0.1875(9)	0.116(11)	0.080(8)	0.067(9)	−0.010(8)	0.017(6)	0.007(8)
O2	0.3688(11)	0.0402(13)	0.0619(8)	0.088(8)	0.143(11)	0.058(8)	0.021(8)	0.020(7)	0.004(8)
O3	0.5000	0.0000	0.3530(8)	0.066(10)	0.095(10)	0.049(10)	0.003(8)	0.000	0.000
O4	0.0000	0.5000	0.1042(8)	0.088(11)	0.091(9)	0.039(9)	0.003(8)	0.000	0.000
P1	0.0000	0.0000	0.0000	0.023	0.023	0.023	0.000	0.000	0.000
P2	0.0000	0.0000	0.5000	0.025	0.025	0.030	0.000	0.000	0.000
P3	0.5000	0.5000	−0.2520(5)	0.024	0.026	0.025	−0.001	0.000	0.000

685(s). Crystals from (B): C 63.95; H 5.57. Calc. for $C_{50}H_{40}P_2S_4$, 6 H_2O: C 63.94; H 5.58. The IR spectrum had, besides those described for (A), medium-strong bands at 3500–3300 and 1630 cm^{-1}. UV spectrum (96% ethanol, nm): λ_{max}: 222 (ε 10^5), 269 (ε 1.4×10^4), 277 (ε 1.7×10^4), 343 (broad, ε 1.6×10^4), 380 (shoulder, ε 7.4×10^3).

X-Ray technique. Symmetry and preliminary cell dimensions were obtained from films. A crystal of $0.1 \times 0.1 \times 0.5$ mm^3 was used for the datacollection on a Packer FACS1 diffractometer with Cu$K\alpha$ radiation. The cell was refined using 12 reflections centred at $\pm 2\theta$. Data for $h,k,l \geq 0$ out $\theta = 60°$ were collected using a step-scan-technique giving 1824 independent reflections. Profile analysis by the Lehmann-Larsen method,[22] Lorentz polarization and absorption corrections were applied.

The structure was solved by means of the MULTAN programme package [23] but not till it was applied to reflections with $l = 2n$ only. Reflections with $h+k+l/2 = 2n$ are 10 times stronger than the other reflections because of the special arrangement of the cations. Refinement by full matrix least squares was only stable when constraints were introduced:[24] The phenyl rings were constrained to be identical and with *mm2* symmetry and each tetraphenylphosphonium ion was treated as a rigid body with extra libration around the P–C bonds giving a total of 138 refineable parameters. The final R-value was 0.062 for 1370 reflections with $I > 3\sigma(I)$.

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Reactions of Benzylic Compounds. Nucleophilicity, Leaving Group Ability and Carbon Basicity of some Ionic Nucleophiles in Acetonitrile. Comments on the Utility of the Finkelstein Reaction in Synthesis

KNUT MAARTMANN-MOE, KNUT A. SANDERUD and JON SONGSTAD

Department of Chemistry, University of Bergen, N-5014 Bergen, Norway

The reactions of some 4-substituted benzylic compounds, 4-Z-$PhCH_2X$, with various ionic nucleophiles, Y^-, (Z = NO_2, H and Me; X and Y = Cl, Br, I, SCN and SeCN) have been studied under homogeneous conditions in acetonitrile at 25.0 °C. All the reactions have been found to proceed through nucleophilic attack at the methylene carbon atom. Isothiocyanates, 4-Z-$PhCH_2NCS$, and isoselenocyanates, 4-Z-$PhCH_2NCSe$, are not formed. The reactions obey second-order kinetics, first order in each of the reactants. The halide ions and the selenocyanate ion show similar nucleophilic strength and are five to ten times at reactive as the thiocyanate ion. The average leaving group ability is $I^- > Br^- \gg Cl^- > NCSe^- > NCS^-$. From the equilibrium constants the average carbon basicity order is $NCSe^- \sim NCS^- \sim Cl^- \gg Br^- > I^-$. The relative basicity of $NCSe^-$, NCS^- and Cl^- is slightly dependent upon Z; in 4-Me-$PhCH_2X$ the order is $NCS^- \geq NCSe^- > Cl$ while in 4-NO_2-$PhCH_2X$ the order is $NCSe^- > NCS^- \sim Cl$.

The tellurocyanate ion, $NCTe^-$, is ten times as reactive as $NCSe^-$ toward 4-NO_2-$PhCH_2Cl$ and no equilibrium is established ($K > 10^4$). The reaction product is an addition compound from the first formed organic tellurocyanate and the displaced chloride ion, the $[4\text{-}NO_2\text{-}PhCH_2TeCN(Cl)]^-$-anion, a Te(II)-tellurate. The results from an X-ray structure determination of $[(Ph_3P)_2N][4\text{-}NO_2\text{-}PhCH_2TeCN(Cl)]$ is presented in brief. In the adduct Cl is *trans* to the cyano group and the Te–Cl bond, 2.923(2) Å, is more than 1 Å shorter than the sum of the van der Waals' radii.

A nucleophilic substitution reaction may be illustrated by the general equation, eqn. (1), where Y

$$Y + AX \underset{k_r}{\overset{k_f}{\rightleftarrows}} AY + X \qquad (1)$$

is the nucleophile, X is the leaving group and A is the substrate. In many cases the leaving group X exhibits some nucleophilic strength toward the product AY and an equilibrium will be established.[1] The ratio of the forward rate constant, k_f, and the reverse rate constant, k_r, is the equilibrium constant, K.

To be able to describe in some detail a reaction system as depicted by eqn. (1), it appears necessary to consider a number of factors: *1.* The nucleophilicity of Y toward the reaction center. *2.* The leaving group ability of X which is in some way related to the energy of the bond to be broken and is dependent upon the structure of the transition state and the nature of the nucleophile Y.[2,3] *3.* The relative element basicity of Y and X as given by the equilibrium constant K which serves as a measure of the possible yield of products. For organic reactions, *i.e.*, when A represents an organic group R, the term carbon basicity applies.[4]

To obtain AY in high yield and of high purity, reaction time and temperature have to be controlled to avoid undesirable by-products due to side-reactions of higher activation energies and from decomposition. A favourable equilibrium constant will first of all be obtained by choosing a leaving group, X, of modest nucleophilicity, but also by experimental conditions by which the nucleophilicity of Y is enhanced [5] and that of X is suppressed or by deactivation or removal of X from the phase in

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which the reaction takes place.

In order to get information about the various factors determining k_f, k_r and thus K for a reaction system the reaction must be well defined, *i.e.* undisturbed by side reactions, and its equilibrium approached with suitable rapidity to allow determination of the rate constants. In the present study we report on some Finkelstein reactions[6] involving various 4-substituted benzylic compounds, eqn. (2).

$$Y^- + 4\text{-Z-PhCH}_2X \underset{k_r}{\overset{k_f}{\rightleftarrows}} 4\text{-Z-PhCH}_2Y + X^- \qquad (2)$$

$Y^- = I^-$, Br^-, Cl^-, NCS^-, $NCSe^-$ and $NCTe^-$

X = I, Br, Cl, SCN and SeCN

$Z = NO_2$, H and Me

Using acetonitrile as solvent and bis(triphenylphosphine)iminium salts, $[(Ph_3P)_2N]Y$, abbreviated [PNP]Y, the reactions are homogeneous. The reactions are sufficiently slow for kinetic measurements and have K-values of suitable magnitude. The use of ^{13}C-pseudohalide ions and the IR-technique allowed exchange reactions involving organic pseudohalides and pseudohalide ions to be studied. A comparable study of 2-substituted-1-phenyl-ethanones (phenacyl compounds) has recently been published.[7]

EXPERIMENTAL

Materials. Solvents. Acetonitrile, Baker Analyzed Reagent, was first distilled from phosphorus pentoxide and then from calcium chloride in an argon atmosphere. The purified solvent was stored over Linde 4 Å molecular sieves in darkness. All the hydrocarbons and diethyl ether were treated with metallic sodium. Acetone, Baker Analyzed Reagent, was used as received. Dichloromethane was fractionated after the usual treatment with concentrated sulfuric acid. All solvents used for reactions involving the tellurocyanate ion were carefully flushed with argon prior to use.

Benzyl compounds. 4-Nitrobenzyl chloride and 4-nitrobenzyl bromide, Fluka *purum*, were crystallized from acetonitrile–diethyl ether and finally from cyclohexane. Benzyl chloride and benzyl bromide, Fluka *puriss*, were flushed for several hours with nitrogen to remove traces of HCl and HBr, then distilled in vacuum with an argon leak. A mid-fraction was used for the kinetic studies. 4-Methylbenzyl bromide, Fluka *purum*, was twice crystallized from cyclohexane.

4-Nitrobenzyl iodide was prepared according to Finkelstein,[6] crystallized from acetone, dried at 0.1 mmHg and stored in darkness. Benzyl iodide was made according to Kumpf,[8] m.p. 25 °C (diethyl ether – pentane) (24 °C [8]). This compound was only used for the recording of its ^1H NMR spectrum, *cf.* Table 1.

4-Nitrobenzyl thiocyanate was prepared according to Bennet and Berry[9] from 4-nitrobenzyl bromide and dry potassium thiocyanate in acetone. After two crystallizations from benzene–diethyl ether the compound was dried at 0.1 mmHg. M.p. 85 – 86 °C (85.5 °C [9]). $M^{\cdot+}$ 194(15); *m/e* 136(100). 4-Nitrobenzyl selenocyanate was prepared and purified in a similar way, m.p. 122 °C (122.5 °C [10]). $M^{\cdot+}$ 240(6) and 242(12), *m/e* 136(100). 4-Nitrobenzyl tellurocyanate was prepared as previously described [11] and crystallized twice from acetone – diethyl ether and finally from dichloromethane, m.p. 123 °C (dec). $M^{\cdot+}$ 292(13), 290(11), 288(7); *m/e* 136(100), 401(3).

Benzyl thiocyanate was made in the same way as 4-nitrobenzyl thiocyanate. After two crystallizations from diethyl ether – pentane the m.p. was 42 °C (41 °C [12]). $M^{\cdot+}$ 149(14); *m/e* 91(100). Benzyl selenocyanate was prepared in a similar way, m.p. 72 °C (diethyl ether) (70 – 71 °C [13]). $M^{\cdot+}$ 195(8), 197(17); *m/e* 91(100).

4-Methylbenzyl chloride was made from the commerically available bromide and tetraphenylarsonium chloride in acetonitrile: To 13.2 g Ph_4AsCl, 0.03 mol, dried at 70 °C for 4 h at 0.1 mm Hg, dissolved in a minimum quantity of acetonitrile, was added 4.6 g 4-methylbenzyl bromide, 0.025 mol, in 25 ml acetonitrile. The mixture was stirred at room temperature for 3 h and left for 1 h at 0 °C. The least soluble salt, tetraphenylarsonium bromide, together with some unreacted chloride was removed by filtration whereupon the solvent was removed in vacuum. The residue was treated with five 50 ml portions of diethyl ether and the combined diethyl ether extracts distilled in vacuum. Yield 2.1 g (60 %), b.p. 92 °C/19 mmHg, (95 °C/20 mmHg [14]). $M^{\cdot+}$ 140(42), 142(14); *m/e* 105(100).

4-Methylbenzyl thiocyanate was made as the 4-nitro-substituted compound. This compound was crystallized from cyclohexane – pentane and purified by sublimation. M.p. 24 °C. $M^{\cdot+}$ 163(7); *m/e* 105(100). The corresponding selenocyanate, a new compound, was obtained in a similar way and crystallized from cyclohexane. The yield was 57 %. M.p. 56 °C. The compound was further purified by sublimation. (Found: C 51.64; H 4.23; N 6.62. Calc. for C_9H_9NSe: C 51.14; H 4.32; N 6.67). $M^{\cdot+}$ 213,211(~1), 209,208,207; *m/e* 105(100).

[PNP]$^+$-salts. These salts were prepared as previously described [7,15] and dried at ~75 °C at 0.1

mmHg to constant weight prior to use. [PNP]Cl m.p. 272–273 °C (273–274 °C[15]), [PNP]Br m.p. 254–255 °C (253–255 °C[15]), [PNP]I m.p. 253–254 °C (252–254 °C[15]), [PNP]SCN m.p. 186–187 °C, (187–188 °C[15]), [PNP]SeCN m.p. 196–197 °C, (182–185 °C[15]), [PNP]TeCN m.p. ~190 °C (dec) (190–193 °C (dec)[15]). The ^{13}C-enriched salts were made from potassium ^{13}C-cyanide,[15] 90.5 % enriched, used as received from Prochem, British Oxygen Co. Ltd.

[PNP]-chlorocyano-4-nitrobenzyltellurate(II). To 0.05 g 4-nitrobenzyl chloride, 0.003 mol, in 40 ml acetone was added 1.73 g [PNP]-tellurocyanate, 0.0025 mol, in 20 ml acetone. The reaction mixture was stirred for 24 h at room temperature. After filtration (to remove traces of Te and TeO_2) the yellow-green solution was evaporated nearly to dryness. Upon addition of a small amount of diethyl ether traces of the white [PNP]-chloride first precipitated and was removed by filtration. The very soluble [PNP]-tellurate, precipitated in close to quantitative yield after 2 days at ~ −10 °C after addition of a considerable amount of diethyl ether. This purification procedure was repeated once to remove traces of [PNP]Cl whereupon a yield of 1.7 g (76%) of yellow-brownish needles was obtained. M.p. ~120 °C (dec.). (Found: C 61.61; H 4.34; N 4.95. Calc. for $C_{44}H_{36}ClN_3O_2P_2Te$: C 61.18; H 4.20; N 4.86). U.V.: Shoulder to the NO_2-peak at ~350 nm, log ε ~3.3. I.R.: The spectrum (KBr-pellet) in the 400–4000 cm^{-1} range appeared as a superposition of the spectra of [PNP]Cl and of 4-nitrobenzyl tellurocyanate except for the peak at ~2100 cm^{-1} due to the the C–N group in nitrobenzyl tellurocyanate which had disappeared.

Crystal structure determination of [PNP][4-$NO_2PhCH_2TeCN(Cl)$]. Elongated prisms, suitable for the X-ray study were obtained from acetone –diethyl ether. A crystal with dimensions 0.25 × 0.07 × 0.08 mm was selected. Space group $P\bar{1}$ (No. 2), $Z=2$, $a=9.833(2)$, $b=14.435(2)$ and $c=16.307(2)$ Å; $\alpha=66.09(1)°$, $\beta=78.63(1)$ and $\gamma=71.77(1)°$; $V=2003$ Å^3, d(calc.) = 1.432 g cm^{-3}. Intensity data were collected on a CAD4 Enraf-Nonius diffractometer using monochromated Mo$K\alpha$ radiation ($\lambda=0.71073$ Å). The number of reflections recorded at room temperature was 7051 of which 3589 with $I>2.0\sigma(I)$ were retained for the structure determination. No decomposition could be observed during X-ray exposure. The structural parameters were determined with the Enraf-Nonius Structure Determination Pack revised in 1980. Full-matrix least squares refinement led to a final conventional R-value of 0.048 ($R_w=0.046$). The estimated standard deviation of the intensity, E.S.D., was 1.136. Tables of observed and calculated structure factors and atomic coordinates *etc.* are available from the authors.

Determination of rate and equilibrium constants. In Table 1 are listed some IR, UV and NMR data for

Table 1. IR, UV and NMR data for benzyl compounds, RX, and some anions, X^-, in acetonitrile.

X^-, RX	ν_{NCX} (cm^{-1})	$\varepsilon_{320\,nm}$	$\varepsilon_{350\,nm}$	δ_{CH_2} (Rel. TMS)
$N^{12}CS^-$	2058			
$N^{13}CS^-$	2011			
$N^{12}CSe^-$	2067			
$N^{13}CSe^-$	2021			
$N^{12}CTe^-$	2081			
4-NO_2PhCH_2Cl		493	236	4.75
4-NO_2PhCH_2Br		754	293	4.66
4-NO_2PhCH_2I			727	4.58
4-NO_2PhCH_2SCN	2159			4.28
4-NO_2PhCH_2SeCN	2150			4.30
4-NO_2PhCH_2TeCN	2157	(Shoulder at ~340 nm, log ε ~3.3)		4.36
[4-$NO_2PhCH_2TeCN(Cl)$]$^-$		(Shoulder at ~350 nm, log ε ~3.3)		4.36
[PNP]Cl		2	0	
$PhCH_2Cl$				4.65
$PhCH_2Br$				4.62
$PhCH_2I$				4.54
$PhCH_2SCN$	2165			4.20
$PhCH_2SeCN$	2158			4.28
4-$MePhCH_2Cl$				4.64
4-$MePhCH_2SCN$	2158			4.28
4-$MePhCH_2SeCN$	2149			4.26

Table 2. Second-order rate constants, k_f, relative rate constants, k_{rel}, (rate constants for RSCN are unity) and equilibrium constants, K, for reactions between 4-Z-PhCH$_2$X and Y$^-$ in acetonitrile at 25.0 °C.

Z	X	Y$^-$	k_f/M^{-1} s^{-1}	k_{rel}	K
NO_2	Br	$NCSe^-$	$2.1(8)\times10^{-1}$ [a]	8.5×10^2	$1.4(5)\times10^3$ [b]
NO_2	Cl	$NCSe^-$	$1.3(3)\times10^{-3}$	3.4	2.6(5)
NO_2	SeCN	$NCSe^-$	$0.9(1)\times10^{-3}$	2.4	1
NO_2	SCN	$NCSe^-$	$3.8(6)\times10^{-4}$	1	2.6(5) [b]
NO_2	Br	NCS^-	$4.5(5)\times10^{-2}$ [c]	2.1×10^3	$3.2(1.2)\times10^2$ [b]
NO_2	Cl	NCS^-	$1.8(3)\times10^{-4}$	8.6	1.4(2)
NO_2	SeCN	NCS^-	$1.2(3)\times10^{-4}$	5.7	0.38(7)
NO_2	SCN	NCS^-	$2.1(4)\times10^{-5}$	1	1
NO_2	I	Cl^-	1.2(2)	1.0×10^4	$1.0(3)\times10^3$ [d]
NO_2	Br	Cl^-	$2.1(4)\times10^{-1}$	1.8×10^3	$3.0(7)\times10^2$ [b]
NO_2	Cl	Cl^-	5.8×10^{-4}	~7 [f]	1
NO_2	SeCN	Cl^-	$4.7(5)\times10^{-4}$	3.9	0.38(6)
NO_2	SCN	Cl^-	$1.2(2)\times10^{-4}$	1	0.8(1)
NO_2	I	Br^-	1.8(3)	1.4×10^4	3.3(5) [b]
NO_2	Cl	Br^-	$6.3(1.4)\times10^{-4}$	4.8	$3.3(7)\times10^{-3}$ [d]
NO_2	SeCN	Br^-	$2.5(1.0)\times10^{-4}$ [g]	1.9	$3.1(1.1)\times10^{-3}$
NO_2	SCN	Br^-	$1.3(0.6)\times10^{-4}$	1	$3.1(1.1)\times10^{-3}$
NO_2	Br	I^-	0.5(0.2) [g]	4.2×10^2 [h]	0.30(0.04) [d]
NO_2	Cl	I^-	$1.2(0.4)\times10^{-3}$ [g]	1 [h]	$1.0(0.3)\times10^{-3}$ [b]
NO_2	Cl	$NCTe^-$	$1.6(2)\times10^{-2}$		$>1\times10^4$
H	I	Cl^-	6×10^{-1} [i]	$\sim\times10^3$ [j]	10^3 [i]
H	Br	Cl^-	1.2×10^{-1} [i]	$\sim1.6\times10^3$ [j]	2.4×10^2 [i]
H	Cl	Cl^-	6.5×10^{-4} [i]	~10 [j]	1
H	SeCN	Cl^-	$2.4(0.4)\times10^{-4}$	4.9	0.40(0.05)
H	SCN	Cl^-	$4.9(0.6)\times10^{-5}$ [k]	1	0.48(0.06)
H	Cl	I^-	6.0×10^{-3} [i]		1×10^{-3} [i]
H	Cl	Br^-	6.0×10^{-3} [i]		1.4×10^{-1} [i]
H	Cl	$NCSe^-$	$6.0(0.7)\times10^{-4}$		2.4(0.4)
H	Cl	NCS^-	$1.2(0.3)\times10^{-4}$		2.3(0.3)
Me	Cl	Cl	4.6×10^{-4} [e]		1
Me	Cl	$NCSe^-$	$9.5(1)\times10^{-4}$		2.4(4)
Me	Cl	NCS^-	$2.1(0.3)\times10^{-4}$		3.0(5)
Me	SeCN	Cl^-	$3.8(0.4)\times10^{-4}$	5.3	0.42(0.06)
Me	SCN	Cl^-	$7.2(0.9)\times10^{-5}$	1	0.36(0.05)

[a] $3.2(2)\times10^{-1}$ M^{-1} s^{-1} in Ref. 16. [b] Calculated from K for the reverse reaction. [c] $5.4(3)\times10^{-2}$ M^{-1} s^{-1} in Ref. 16. [d] Determined by ^{1}H NMR. [e] At 20.0 °C, Ref. 17. [f] Assuming the rate constant at 25.0 °C to be 50% higher than at 20.0 °C. [g] Calculated from K and k_r. [h] Rate constant of RCl defined as unity. [i] At 30.0 °C, Ref. 18. [j] Assuming the rate constant at 25.0 °C to be 30% lower than at 30.0 °C. [k] $k_f = 5.4\times10^{-5}$ M^{-1} s^{-1} at 30.0 °C, Ref. 18. [l] From rate constant at 20.0 °C, $k_f = 3.1\times10^{-4}$ M^{-1} s^{-1} +50%, Ref. 17.

the various compounds which formed the basis for the analytical methods applied for the determination of rate and equilibrium constants. The rate of the reactions between the organic halides and the halide ions were determined by UV; for reactions involving the pseudohalide ions the IR technique was used throughout. For the majority of the reactions the equilibrium constants, K, were determined from the ratio between the forward and the reverse rate constants, k_f and k_r, and checked by UV and IR of kinetic runs at infinite time or by NMR of more concentrated solutions. Some rate constants could only be determined by the ratio between K and k_f or between K and k_r, *cf.* footnotes in Table 2.

The rate constants were calculated from kinetic runs performed under pseudo first-order or second-order conditions, depending upon rate and

equilibrium constants. The concentration of the ionic nucleophiles was in the $(2-10)\times10^{-3}$ range. When calculating the equilibrium constants the activity coefficients of the two competing anions were assumed to be the same. No decomposition could be observed during any of the studied reactions and the linearity of the rate plots was generally satisfactory for several half-lives suggesting the reactions to be clean second-order reactions, first order in each of the reactants. For further details with regard to the experimental procedures, *cf.* Ref. 7.

Calculations. From the general equation, eqn. (3),

$$RA + B^- \underset{k_r}{\overset{k_f}{\rightleftarrows}} RB + A^- \qquad (3)$$

where k_f and k_r are the second-order rate constants, one obtains the integrated rate equations (4)–(7) when $K_r = k_r/k_f$, a and b are the initial concentrations of the reactants and x is the concentration of product at time t.

$a \neq b, \quad K_r \neq 0$:

$$Z^{-\frac{1}{2}} \ln \frac{2ab - x(a+b-Z^{\frac{1}{2}})}{2ab - x(a+b+Z^{\frac{1}{2}})} = k_f t \qquad (4)$$

where $Z = (a-b)^2 + 4abK_r$

$a = b, K_r \neq 0$:

$$(2aK_r)^{-1} \ln \frac{a - x(1-K_r^{\frac{1}{2}})}{a - x(1+K_r^{\frac{1}{2}})} = k_f t \qquad (5)$$

$a = b, K_r \ll 1$:

$$\frac{x}{a(a-x)} = k_f t \qquad (6)$$

$a \gg b, K_r \neq 0$

$$M^{-1} \ln \frac{2ab - x(a-M^{\frac{1}{2}})}{2ab - x(a+M^{\frac{1}{2}})} = k_f t \qquad (7)$$

where $M = a^2 + 4abK_r$

For the isotope exchange reactions, *i.e.*

$$RXCN + X^{13}CN^- \xrightarrow{k_f} RX^{13}CN + XCN^-,$$

employing $X^{13}CN^-$ of 90.5 % isotopic purity in ^{13}C, the rate equation (8) is valid since K is necessarily

$$\frac{1}{a+b} \ln \frac{0.905\,ab}{(a+b)x - 0.905b^2} = k_f t \qquad (8)$$

$$\frac{1}{2a} \ln \frac{0.905\,a}{2ax - 0.905a} = k_f t \qquad (9)$$

unity. For equal concentrations of reactants, $a = b$, this equation reduces to eqn. (9). A general program was written and used for the evaluation of the rate constants according to eqns. (4)–(9).

Instrumental. The UV measurements were performed with a Perkin Elmer 555 Spectrophotometer employing 1 and 0.1 cm quartz cells. A Perkin Elmer 399B Infrared Spectrophotometer and 0.05 cm liquid cells were used for the IR measurements. A Varian EM 360 A NMR Spectrometer was used for the determination of the ^1H NMR data.

All rate and equilibrium constants determined by IR and UV were calculated from measurements performed at 25.0(1) °C. Up to 10 aliquots were withdrawn periodically for each kinetic run. The NMR experiments were performed at ambient temperature, 25(2) °C.

RESULTS

In Table 2 are listed the second-order rate constants, k_f, the equilibrium constants, K, and the relative rate constants, k_{rel}, obtained in the present study. (The rate constants for RSCN are defined as unity.) Table 2 also includes some rate constants of $RCl + X^-$ and of $RX + Cl^-$ at 20.0 °C and at 30.0 °C determined by Hayami and co-workers using ^{36}Cl.[17,18] Their rate constants are in general agreement with the present data with the exception of the rate of the reactions between benzyl chloride and ionic bromide and iodide which tend to be far too high as compared with the rate constants for the corresponding reactions of 4-nitrobenzyl chloride determined in the present study. We cannot offer a satisfactory explanation for this discrepancy. In order to make a comparison with our rate constants at 25.0 °C the rate constants at 20.0 °C[17,18] were given an increment of 50 % while the rate constants at 30.0 °C[17,18] were reduced with 30 %.

Admittedly, some of the rate constants and especially some of the equilibrium constants are of rather modest accuracy. Equilibrium constants outside the $10^{-2}-10^2$ range were particularly difficult to determine accurately. Likewise, rate constants for reactions which progressed only to a small extent ($K \ll 1$) could only be determined with very limited accuracy. (Example: $RSCN + Br^- \xrightarrow{k_f} RBr + NCS^-$.)

In none of the reactions studied could any significant amounts of by-products be detected by IR or by NMR, even after prolonged reaction times. Owing to the large extinction coefficients of RNCS and RNCSe in the $2000-2200\ cm^{-1}$ region even trace amounts of these compounds should be possible to detect by IR. Previously it has been shown that both in the benzyl[18,21] and in the phenacyl series[7] only esters of the thiocyanic acid, RSCN, and of the selenocyanic acid, RSeCN, are formed by substitution reactions in acetonitrile. Toward benzyl halides in acetonitrile the sulfur end of the potentially ambident thiocyanate ion is known to be three orders of magnitude more reactive than is the nitrogen end.[22] No data are presently available for the Se/N ratio for the selenocyanate ion but in view of the high reactivity of the selenium atom, *cf.* Table 2, this ratio may actually be even higher than 10^3 as observed for the S/C ratio in the case of the thiocyanate ion.

Benzylic compounds are also known to act as ambident species, *i.e.* other atoms than the methylene carbon atom may be attacked by nucleophilic species. Of special interest are the sulfur and the selenium atoms in reactions as depicted by eqn. (10) (in the case of Se). No such

$$RSeCN + NCSe^- \rightarrow RSeSeCN + CN^- \qquad (10)$$

products could be detected in any significant amounts by IR and the anticipated yellow colourization of the reaction solution due to RSeSeCN could not be observed. Schiavon[22] has shown by isotopic exchange studies in acetonitrile that the rate of the reaction according to eqn. (10) is only $\sim 1\,\%$ of the rate of the reaction which proceeds through attack at the methylene carbon atom. The sulfur atom in RSCN is an even less reactive electrophilic atom.[23]

The majority of the experiments in the present study were performed with 4-nitro-substituted compounds ($Z = NO_2$). This choice was governed by the fact that all 4-nitro-substituted benzyl compounds are well-crystalline and most stable substances and thus simple to purify by crystallization. Furthermore, these compounds are known to be the classic substrates with regard to the S_N2 mechanism without contributions from S_N1-like mechanisms. The high reactivity of the methylene carbon atom[16] causes side-reactions as the one depicted by eqn. (10) to be kinetically of negligible importance.[22] However, the fairly high acidity of the methylene protons in this class of compounds[24] prevents the use of nucleophiles of some hydrogen basicity in this type of study, particularly in dipolar aprotic solvents.[5]

As a final test of the equilibrium constants determined in the present study and thus also of the rate constants listed in Table 2, the equilibrium constants were the subject of an internal consistency analysis as described by Hine and Weimar.[25] From an equilibrium constant $K_{A/B}$ for a reaction $RB + A \rightleftarrows RA + B$ and $K_{B/C}$ for $RC + B \rightleftarrows KB + C$, $K_{A/C}$ for $RC + A \rightleftarrows KA + C$ is given by the ratio between $K_{A/B}$ and $K_{B/C}$ and can be compared with the experimentally determined value of $K_{A/C}$. When taking the estimated uncertainties into account, no discrepancies between calculated and experimentally determined equilibrium constants could be observed.

The listed equilibrium constant for the reaction between the tellurocyanate ion and 4-NO_2-$PhCH_2Cl$, $K > 10^4$, is only a crude estimate. No trace of ionic tellurocyanate could be detected by IR from the reaction between the tellurate ion, [4-NO_2-$PhCH_2TeCN(Cl)]^-$, and a large excess of ionic chloride. Fig. 1 shows the structure of this tellurate anion from the X-ray study of the [PNP]-salt; the Cl

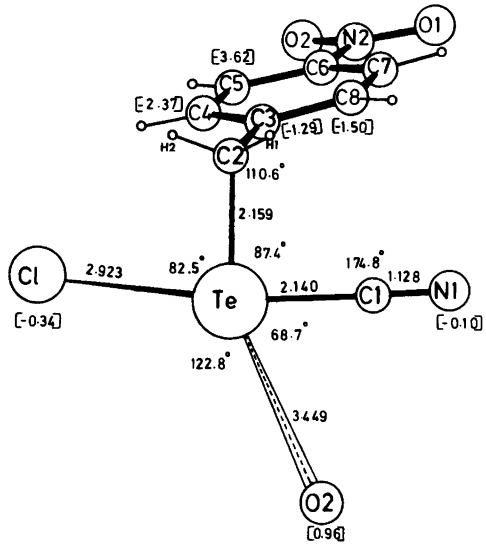

Fig. 1. The structure of the anion in $[Ph_3P)_2N][4\text{-}NO_2\text{-}PhCH_2Te(CN)Cl]$ as determined by X-ray crystallography. The C2, Te and Cl atoms are in the plane of the paper. The numbers in brackets indicate the distance of the various atoms from this plane.

–Te–C2 part of the ion is in the plane of the paper. The Te–Cl bond in the adduct is approximately *trans* to the Te–C(CN) bond. The Te–Cl bond distance is 2.923(2) Å and is somewhat longer than a Te–Cl single bond, 2.36 Å, but is considerably shorter than the sum of the van der Waals' radii of Te and Cl, 4.00 Å.[25] The Te–Cl bond formation causes a significant elongation of the Te–C(CN) bond length from 2.060(4) Å in 4-nitrobenzyl tellurocyanate[11] to 2.140(10) in the Te(II)anion. The short Te–Cl bond distance and the elongation of the Te–C(CN) bond length suggest that the anion may be considered as yet another example of a three-center two-electron Te-complex.[26] The [PNP]-cation has the expected non-linear form with a P–N–P bond angle of 137.1(4)°. A detailed structural report on [PNP][4-NO_2-$PhCH_2TeCN(Cl)$] and the corresponding bromo and iodo compounds will be published shortly. No evidence for the formation of similar selenium complexes could be observed.

DISCUSSION

It has become increasingly clear in recent years that the classical organic reactions of the Finkelstein type[6] (charged nucleophiles) and of the Menschutkin type[27] (uncharged nucleophiles) are reversible reactions. Since equilibrium constants are in principle measurable quantities, a number of studies have appeared attempting to relate the kinetics and the thermodynamics of these alkyl transfer reactions in the same manner as the Brønsted equation treats protolysis; for recent surveys Refs. 28 and 29. Unfortunately, the relatively high activation barriers for these reactions is a serious obstacle to obtaining data of both rates and equilibria of sufficient accuracy to allow reliable comparisons to be made between the kinetic property, carbon nucleophilicity, and the thermodynamic property, carbon basicity.

Since benzylic compounds undergo substitution reactions with a number of nucleophiles fairly rapidly[16,30] with clean second-order kinetics, this class of compounds is well-suited for this type of study. Furthermore, by the use of substituents, rates and equilibria may be correlated with Hammett substituent constants. However, it should be emphasized that conclusions derived from studies using ionic nucleophiles as in the present study may not be valid for Menschutkin reactions and *vice versa* since the structure of the transition state for these two reactions is apparently quite dissimilar. For Finkelstein reactions the transition state is "tight" and fairly symmetrical[31] while for the latter reactions the transition state is fairly product-like.[29,30] Furthermore, it is well known that charged and uncharged nucleophiles are responding most differently to donor and acceptor substituents in the vicinity of the reacting carbon atom.[16,33]

The choice of acetonitrile as the solvent in the present study was governed by the fact that several relevant studies have been performed in this solvent[7,17,18,34–38] and thus allowing valid comparisons to be made.[4,5,39] By using the very soluble and well-dissociated [PNP]-salts as the source of the nucleophiles, experimental difficulties caused by precipitation or variations in the degree of dissociation were eliminated. Unfortunately, when using charged nucleophiles attention cannot be focused on the kinetics–thermodynamics relationship since the nucleophilic atom will be altered from one nucleophile to another which is not necessarily the case in Menschutkin reactions.[29,40]

The nucleophilicity of the anions, The data in Table 2 indicate that I^-, Br^-, Cl^- and $NCSe^-$ exhibit approximately the same nucleophilicity and these ions are slightly but significantly more nucleophilic than is the thiocyanate ion, NCS^-. The tellurocyanate ion, $NCTe^-$, is by far the more nucleophilic one being ten times as reactive as is the selenocyanate ion. This observation agrees with the results from a previous kinetic study employing benzyl bromide as the substrate.[41]

Table 3 presents some ratios between second-order rate constants for the various reactions studied. For comparison the range of these ratios from a similar study on 2-substituted-1-phenylethanones (phenacyl compounds), $PhC(O)CH_2X$, is included in the Table. It is apparent that none of the nucleophiles show any preference for any of the leaving groups, even though the reactivity of the various substrates spans over several powers of ten, *cf.* Table 2. These rate ratios are also, as can be seen from Table 3, quite independent upon the substituents in the 4-position. The $k_f(NCSe^-)/k_f(Br)$ ratios may suggest that the selenocyanate ion prefers a benzylic carbon atom to a phenacylic carbon atom as compared with the bromide ion, but the effect is not very pronounced.

When comparing the rates of the various substrates as a function of the 4-substituents, Me, H

Table 3. The ratio between second-order rate constants for various nucleophiles reacting with 4-Z-PhCH$_2$X in acetonitrile at 25.0 °C.

Z	X	$\frac{k_f(NCSe^-)}{k_f(NCS^-)}$	$\frac{k_f(NCSe^-)}{k_f(Cl^-)}$	$\frac{k_f(NCSe^-)}{k_f(Br)}$	$\frac{k_f(Br^-)}{k_f(Cl^-)}$	$\frac{k_f(I^-)}{k_f(Br^-)}$
NO_2	I				1.5	
NO_2	Br	7	1.5			
NO_2	Cl	7	1.5[a]	2.1	0.7[a]	~2
NO_2	SeCN	7	1.9	3.6	0.5	
NO_2	SCN	18	3.2	2.9	1.1	
H	Cl	5	1.5[b]		(9)[c]	1[c]
Me	Cl	5	2			
PhC(O)CH$_2$	X[d]	8 – 20	0.6 – 2.5	0.3 – 1.1	~2.2	

[a] Rate constant for Cl^- exchange at 20.0 °C, *cf.* Table 2. [b] Rate constant for Cl^- exchange at 30.0 °C, *cf.* Table 2. [c] From rate constants at 30.0 °C, Refs. 17 and 18. [d] Range observed for reactions with phenacyl compounds, X = Br, Cl, SeCN and SCN from Ref. 7.

and NO_2, the usual *V*-shaped Hammett plot is observed as for numerous other Finkelstein reactions.[16,42] The small variations in the rate ratios with substitutents as listed in Table 3 may imply that the form of the Hammett plot is not very dependent upon either the nucleophile or the leaving group.

Hayami and co-workers[37,38] have concluded from NMR-studies that benzylic halides and related substances form addition compounds *via* the methylene protons to halide ions in acetonitrile. These authors have suggested that the formation of these adducts may be part of the mechanism for the substitution process and thus to be taken into account when calculating the rate constants. However, since the ions used in the present study differ considerably in hydrogen basicities and as the acidities of the methylene protons in the benzylic compounds are well known to be significantly dependent upon the substituents,[24] the small variations in the ratios in Table 3 point against a pre-equilibrium step in the substitution process to be of any kinetic significance.

In Table 4 a summary is made of the average nucleophilicities of the various ions as observed in the present study and a comparison is made with data obtained on 2-substituted-1-phenyl ethanones. The values are given as relative to the thiocyanate ion. As can be seen from the data, only small variations are observed; the bromide ion is apparently somewhat less nucleophilic toward 4-

Table 4. A comparison between the average nucleophilicity, the average leaving group ability and the carbon basicity of I^-, Br^-, Cl^-, $NCSe^-$ and NCS^- for reactions with 4-NO_2-PhCH$_2$X (this study) and for reactions with Ph(O)CH$_2$X (Ref. 7) in acetonitrile at 25.0 °C.

	I^-	Br^-	Cl^-	$NCSe^-$	NCS^-
Nucleophilicity					
4-NO_2-PhCH$_2$X	6	4	4	8	1
PhC(O)CH$_2$X		15	7	10	1
L group ability					
4-NO_2-PhCH$_2$X	1×10^4	1.5×10^3	6	3	1
PhC(O)CH$_2$X		2×10^4	75	10	1
Carbon basicity					
4-NO_2-PhCH$_2$X	6×10^{-4}	2.5×10^{-3}	0.9	2.8	1
PhC(O)CH$_2$X		1.4×10^{-3}	0.14	1.2	1

nitrobenzylic compounds than toward phenacyl compounds. A similar comparison for the average leaving group ability and the carbon basicity is also presented in Table 4.

The leaving group ability. The iodides are clearly the most reactive among the 4-nitrobenzylic compounds. This observation is in general agreement with all other alkyl halides undergoing substitution reactions by the S_N2 mechanism.[43] Likewise, the bromides are far more reactive than are the chlorides; the average rate ratio between RBr and RCl being ~200 which is of the expected order of magnitude.[7] It is notable from the data in Table 4 that relative to the thiocyanate ion, the leaving group ability of $NCSe^-$ and especially the halide ions is far less in the benzyl series than in the phenacyl series. Apparently, in 2-thiocyanato-1-phenylethanone the carbon–sulfur bond is exceptionally strong.

The carbon basicity. The iodide ion is the least basic one, but the difference between the iodide ion and the bromide ion is somewhat less than could be concluded from the leaving group ability of these two ions owing to the slightly higher nucleophilicity of the iodide ion. The chloride ion is far more basic and is comparable with the thiocyanate ion. As in the phenacyl series [7] the selenocyanate ion is both a better leaving group and a better nucleophile than is the thiocyanate ion; the net effect causes the selenocyanate ion to be a better carbon base than is the thiocyanate ion. Since the leaving group ability of the selenocyanate ion relative to that of the thiocyanate ion is somewhat less in the benzyl series than in the phenacyl series the result is a slight increase in the carbon basicity of the selenocyanate ion.

In Table 5 a comparison between the carbon basicity of Cl^-, $NCSe^-$ and NCS for various benzylic substrates is made. In the case of the 4-methyl substituted compounds the basicity order is $NCS^- > NCSe^- > Cl^-$ while in the compounds containing the electron-attracting NO_2-group in the 4-position the order is $NCSe^- > NCS^- \sim Cl^-$; the order being intermediate in the unsubstituted compounds. The data in Table 2 reveal that this change in basicity order with substituents is due to a slight preference of the thiocyanate ion for substrates with electron-donating substitutents relative to $NCSe^-$ and Cl^- with regard to nucleophilicity which is not sufficiently compensated by a similar effect upon the leaving group ability of NCS^-. Such variations in carbon basicity with changes in substrate are highly expected and it has previously been argued that no absolute order of carbon basicity of nucleophiles can be arrived at.[7] In Table 6 is listed some equilibrium constants for reactions between some organic iodides and ionic bromide in acetonitrile. When making such comparisons it is obvious that only results from studies in one solvent and preferably at one temperature can be used,[4,35] *cf.* Table 7 for the effect of the solvent upon the equilibrium constant for the methyl iodide–bromide ion reaction. Specific effects of cations may also exert a profound influence

Table 5. The carbon basicity of Cl^-, $NCSe^-$ and 4-Z-NCS^- ($NCS^- = 1$) for reactions of 4-Z-$PhCH_2X$ in acetonitrile at 25.0 °C.

Z	Cl^-	$NCSe^-$	NCS^-
CH_3	0.35	0.8	1
H	0.46	1.1	1
NO_2	0.9	2.8	1

Table 6. The equilibrium constants for the reaction between organic iodides and ionic bromide, $RI + Br^- \overset{K}{\rightleftarrows} RBr + I^-$ in acetonitrile.

R	4-Nitrobenzyl	Methyl	Ethyl
K	3.3(5)[a]	3.7[b]	17.7[c]

[a] This study, 25.0 °C. [b] From Ref. 35, 30.0 °C. [c] From Ref. 36, 30 °C.

Table 7. The equilibrium constants for the reaction between methyl iodide and ionic bromide in various solvents.

	MeCN	Acetone	H_2O	MeOH	EtOH	2-PrOH	*t*-AmOH
K	3.7[a]	12[b]	0.08[b]	0.08[c]	0.1[c]	0.14[c]	0.25[c]

[a] Ref. 35, 30.0 °C. [b] Ref. 4, 25.0 °C. [c] Ref. 36, 30. °C.

upon carbon basicities, even for reactions which apparently are completely homogeneous. Schiavon[22] has found the rate constant for the exchange reaction between 4-nitrobenzyl selenocyanate and potassium [^{14}C]-selenocyanate in acetonitrile at 25.0 °C to be 1.6×10^{-2} M^{-1} s^{-1} which is nearly 20 times as high as the one determined in the present study, *cf.* Table 2, even though KSeCN is extensively associated in acetonitrile.[44] Presumably, the leaving group ability of the selenocyanate ion is enhanced in the presence of potassium ions through interaction between the potassium ion and the nitrogen end of the selenocyanate group in RSeCN.[44]

It has become customary to use the thiocyanate ion as a reference ion when making comparisons of carbon basicity in various solvents, *cf.* Refs. 4 and 7 and Tables 2, 4 and 5. However, the data, especially in the two latter tables, may indicate that this choice of reference ion may not be a good one. It is highly possible that the strength of the sulfur $-$carbon(CH_2) bond is more dependent upon the organic group than is any of the other X$-$C(CH_2) bonds owing to the presence of some double-bond character of the sulfur$-$carbon(CN) bond as represented by the following canonical structure:

$$R-CH_2-\overset{\delta+}{S}=C=\overset{\delta-}{N}$$

Table 8 summarizes some data from a recent X-ray structural investigation on 4-NO_2-PhCH_2XCN (X = Te, Se and S).[45] The results from this study indicate a distinct shortening of the X$-$C(CN) bond when ascending the VI main group. This is in agreement with the suggestion of some double-bond character of the S$-$C(CN) bond.

The tellurocyanate ion is by far the most basic one of the ions examined which is highly surprising in view of the reputed weakness of the tellurium $-$carbon bond.[46,47] However, this observation is entirely due to the fact that the displaced halide ion is trapped by the tellurium atom in the first formed organic tellurocyanate forming a Te(II) complex ion, *cf.* Fig. 1, and thus preventing the reverse reaction to take place.

Table 8. A comparison between the X$-$C(CN) and the X$-$C(CH_2) bond lengths in 4-NO_2-PhCH_2XCN.[45] (X = Te, Se and S) (Bond lengths in Å).

	RTeCN	RSeCN	RSCN
X$-$C(CH_2)	2.167(3)	1.972(3)	1.822(2)
X$-$C(CN)	2.060(4)	1.844(3)	1.680(2)
Difference	0.107	0.128	0.142
ΣRcov.X$-$C[a]	2.14	1.94	1.81

[a] Based upon values from Ref. 25.

Comments on the utility of the Finkelstein reaction in synthesis. Although the data on rates and equilibria for Finkelstein reactions made available in recent years suggest considerable dependence of nucleophilicity, leaving group ability and carbon basicity upon the structure of the organic group and also the cation present, some general conclusions can still be made. Together with suitable information on solubilities of alkali and onium salts in both protic and aprotic solvents, these data allow a closer scrutiny on the utility of the Finkelstein reaction in synthesis.

First of all, all rate constants, and not to be diregarded, also all rate constants for the reverse reactions, are considerably higher in dipolar aprotic solvents than in protic ones. Since small nucleophilic atoms are discriminating far more between protic and aprotic solvents than large ones, dipolar aprotic solvents are levelling carbon basicities.[4] Variations in equilibrium constants of several orders of magnitude may therefore be experienced; *cf.* Table 7 for a case where only modest variations are observed since the two competing anions, the bromide ion and the iodide ion, are fairly similar in size.

The Finkelstein reaction is usually applied for the preparation of RY from the commercially available chlorides and bromides, RX, eqn. (11). Both from a

$$RX + Y^- \rightarrow RY + X^- \qquad (11)$$

X = Cl, Br, $Y^- = I^-$, NCS^-, $NCSe^-$ $NCTe^-$, F^-, N_3^-, ROO^-, RS^- *etc.*

kinetic and a thermodynamic point of view it is apparent that the bromides are superior to the chlorides, *cf.* Table 4 for acetonitrile as solvent and Ref. 48 for water as solvent. Thus, any RY, where Y^- exhibits a higher carbon basicity than the bromide ion may in principle be prepared in homogeneous reactions, particularly in a dipolar aprotic solvent to avoid parallel or consecutive solvolysis reactions. However, the limited solubility of most alkali salts

(MY) in dipolar aprotic solvents often necessitates the use of onium salts as the source of the anion. Furthermore, the considerable hydrogen basicity of many anions in dipolar aprotic solvents, notably NC^-, N_3^-, OCN^-, OR^- and F^-, may promote undesirable elimination reactions.[49,50] Substitution reactions with these nucleophiles may therefore better be performed in protic solvents but at a high kinetic price.[5] Alternatively, one has to apply various synthetic procedures based upon phase transfer catalysis.[51] The carbon basicity of all these ions are known to be superior to Br^- in in all solvents and in most cases to Cl^- [18,48] preventing the reverse reactions to take place. It should be emphasized that RF cannot be prepared in good yield from RCl, even in dipolar aprotic solvents, without allowing the product to escape, owing to the fairly similar carbon basicity of F^- and Cl^-.[18,48] The fluoride ion is well solvated also in dipolar aprotic solvents preventing this ion to exhibit the anticipated powerful nucleophilicity in this class of solvents.[52]

The data in Tables 4 and 5 indicate that RSCN and RSeCN cannot be prepared from the corresponding chlorides in good yield and of high purity in homogeneous reactions in dipolar aprotic solvents. Thus, either the bromides, RBr, are to be used or, in the case of the chlorides, RCl, alkali salts, MY, have to be the source of NCS^- and $NCSe^-$ whereby the displaced chloride ions are trapped as their fairly insoluble alkali salts in both protic and aprotic solvents, eqn. (12).

$$RCl + MY \rightarrow RY + MCl\downarrow \qquad (12)$$

Eqn. 12 forms the basis for the preparation of organic tellurocyanates, RTeCN, in dimethyl sulfoxide as outlined by Cava and co-workers.[47] In the presence of small alkali ions the displaced chloride ion is trapped by the alkali ion allowing the organic tellurocyanate to be isolated. In the presence of onium cations in a homogeneous reaction the chloride ion is trapped by the organic tellurocyanate and the Te(II)-complex anion is formed,[46] *cf.* Fig. 1.

Both from a kinetic and a thermodynamic point of view, organic iodides, RI, are better reagents than are the corresponding bromides, *cf.* Table 4. However, it should be emphasized that the data readily suggest that the advantage of the iodides to the bromides is generally greatly overestimated. Both in protic and in aprotic solvents the gain in rate is only 5 to 10-fold, *cf.* Table 4 and Refs. 16, 43, 48 and 53 for Finkelstein reaction; the same applies to Menschutkin type reactions.[43] When considering their cost and, in most cases, their limited availability, their thermic and photolytic instability and their susceptibility to undergo elimination reactions and to be attacked at the iodine atom by strongly halophilic reagents,[54,55] it is doubtful that the slight increase in rate from the bromides justifies any extensive use of alkyl iodides in either Finkelstein or Menschutkin reactions. Furthermore, owing to the high solubility of most alkali iodides in both protic and aprotic solvents an increase in yield cannot be accomplished by precipitation as outlined in eqn. 12 for the chlorides. Admittedly, toward weakly basic nucleophiles as Me_2S and related compounds,[56] alkyl iodides are to be preferred to the alkyl bromides, particularly in aprotic solvents, the preference being due to the lower basicity of the iodide ion, *cf.* Table 7. Alkyl iodides are also well known to be superior to the corresponding bromides in insertion reactions and in many-center reactions.[27]

Alkyl iodides present a particular problem owing to their generally difficult preparation. From Table 4 it is seen that RI cannot be prepared from RCl in homogeneous reactions; only when applying alkali salts is the equilibrium shifted in the desired direction, according to eqn. (12); *cf.* Experimental. In the case of RBr being the substrate, the problem is more complex since alkali bromides and alkali iodides have fairly comparable solubilities in most solvents preventing the reaction according to eqn. (12) to take place. According to equilibrium constants listed in Table 7 the equilibrium is shifted in the right direction in protic solvents. The considerably lower rate constant in this class of solvents combined with the unavoidable solvolysis reactions, particularly when the alkyl group is a secondary or a tertiary one, leads quite often to a less amenable reaction mixture than desired. Organic iodides can, therefore, in practice only be prepared from the corresponding chlorides in heterogeneous reactions in dipolar aprotic solvents or in homogeneous reactions from compounds with leaving groups of very low carbon basicity as $ROSO_2F$, $R_3O^+X^-$ and related compounds. Recently some entirely new methods for the preparation of RI have been described [59,60] which are not based upon the Finkelstein reaction.

As outlined above, the bromides and the chlorides

appear to be the most convenient starting compounds in substitution reactions. However, for many organic groups only one of the organic compounds is commerically available or is conveniently synthesized in the laboratory. In order to increase rate and yield, the bromides are to be preferred to the chlorides, *cf.* Table 4. For reactions with very powerful nucleophiles the chlorides may be sufficiently reactive. Furthermore, the chlorides are generally more stable compounds and less susceptible to undergo solvolysis reactions and to be attacked at the halogen atom by halophilic nucleophiles. Thus, for practical purposes one needs simple and clean procedures for the conversion of chlorides to bromides and *vice versa.*

Organic bromides may, in principle, be prepared from the chlorides by heterogeneous Finkelstein reactions taking advantage of the low solubility of alkali chlorides in most solvents, *cf.* eqn. (12). The yields are generally low, the maximum conversion reported is 84% when varying the substrate, the cation and the solvent.[61] However, by adding ethyl bromide to the reaction mixture whereby the competing ions are removed from the reaction by forming the very volatile ethyl chloride the conversion is close to being quantitative.[61]

When converting bromides to chlorides the procedure is based upon the significantly higher carbon basicity of the chloride ion in homogeneous reactions in dipolar aprotic solvents, *cf.* Table 4. The high nucleophilicity of the chloride ion in this class of solvents[5,33] causes these reactions to be kinetically favourable. Since high molecular weight onium chlorides are necessary for this reaction in order to maintain the ionic chloride soluble, this reaction is hardly recommendable for large scale synthesis of organic chlorides. However, for the preparation of small quantities of organic chlorides this reaction appears as a valuable method. In the present study 4-methylbenzyl chloride was readily obtained from the commercially available bromide using tetraphenylarsonium chloride as the source of the chloride ions, eqn. (13), *cf.* Experimental. Owing

$$\text{4-Me-PhCH}_2\text{Br} + \text{Ph}_4\text{AsCl} \rightarrow \text{4-Me-PhCH}_2\text{Cl} + \text{Ph}_4\text{AsBr} \qquad (13)$$

to the lower solubility of Ph_4AsBr than of Ph_4AsCl in acetonitrile the equilibrium is forced in the desired direction by cooling of the reaction mixture. [PNP]Cl, being non-hygroscopic,[15] is an even more convenient source of chloride ions than is Ph_4AsCl for the reaction.[62] Any trace of water present will reduce the nucleophilicity of the chloride ions and thus the rate of the reaction and invariably lower the yield since the equilibrium will be shifted in the undesired direction.

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Mechanisms of the Electrohydrodimerization of Activated Olefins. VI.* Cyclohydrodimerization of *p*-Methylbenzylidene Malononitrile Anion Radical

OLAV LERFLATEN and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The kinetic and activation parameters were obtained for the electrocyclohydrodimerization of *p*-methylbenzylidene malononitrile (MBM) anion radical in acetonitrile (AN), *N*,*N*-dimethylformamide (DMF) and in AN containing acetic acid. In solution in the absence of acid, hydrodimerization is accompanied by the formation of base which reacts with substrate giving rise to complex kinetic behaviour. In the presence of acetic acid the proton donor does not become involved until after the dimer forming reactions and the kinetics are simplified. Under conditions where the electrogenerated base does not influence the kinetics, rate law (i) is followed. Activation energies at low substrate concentration

$$\text{Rate} = k_{app}|\text{MBM}^{\cdot -}|^2 \quad \text{(i)}$$

were observed to be 0.94 (AN) and 1.6 (DMF) kcal/mol with corresponding entropies of activation equal to −25 and −28 cal/K mol, respectively. The mechanism is proposed to involve a reversible dimerization of anion radicals followed by protonation of the dimeric dianion and cyclization of the resulting carbanion. The evidence is examined with relation to previous reports of the electrohydrodimerization of MBM.

The question of the mechanism of the electrohydrodimerization (EHD) of activated olefins has been reopened after nearly a decade of acceptance of the anion radical dimerization (1) as the key step

$$2R^{\cdot -} \rightarrow R^- - R^- \quad (1)$$

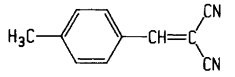
in the mechanism by most workers in the field.[1–5] The literature in this area was surveyed in another part of this series.[3] The recent work [1–5] has demonstrated that the anion radical–substrate coupling mechanism (2)–(3) can play an important and in

$$R^{\cdot -} + R \rightarrow R^- - R^{\cdot} \quad (2)$$

$$R^- - R^{\cdot} + R^{\cdot -} \rightarrow R^- - R^- + R \quad (3)$$

some cases a predominant role in the overall reaction.

Reports of the electrohydrodimerization of *p*-methylbenzylidene malononitrile (*1*) has attracted



1 = MBM

our attention.[6–8] The reaction was first studied by linear sweep voltammetry (LSV) and convolution potential sweep voltammetry in acetonitrile at millimolar concentrations.[6,7] Excellent fits of the experimental to theoretical data for the anion radical dimerization (1) was found.[6,7] The peak potential during LSV was observed to be dependent upon the water concentration but the changes were related to those of the reversible potential which indicates that water is not kinetically involved in the reaction. The reaction was later studied by chronopotentiometry at high concentrations in order to test for a change in mechanism with increasing concentration.[8] The conclusion of this study was that the

*See Refs. 1–5 for other parts in this series.

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reaction remained of the anion radical dimerization type but that water playned a significant role when the concentrations of $MBM^{\cdot -}$ were increased. The effect of water was rationalized as follows; When the concentrations of MBM and water are of the same order of magnitude, the preferential solvation of the anion radical, $A^{\cdot -}$, by water was considered essentially as the formation of an adduct and the reactions rationalized as (4)–(7) where TH is water, $AS^{\cdot -}$ is the anion radical–water adduct, and D^{2-} is the dimer dianion. It was concluded that since coulombic repulsions are reduced in $AS^{\cdot -}$,

$$A^{\cdot -} + TH \underset{}{\overset{K_4}{\rightleftharpoons}} AS^{\cdot -} \quad (4)$$

$$2AS^{\cdot -} \xrightarrow{k_5} DH_2 + 2T^- \quad (5)$$

$$AS^{\cdot -} + A^{\cdot -} \xrightarrow{k_6} DH^- + T^- \quad (6)$$

$$2A^{\cdot -} \xrightarrow{k_7} D^{2-} \quad (7)$$

the dimerization (5) can be considered to play a major role in the overall reaction.

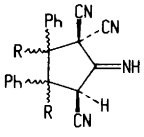
We find the proposals by Nadjo and Savéant[8] concerning the mechanism of the electrohydrodimerization of MBM unacceptable from a number of considerations. (i) The fact that base is generated (T^-) was taken into account but the fact that MBM is readily attacked by hydroxide ion, as in (8), was neglected. A similar reaction has been shown[3]

$$HO^- + MBM \rightarrow PhCH(OH)-\bar{C}(CN)_2 \quad (8)$$

to be responsible for the low coulometric n values observed during electrohydrodimerization of diethylfumarate.[9]

(ii) If equilibrium (4) is important at high MBM concentrations it should also be important when MBM is in the millimolar range and the water concentration is increased. This was not observed.

(iii) Work by Avaca and Utley has shown that the product of hydrodimerization of MBM in DMF containing acetic acid is not the normal hydrodimer as assumed by Savéant and co-workers[6-8] but rather the cyclic structures (*2*) and (*3*).[10-11]



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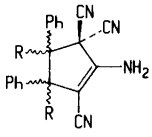


3

The points raised in the previous paragraph prompted us to undertake a reinvestigation of the electrohydrodimerization of MBM in order to more clearly define the effect of proton donors, to find the effect of base on the overall reaction pathway and to compare the mechanisms in the two solvents previously used, AN[6,7] and DMF.[8,10,11]

RESULTS

Kinetic method. We found that the kinetics of the dimer forming reactions of $MBM^{\cdot -}$ could be studied by derivative cyclic voltammetry (DCV)[12,13] in both AN and DMF. The data were treated by the reaction order approach recently described.[14] The essential feature of the method is embodied in eqns. (9) and (10). The reaction order $R_{A/B}$ refers to the sum of the order in substrate (A) and inter-

$$R_{A/B} = 1 + z \quad (9)$$

$$v_{\frac{1}{2}}/C_A^z = \text{constant} \quad (10)$$

$$A \pm e^- \rightleftarrows B \rightarrow \text{products} \quad (11)$$

mediate (B) reacting in process (11). The quantity z is the power to which C_A must be raised in order for relationship (10) to hold where $v_{\frac{1}{2}}$ is the voltage sweep rate necessary for the derivative peak ratio to equal 0.500. For the simple dimerization mechanism (1) the reaction order in substrate is 0 and that in the intermediate anion radical is 2 which results in $R_{A/B}=2$ and $z=1$. On the other hand, the anion radical–substrate coupling mechanism (2)–(3) follows rate law (12) which corresponds to $R_{A/B}=3$ and $z=2$. Further detail and discussion of the method can be found in Ref. 14.

$$\text{Rate} = k_{app}|R^{\cdot -}|^2|R| \quad (12)$$

The kinetics in acetonitrile. Data for two series of measurements carried out in AN at −39.8 °C are summarized in Table 1. The reaction was observed to be quite rapid and required the use of high v. The column headed $v_{\frac{1}{2}}/C_A$ ahows the test for the dimerization of anion radicals (1) which is characterized by $z=1$ and $R_{A/B}=2$. Adherence to this mechanism requires that $v_{\frac{1}{2}}/C_A$ remains constant while C_A is varied. The data show a decreasing trend as C_A was increased. The next two columns test $z=0.75$ and $z=0.5$. The values of $v_{\frac{1}{2}}/C_A^z$ were averaged

Table 1. Reaction order analysis of the electrohydrodimerization of MBM in acetonitrile.[a]

C_A/mM	$\|H_2O\|$[b]/M	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A$	$v_{\frac{1}{2}}/C_A^{0.75}$	$v_{\frac{1}{2}}/C_A^{0.5}$
0.25	0	165	660	466	330
0.50	0	250	500	420	354
1.00	0	440	440	440	440
			533(114)	442(23)	375(58)
0.25	0.1	160	640	452	320
0.50	0.1	250	500	420	354

[a] Data measured in solvent containing Bu_4NBF_4 (0.1 M) at a mercury electrode at −39.8 °C. [b] Refers to the concentration of added water.

Table 2. Reaction order analysis of the electrohydrodimerization of MBM in DMF.[a]

C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A$	% Deviation[b]
0.10	14.8	148	6.9
0.20	32.2	161	1.3
0.30	48.1	160	0.6
0.50	77.6	155	2.5
0.70	118	169	6.3
1.00	166	166	4.4
2.00	304	152	4.4

[a] Measurements in solvent containing Bu_4NBF_4 (0.1 M), H_2O (0.2 M) at 298 K at a mercury electrode. [b] The percent deviation from the mean value.

for the first set of experiments. The standard deviations, in parentheses, are a measure of the best fit to eqn. (10). In this case, the best fit is clearly with $z=0.75$. Nearly identical results were obtained for the reaction in the presence of water (0.1 M) and the data for the two concentrations give a best fit to relationship (10) when z is midway between 0.5 and 0.75. The data indicate significant deviations from $R_{A/B}=2$ required for the simple irreversible dimerization of anion radicals (1).

The kinetics in DMF. The rate of the reaction of $MBM^{\cdot -}$ was observed to be considerably lower in DMF than in AN under comparable conditions. Data from measurements at 25 °C are summarized in Table 2. In this case, little deviation was observed

Table 3. The effect of water on the rate of electrohydrodimerization of MBM in AN and DMF.[a]

Solvent	T/K	$\|H_2O\|$/mM[b]	v_c^c/V s^{-1}	% Deviation[d]
AN	255	0	159	
AN	255	100	167	2.5
AN	255	200	162	
DMF	255	0	129	
DMF	255	100	135	2.6
DMF	255	200	135	
DMF	273	0	194	
DMF	273	100	200	3.2
DMF	273	200	207	
DMF	285	0	81.6	
DMF	285	50	91.6	9.4
DMF	285	100	99.5	
DMF	285	200	100.4	

[a] Measurements in solvent containing Bu_4NBF_4 at a mercury electrode. $E_{switch}-E_{rev}=500$ mV. [b] Water added to the solutions. [c] In AN c refers to 0.3 and in DMF to 0.5. [d] Percent deviation from the mean value in each set.

Table 4. Reaction order analysis of the electrohydrodimerization of MBM in AN containing acetic acid.[a]

C_A/mM	T/°C	|HOAc|/mM	$v_{0.3}$/V s^{-1}	$v_{0.3}/C_A$	% Dev.[b]
0.25	12	22	42.8	171.2	5.3
0.50	12	22	92.4	184.8	2.3
0.75	12	22	138.0	184.0	1.8
1.00	12	22	182.7	182.7	1.1
0.50	−26	0	88.9	177.8	3.9
0.50	−26	4.4	86.3	172.6	6.8
0.50	−26	8.8	97.5	195.0	5.3
0.50	−26	22	98.0	196.0	5.9
0.50	−26	44	92.0	184.0	0.6

[a] In solvent containing Bu_4NBF_4 (0.1 M) and water (0.1 M). [b] Percent deviation from the mean value.

in $v_{\frac{1}{2}}/C_A$ over a 20-fold C_A range. The data indicate that $R_{A/B}$ is 2 and that the kinetics are consistent with the irreversible dimerization reaction (1).

Tests for effect of water on the kinetics. Several series of experiments were carried out at constant C_A while the concentration of water was varied from 0 to 200 mM. The last column in Table 3 shows the standard deviations in each series expressed in percent of the mean value. Measurements at 255 K in either AN or DMF showed no dependence of the apparent rate constant on the water concentration. In DMF at 273 and 285 K, a small but definite increase of $v_{\frac{1}{2}}$ with increasing water concentration was apparent.

Kinetic analysis of reactions carried out in the presence of acetic acid. In the presence of HOAc (22 mM) in AN, $R_{A/B}$ was observed to be 2 as is evident from the small deviations in $v_{\frac{1}{2}}/C_A$ listed in the last column of Table 4. The quantity measured was $v_{0.3}$ which required considerably lower sweep rates and allowed the analysis to be carried out at higher temperature. The subscript 0.3 refers to a derivative peak ratio of 0.300. The set of experiments carried out at $C_A = 0.5$ mM summarized in the lower half of the table show that the apparent rate constant is essentially independent of the acetic acid concentration in the range, 0 to 44 mM. The deviations from the mean shown in the last column indicate that the

Table 5. Reaction order analysis of the electrohydrodimerization of MBM in DMF containing acetic acid.[a]

C_A/mM	|HOAc|/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A$	$v_{\frac{1}{2}}/C_A^{0.75}$
0.25	22.0	18.8	75.4	53.3
0.50	22.0	51.1	102.3	86.0
0.75	22.0	75.7	100.9	93.9
1.00	22.0	93.8	93.8	93.8
1.50	22.0	111.6	74.4	82.3
0.50	0	56.3	–	–
0.50	4.4	61.4	–	–
0.50	8.7	61.2	–	–
0.50	13.1	59.0	–	–
0.50	21.9	57.0	–	–
0.50	43.7	57.6	115.2	96.8
0.75	43.7	78.7	105.0	97.7
1.00	43.7	94.5	94.5	94.5
1.50	43.7	112.3	74.9	82.9

[a] In solvent containing Bu_4NBF_4 (0.1 M) and water (278 mM) at 11.5 °C.

apparent rate constant varied by about ±5 % in the series of measurements.

Data obtained for the EHD of MBM in DMF containing acetic acid are summarized in Table 5. In this case either at |HOAc| equal to 22 or to 44 mM, $v_{\frac{1}{2}}/C_A^{0.75}$ gave the best fit to relationship (10) indicating that $R_{A/B}$ is about 1.75. On the other hand experiments carried out at constant C_A (0.5 mM) indicate that the apparent rate constant is independent of the acetic acid concentration.

The effect of temperature on the apparent rate constants for the EHD of MBM. Since most of the data discussed so far indicate that the kinetic behaviour of the reaction approximates the simple dimerization of anion radicals (1) this mechanism was taken as the basis for the calculation of rate constants in order to evaluate apparent activation parameters. Rate constants can be evaluated from theoretical data using eqn. (13).[13] The constants c and m are dependent upon the mechanism and the

$$k=(F/R)\,(v/C_A^z T)\exp\left[(\ln R_I'-c)/m\right] \quad (13)$$

difference between the switching and reversible potentials, $E_{sw}-E_{rev}$, and R_I' is the derivative peak ratio. The appropriate values of c and m can be obtained from Ref. 13. For the simple dimerization mechanism (1) with $R_I'=0.500$ and $E_{sw}-E_{rev}=$ 300 mV eqn. (13) reduces to (14).

$$k=1362\,v_{\frac{1}{2}}/C_A T \quad (14)$$

The results of two sets of experiments on the EHD of MBM in AN are summarized in Table 6. In the first set of measurements C_A was 0.25 mM. The last column in the table gives rate constants calculated from the Arrhenius correlation of the experimental values listed in the previous column. The deviations from the experimental values were in all cases quite small. The second set of experiments were at C_A equal to 0.50 mM and |HOAc| was 22 mM. Once again, a very close correspondence was observed between the experimental rate constants and those obtained from the Arrhenius correlation.

Three sets of data for the temperature dependence of the apparent rate constant for the EHD reaction in DMF are summarized in Table 7. Data were obtained at C_A equal to 0.50, 1.00 and 4.00 mM and in all cases the correlations were very good.

Correlation coefficients for all of the data in Tables 6 and 7 were greater than 0.99. We find the comparison of the experimental values with those obtained from the correlation more instructive than the correlation coefficients.

The activation parameters obtained from the Arrhenius correlations of the data in Tables 6 and 7 are summarized in Table 8. The most striking features of the data are the low activation energies,

Table 6. The effect of temperature on the apparent second order rate constants for the electrohydrodimerization of MBM in AN.[a]

|HOAc|/mM	T/K	v_c[b]/V s^{-1}	$10^{-6}k_{app}$/M^{-1}s^{-1}	$10^{-6}k_{corr}$[c]
$C_A=0.25$ mM				
0	234	170	6.88	6.99
0	240	190	7.49	7.35
0	246	200	7.70	7.71
0	253	215	8.05	8.13
0	260	240	8.74	8.55
0	271	260	9.09	9.21
$C_A=0.50$ mM				
22	261.2	70.5	3.88	3.92
22	266.2	75.6	4.09	4.10
22	271.7	82.9	4.39	4.32
22	277.2	88.0	4.57	4.53
22	283.2	93.9	4.77	4.77
22	288.7	99.0	4.93	4.99

[a] In solvent containing Bu_4NBF_4 (0.1 M) and H_2O (0.1 M) at a mercury electrode. $E_{switch}-E_{rev}=300$ mV. [b] At $C_A=0.25$ mM c refers to 0.5 and $E_{sw}-E_{rev}=200$ mV while at C_A equal 0.50 mM, c refers to 0.3 and $E_{sw}-E_{rev}$ was 300 mV. [c] The apparent rate constant obtained from the Arrhenius correlation lines.

Table 7. The effect of temperature on the apparent second order rate constants for the hydrodimerization of MBM in DMF.[a]

T/K	$v_{\frac{1}{2}}$/V s^{-1}	$10^{-5}k_{app}$[b]/M^{-1}s^{-1}	$10^{-5}k_{corr.}$[c]/M^{-1}s^{-1}
C_A=0.50 mM			
257	40.5	4.29	4.38
262	45.7	4.75	4.66
268	49.1	4.99	4.99
273	53.2	5.31	5.27
279	58.1	5.67	5.62
287	63.3	6.01	6.09
C_A=1.00 mM			
268.2	23.3	1.18	1.20
274.7	30.5	1.51	1.47
282.7	38.6	1.86	1.88
C_A=4.00 mM			
263.2	67.6	0.875	0.864
268.2	80.6	1.02	1.04
274.7	107.0	1.33	1.32

[a] In solvent containing Bu_4NBF_4 (0.1 M) and water (0.2 M) at a mercury electrode. [b] Calculated from theoretical data assuming an EC(dim) mechanism as described in the text. [c] Calculated from the Arrhenius correlation.

ranging from 1 to 5 kcal/mol and the clear dependence of the value of E_a in both solvents upon the substrate concentration. This is a clear indication that the reactions are more complex than implied by the simple irreversible dimerization (1). It is also of interest to note that the apparent rate constants are significantly greater in AN and that the latter are lower in the presence of HOAc. The value of k_{298} in DMF when C_A was 0.50 mM appears to be larger by a factor of about 2 than expected from the other values obtained at higher C_A. The reason for this discrepancy is not clear. The series of measurements were repeated with nearly identical results.

LSV analysis of the EHD of MBM in acetonitrile. The dependence of the LSV peak potential on v and C_A can be analyzed by means of eqns. (15) and (16).[15] The lower case letters refer to reaction orders in A (a), B (b) and I (i), a species generated during the process which further participates. For the EC(dim) mechanism (1), $dE^p/d\log v$ is predicted by (15) to

$$dE^p/d\log v=[\ln 10/(b+1)]RT/nF \quad (15)$$

$$dE^p/d\log C_A = [(a+b+i-1)\ln 10/(b+1)]RT/nF \quad (16)$$

equal (ln 10) $RT/3F$ and eqn. (16) results in a number of the same magnitude for $dE^p/d\log C_A$ if both a and i are 0. Data along with the theoretical values for the EC(dim) mechanism for measurements in AN

Table 8. Activation parameters for the electrohydrodimerization of MBM.[a]

Solvent	C_A	E_a/kcal mol^{-1}	$\Delta S^{\ddagger}_{298}$/cal K^{-1} mol^{-1}	$k_{298}10^{-5}$/M^{-1} s^{-1}
AN	0.25	0.94	−25	108
AN (HOAc)[b]	0.50	1.3	−25	63.6
DMF	0.50	1.6	−28	6.8
DMF	1.00	4.7	−20	2.9
DMF	4.00	5.3	−18	2.8

[a] Data from Tables 6 and 7. The entropies of activation were calculated assuming that the frequency factor can be equated to $kT/h\ \exp(\Delta S/R)$ and that ln k varies linearly with $1/T$. [b] 22 mM.

Table 9. Linear sweep voltammetry peak potential dependence on substrate concentration and voltage sweep rate.

Conditions	$dE^p/d \log v$[a]	$dE^p/d \log C_A$[b]	Theory[c]
AN–HOAc[d,e]	15.1(0.4)↑	−17.3(0.5)↓	±18.9
AN[d]	14.1(0.7)↑	−14.4(1.0)↓	±16.9

[a] Measurements at 100, 200, 300 and 1000 mV s^{-1} and expressed in mV/decade. The numbers in parentheses are the standard deviations for measurements at concentrations ranging from 0.25 to 1.00 mM. The arrows indicate an increasing trend as C_A was increased. [b] Measurements at C_A ranging from 0.25 to 4.00 mM and expressed in mV/decade. The numbers in parentheses are the standard deviations for measurements at 100 to 1000 mV/s. The arrows indicate a decreasing trend as v was increased. [c] The theoretical value for the EC(dim) mechanism taking into account the temperature. [d] In solvent containing Bu_4NBF_4 (0.1 M) and water (200–278 mM). [e] HOAc concentration = 22 mM.

are summarized in Table 9. Either in the presence or absence of HOAc, the slopes are all too low and trends were observed. The trends are designated by the arrows, in the case of $dE^p/d \log v$, an increase in C_A brought about an increase in the slope while an increase in v was in both cases accompanied by a decrease (numerically) in $dE^p/d \log C_A$. The values, especially those for $dE^p/d \log v$ deviate significantly from the theoretical values and the deviations are more serious in the absence of HOAc.

The reaction of MBM with hydroxide ion in acetonitrile. The data in Table 10 indicate that MBM is consumed nearly stoichiometrically upon the addition of Bu_4NOH to a 1.00 mM solution in AN. The reaction apparently occurs as rapidly as mixing takes place and attempts to measure the rate of the reaction by cyclic voltammetry were not successful. The data listed are for peak potential measurements 3 s after mixing was begun and only very small changes could be observed after that time.

DISCUSSION

In formulating a mechanism for the EHD of MBM, the following conclusions based upon the kinetic studies must be taken into account: (i) The reaction, under most conditions is very nearly second order in anion radical and there is no evidence for the involvement of substrate. (ii) Proton donors, either water or acetic acid, have a small or negligible effect on the rate of the reaction. (iii) The apparent activation energy is small and concentration dependent in both AN and DMF. (iv) Base is generated in the EHD and MBM very rapidly reacts with base.

Table 10. The reaction of MBM with hydroxide ion in acetonitrile.

$\lvert Bu_4NOH \rvert$/mM[a]	% MBM Consumed[b]
0.20	25.7
0.40	50.4
0.60	74.2
0.80	95.4

[a] The concentration after addition to a solution of MBM (1.00 mM) in AN containing Bu_4NBF_4 (0.1 M) at 12 °C. [b] Determined by the measurement of the LSV peak current 3 s after mixing was begun.

If we make the assumption that the deviations from the theoretical relationships for the simple dimerization mechanism (1) are primarily caused by the interference of the side reaction between MBM and the base generated during EHD, we can formulate a relatively simple mechanism which takes into account the kinetic observations. Equilibrium (17), which can be viewed as the reversible formation of a dimeric complex of the anion radical, followed by bond forming reaction (18) results in rate law (19). The pre-equilibrium (17) is required

$$2MBM^{\cdot -} \overset{K_{17}}{\rightleftharpoons} MBM^{\cdot -}/MBM^{\cdot -} \quad (17)$$

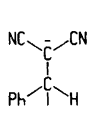
$$MBM^{\cdot -}/MBM^{\cdot -} \xrightarrow{k_{18}} \underset{4}{\text{NC(NC)C(Ph)–CH(Ph)–C(CN)(CN)}} \quad (18)$$

$$\text{Rate} = k_{18}K_{17}|MBM^{\cdot -}|^2 \quad (19)$$

in order to take into account the concentration dependent, very low activation energy observed in both AN and DMF. The concentration dependence of the activation energy reflects the decreasing importance of equilibrium (17) as the concentration

increases in determining the overall activation energy. It is conceivable that at high concentrations equilibrium (17) lies further to the right and the activation energy is almost completely that for reaction (18). This is also supported by the data in Table 8. The greatest changes in E_a are observed at substrate concentrations lower than 1 mM. Between C_A equal 1.0 and 4.0 mM, E_a was observed to increase only slightly from 4.7 to 5.3 kcal/mol.

The kinetic data observed for this system are not unique. It is of interest to compare the structural features of dimeric dianion *4* with those derived from two other anion radicals which dimerize by mechanisms identical to (17)–(18). The two other systems are the anion radicals derived from 9-diazofluorene[16] and anthracenes substituted with electron withdrawing substituents.[17] The observation of activation energies too low for even diffusion controlled reactions required the postulation of equilibria of the type of (17) for both of these systems. A comparison of the partial structures *5*, *6* and *7*



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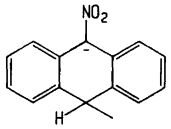

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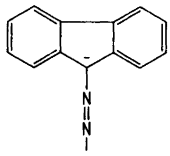

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reveals that in all three cases, the dimeric dianions have strongly stabilizing structural features, the dicyanomethyl anion (*5*), the acinitro anion (*6*) and the fluorenyl anion (*7*). The kinetics of the three systems are practically identical in most respects. The simplest case involves the dimerization of 9-nitroanthracene anion radical since in this case the product is the stable dianion related to half structure *6*.[17] The apparent activation energy in that case was found to be approximately zero. The rate of dimerization in the latter case was observed to be independent of the water concentration in DMF. These observations led to the conclusion that the mechanism is described by eqn. (20) which is identical to (17)–(18) with the exception that the formation of the dimer dianion was considered to be reversible.

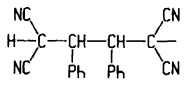
$$2AN-NO_2^{\cdot -} \rightleftarrows (AN-NO_2^{\cdot -})_2 \rightleftarrows (O_2N-AN-AN-NO_2)^{2-} \quad (20)$$

The MBM$^{\cdot -}$ dimerization differs from that of the 9-nitroanthracene anion radical in that the dimeric dianion undergoes further reactions. In view of the product studies by Avaca and Utley[10,11] the most likely reactions following (18) are (21) to (23). These reactions are apparently fast and do not contribute to the rate of the dimerization.

$$4+ROH \rightarrow 8+RO^- \quad (21)$$

$$8 \rightarrow 9 \quad (22)$$

$$9+ROH \rightarrow (2 \rightleftarrows 3)+RO^- \quad (23)$$



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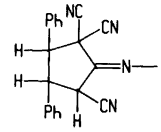


9

We can now examine the conclusions of Nadjo and Savéant[8] in more detail. They concluded that the mechanism does not change when passing from the millimolar to the decimolar concentration range of MBM during the EHD in DMF. They also concluded that residual water plays an important role in the overall reaction and that the formation of an anion radical–water adduct is an essential step as designated by eqn. (4). The dimerization of this anion radical–water adduct (5) was then proposed to play a major role in the formation of hydrodimer. The second conclusion is in sharp contrast to both the kinetic results presented in this paper and to those reported earlier from the same laboratory.[6,7]

First, we can consider the kinetic implications of adduct formation (4) followed by rate determining dimerization (5). The two extreme kinetic cases are when $K_4 \ll 1$ and when $K_4 \gg 1$. The first case gives rise to rate law (24) and the second to (25). In the

$$\text{Rate} = k_{app}|A^{\cdot -}|^2|H_2O|^2 \quad (24)$$

$$\text{Rate} = k_{app}|AS^{\cdot -}|^2 \quad (25)$$

later case the apparent rate constant would be independent of the water concentration as long as the latter is in excess. On the other hand, rate law (24) predicts a second order dependence on the water concentration. Thus, the case where $K_4 \ll 1$ is immediately ruled out on the basis of the kinetic data. However, we cannot rule out the case where $K_4 \gg 1$ on the basis of kinetics alone since the experimental rate laws are indistinguishable.

Table 11. The effect of the water concentration on the peak potential for the reduction of MBM in DMF.[a]

$\|H_2O\|$/mM	R_I'[b]	$-E^p$/mV[c]
<1[d]	0.583(0.002)	1515.0(0.2)
69.5	0.571(0.003)	1510.8(0.2)
139	0.571(0.003)	1512.1(0.4)
278	0.558(0.003)	1514.9(0.2)

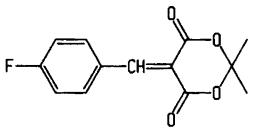
[a] Measurements by derivative cyclic voltammetry at a voltage sweep rate of 97.9 V s^{-1} in solvent containing Bu_4NBF_4 (0.1 M) at 14 °C. $E_{sw}-E_{rev}=300$ mV. [b] The derivative peak ratio. Numbers in parentheses are standard deviations in five measurements. [c] The peak potential *vs.* Ag/Ag^+ measured at a mercury electrode. The numbers in parentheses are standard deviations in five replicates. [d] The solvent electrolyte had been passed through neutral alumina before beginning measurements.

However, the later situation can be evaluated by a consideration of the effect of the water concentration on the reversible potential for the reduction of MBM. The change in reversible potential expected when $K_4 \gg 1$ is given by eqn. (26). When $K_4|H_2O| \gg 1$ the reversible potential is predicted

$$\Delta E^{rev} = (\ln 10)RT/F \log(1+K_4|H_2O|) \quad (26)$$

to shift 59.2 mV in the positive direction for each 10-fold increase in the water concentration at 298 K. The data in Table 11 were collected in order to test the effect of water concentration on the reversible potential for the reduction of MBM. The water concentration was varied by more than a factor of 278 with only small variations in the peak potential for the reduction being apparent. The expected result if $K_4|H_2O|$ is large is the E^p should have been shifted by more than 145 mV in the positive direction by the 278-fold increase in $|H_2O|$. Since this was not observed, the involvement of water in the EHD of MBM can be ruled out.

It is not surprising that water is not involved kinetically in the EHD of MBM since we find that HOAc, a very much better proton donor, also only becomes involved after the rate determining steps in the reaction. A similar situation has recently been reported during the EHD of *10* in acetonitrile.[5] This substrate was observed to undergo anion radical–substrate coupling predominantly and the rate of the reaction was not observed to be affected either by HOAc or H_2O. It was proposed that the anion radical is not very basic and this was related



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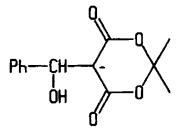
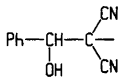


11

to the fact that at pH 5.4, benzylidene Meldrum's acid is half converted to *11*.[18-20]

We suggested earlier that the small deviations from second order kinetics during the EHD of MBM could be due to the reaction of the substrate with base. The most likely structure of the hydroxide adduct in analogy to *11* is *12*. The rapid consumption of substrate by the base generated during



12

EHD could be responsible for the deviations which Nadjo and Savéant attributed to the involvement of water in the kinetics. In any case, the experimental electrode response when this reaction is taking place could not be expected to be identical to the theoretical response for the simple dimerization mechanism. We clearly see deviations in the LSV and the derivative cyclic voltammetric response. It is somewhat surprising that the convolution potential sweep voltammetry analysis[7] did not show any deviations from the expected response for the simple dimerization in the absence of any complications.

EXPERIMENTAL

The cells, electrodes, instruments and data handling procedures were the same as used in other papers in this series.[1-5] MBM was prepared by a standard procedure.[21]

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Studies of α-Phenyl-β-amidoethanols. 1. Solution Conformations and Isomeric Distribution of the *N*-Acylamino Derivatives

BO NORDÉN and ULF EDLUND

Department of Organic Chemistry, Umeå University, S-901 87 Umeå, Sweden

A study of the solution conformations of a series of *p*-substituted α-phenyl-β-(*N*-methylacetamido)ethanols is presented. The ratio between the *E* and Z isomers has been determined under polar and nonpolar conditions using ^{1}H NMR. The *Z* isomer, which is the favoured rotamer in nonpolar solvents, is stabilized by a hydrogen bonded seven-membered ring. Electron-withdrawing substituents in the *para* position and dilute conditions favour the *Z* isomer. A qualitative discussion concerning the isomer distribution is presented.

Amino alcohols have received a lot of attention due to their use as adrenergic agents but also as model compounds for serine enzymes. Especially the multiplicity of NH and OH groups to act as hydrogen bond donors and acceptors has received a considerable interest. However, for studying the dynamic behaviour of peptides, the *N*-acylated derivatives should be more appropriate models, since these derivatives then have similarities to serine residues linked in a peptide chain.

In this paper we report an NMR study of such model compounds, α-phenyl-β-(*N*-acylamino)ethanols. We will especially focus our interest on the complex hydrogen bonding situation by investigating the equilibria between folded and stretched conformations as well as the overall association equilibrium. Besides intermolecular aggregation, one of the stable folded forms of the α-phenyl-β-(*N*-acylamino)ethanols will be intramolecularly hydrogen bonded by a seven-membered ring formed between the OH and the amide carbonyl. Such seven-membered hydrogen bond structures are prevalent in many biological systems[1–3] having an NH as a proton donor (γ-turns). A representative work in this area is the quantitative study of proline containing peptides that recently was reported.[4] Using Ac-Pro-NHMe as a model for proline residues in proteins and peptides, it was concluded that the *cis/trans* (*E/Z*) ratio is highly dependent on the solvent used and that an increased ratio is observed in more polar media. From this ^{1}H NMR study it was also stated that the dominant mode of aggregation is self-aggregation of the *cis* isomer as well as mixed association of the *cis* and *trans* isomers. This latter conclusion has been questioned in a reinvestigation but without conclusive evidence.[5] Unfortunately, such association studies in the Ac-Pro-NHMe system are difficult because of an undetectable *cis* population at low total concentrations. In our system, β-(*N*-acylamino)ethanols, the sensitivity requirement should be less serious since the *cis/trans* conformations are more equally populated in the concentration range studied.

Moreover, by changing *para* substituents of the phenyl ring, we will be able to model the effect of changes in H donating ability and field/inductive effects that could be induced in systems of biological importance.

RESULTS AND DISCUSSION

Since the model compounds contain an amide bond, one expects in an NMR study to find two rotamers within the slow exchange limit, the *E* and *Z* rotamers.

In a nonpolar solvent like chloroform, it is found that the *Z* isomer is favoured due to an intramolecular hydrogen bond developed between the hydroxyl group and the carbonyl oxygen of the amide group. By changing the medium to an aprotic dipolar solvent a shift of the *E/Z* equilibrium towards the

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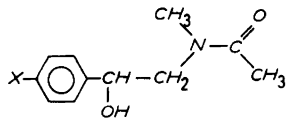

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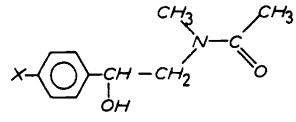

Z

E isomer is observed. The medium change also causes an overcrossing of the OH and NCH_3 resonances in ^1H NMR as proven by addition of minute amounts of DMSO to the chloroform solution. This fact facilitates the ^1H chemical shift assignment and a typical ^1H NMR spectrum of α-phenyl-β-(*N*-methylacetamido)ethanol is shown in Fig. 1. The assignment of the hydroxyl, the methine and the methylene resonances has been verified by homospin decoupling experiments.

As shown by the appearance of doublets in the proton spectrum the exchange of the hydroxyl protons is slow on the NMR time scale. This observation suggests that there is no interaction between hydroxyl groups but rather between the hydroxyl and the carbonyl function.

The different forms of molecular aggregates and conformational changes in the β-amidoalcohol system reveal a number of equilibria [eqn. (1)].

$$E_{\text{free, inter}} \leftrightarrows Z_{\text{free, inter, intra}} \quad (1)$$

Thus both conformers can be free or intermolecularly bonded. In addition, the *Z* isomer can maintain a folded, intramolecularly bonded form. By decreasing the concentration it is observed that the hydroxyl doublet of the *Z* isomer undergoes a much smaller high field shift as compared to the *E* isomer (Fig. 1). Moreover, a ^1H chemical shift of $\delta = 4.47$ of the *Z* isomer at 1 mM is a clear indication of the proposed intramolecular chelation. The hydroxyl resonance of the *E* isomer appears at $\delta = 2.05$ at this concentration. This shift value is 1 ppm low field to the infinite dilution shift found for

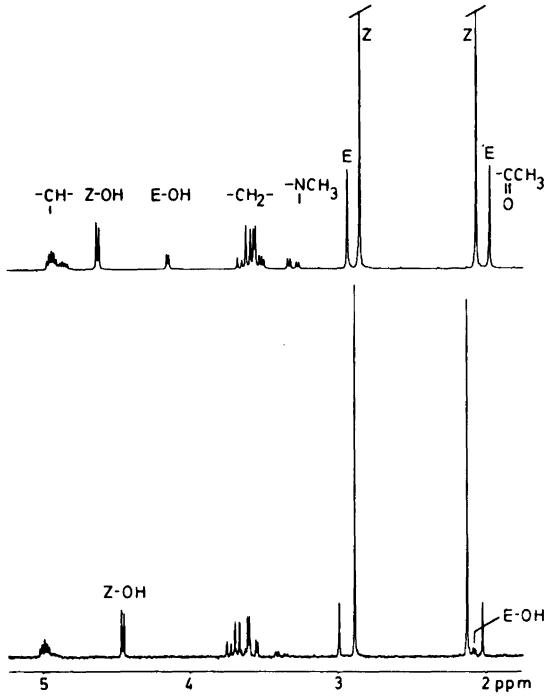

Fig. 1. Portion of a ^1H NMR spectrum of α-phenyl-β-(*N*-methylacetamido)ethanol at two concentrations in $CDCl_3$, 0.32 M (upper) and 0.0032 M.

benzyl alcohol,[6] a difference that could mainly be ascribed to the anisotropy effect of the carbonyl function.

The chemical shift difference in the reported concentration interval is an order of magnitude smaller for the *Z* structures which reflects a stronger tendency of the *Z* rotamer to exist in monomeric forms, either as free or intramolecularly bonded species. This ability is also probed by the observed *E*/*Z* ratio at the two concentrations. At 0.38 M ($CDCl_3$) the fraction of the *Z* isomer is 70 % whereas as at 1 mM the fraction of the *Z* isomer has reached 83 %.

It would also be interesting to know how an induced dipole at the phenyl moiety would affect the *E*/*Z* ratio. One could expect that electron-withdrawing groups would increase the H donating ability of the OH group and consequently favour inter- and intramolecular chelation. Thus a larger fraction of intermolecularly bonded forms would be expected for both isomers, including hybrid *E*−*Z* association. However, within the monomeric fraction of the *Z* rotamer we would also expect a larger share of intramolecularly bonded species. If an induced dipole has an influence on the H accepting ability of the carbonyl group, this polarizing effect will be most obvious for the stretched forms and decrease the H accepting ability. Since folded forms are more likely for the *Z* isomer, one would expect that such a polarizing effect, if existent, would increase the total *Z* fraction. Either of these explanations or both combined would thus account for a larger portion of the *Z* isomer, if electron-withdrawing groups are attached at the *para* position.

Table 1 shows the substituent induced changes on the *E*/*Z* ratio in two solvents and at three different concentrations. In chloroform we observe a decreased *E*/*Z* ratio for all compounds as the concentration is lowered. A few representative compounds were also examined in carbon tetrachloride at the lowest concentration but no significant changes of the *E*/*Z* ratio were observed relative to the chloroform experiments. Thus in this case any influence due to chloroform acting as an H donor can be excluded. In an aprotic polar solvent like dimethyl sulfoxide we observe as expected a large increase of the *E* fraction. The strong hydrogen bond accepting capability of this medium, means that DMSO competes effectively with the carbonyl as a hydrogen bond acceptor. Thus a decreased association of the amidoalcohol is revealed, especially obvious for intermolecular aggregation (*vide infra*). As could be seen from Table 1, the *E*/*Z* ratio is concentration independent in this range. This implies that only solvated monomers are present in DMSO solutions, an assumption in agreement with the results obtained for the Ac-Pro-NHMe system.[4]

Table 1. Substituent induced changes on the *E*/*Z* ratio in different solvents.

p-X	Conc./M	*E*/*Z* ratio	
		DMSO-d_6	$CDCl_3$
H	0.38	1.47	0.42
	0.10	1.48	0.30
	0.002	1.50	0.21
Br	0.38	1.33	0.34
	0.10	1.34	0.22
	0.002	1.42	0.15
Cl	0.38	1.31	0.35
	0.10	1.29	0.22
	0.002	1.38	0.14
F	0.38	1.36	0.37
	0.10	1.38	0.26
	0.002	1.30	0.18
NO_2	0.14[a]	1.11	0.19
	0.10	1.11	0.13
	0.002	1.01	0.07
Ph	0.38	1.40	0.42
	0.10	1.48	0.28
	0.002	1.35	0.20
OMe	0.38	1.53	0.47
	0.10	1.56	0.33
	0.002	1.64	0.21

[a] Saturated solution, 0.14 M ($CDCl_3$).

With the presence of electron-withdrawing *para* substituents, we would expect, by lowering the concentration and thus increasing the monomer population of each isomer, that the fraction of folded, intramolecularly bonded *Z* isomer would increase relative to the situation using the parent *p*-H compound. Hence, the *Z* conformer will be more favoured at low concentrations having *para* substituents like NO_2 attached compared to *p*-H or *p*-OMe. This is also observed. For α-(*p*-nitrophenyl)-β-(*N*-methylacetamido)ethanol (2 mM, $CDCl_3$) we note that almost all molecules exist in the *Z* conformation (93 %) while for α-(*p*-methoxyphenyl)-β-(*N*-methylacetamido)ethanol this portion has shifted to 83 %. Interestingly, this difference is maintained in DMSO-d_6, 50 % of *Z* for the *p*-NO_2 compound and 38 % of *Z* for the *p*-OMe compound. This strongly indicates that the intramolecular hydrogen bonded *Z* structure still exists in this

medium while no intermolecular aggregation can be proven. Similar results have been noted for γ-turns of the Ac-Pro-NHMe molecule in DMSO-d_6.[4]

Further information can be obtained by examining the ^{13}C chemical shifts of the carbonyl carbons in these experiments. The chemical shift difference for the two extremes, $\Delta\delta(\delta_{CO,OMe} - \delta_{CO,NO2})$, is −0.29 for the *E* isomer but −0.63 for the *Z* isomer when measured as 0.1 M solutions in $CDCl_3$. A low field shift is expected by going to the nitro compound for both isomers. This is due to an increased aggregation *i.e.* the fraction of hydrogen bonded carbonyl structures increases. The larger downfield shift noticed for the *Z* isomer is only compatible with a model having an intramolecular bond that is stronger than the intermolecular one or that the fraction of hydrogen bonded carbonyls is larger for the *Z* conformation than for the *E* isomer.

To summarize, the present study shows results that are very similar to those observed for the peptide derivatives[4,5] thus confirming the relevance of β-amidoalcohols as model systems for the study of peptide dynamics. Using α-phenyl-β-(*N*-acylamino)ethanols we observe a change of the overall *E*/*Z* equilibrium towards the *Z* isomer by:

A. Decreasing the amidoalcohol concentration in a nonpolar solvent.

B. Going from polar aprotic solvents to nonpolar media.

C. Having electron-withdrawing *para* substituents attached.

Quantitative variable concentration and variable temperature studies are underway.

EXPERIMENTAL

Measurements. The ^1H and ^{13}C NMR spectra were obtained on a Bruker WM-250 (5.875 T) using 16K input data points and internal ^2H lock ($CDCl_3$, DMSO-d_6). The spectral widths were 2200 Hz for the ^1H experiments and 15000 Hz for the ^{13}C NMR runs. The most dilute ^1H NMR runs required 400 transients using a 90° pulse width. The chemical shifts were measured at 26 °C using TMS as internal standard. The fractions of the *E* and *Z* isomers were measured by integration of the N-methyl and CO-methyl resonances (^1H NMR).

IR spectra were recorded on a Perkin-Elmer Model 681 ratio recording instrument using KBr plates.

Solvents. Chloroform-*d* was washed several times with water and predried over $CaCl_2$. After distillation, $CDCl_3$ was stored over molecular sieves (4A) and stabilized with Ag foil. DMSO-d_6 was dried twice over activated molecular sieves (4A).

Table 2. Melting points and IR bands.

p-X	M.p./°C[a]	IR bands/cm^{-1}
H	81–82[b]	1618, 1634, 3235, 3319, 3458
Br	109–110	1618, 1630, 3300
Cl	104–105	1618, 1631, 3328
F	111–113	1620, 1635, 3329
NO_2	135–136	1600, 1616, 1631, 3300
Ph	144–146	1617, 1632, 3352
OMe	90–92	1583, 1613, 3266

[a] Melting points were determined for *E*/*Z* isomer mixtures (recrystallized from CCl_4 or CCl_4/EtOH). [b] Lit.[10] 83–84 °C, pure *E* isomer.

Compounds. The *para* substituted α-phenyl-β-(*N*-methylamino)ethanols have been prepared according to known synthetic routes[7,8] using *para* substituted ω-bromoacetophenones as starting materials. For the *p*-nitro derivative a somewhat modified procedure has been used. *p*-Nitrostyrene oxide was reacted with an excess of methylamine (40 % solution in H_2O), using dioxane as solvent. The reaction was completed in a sealed ampoule at 120 °C for 1.5 h. The reaction mixture was diluted with chloroform and the product was extracted by dilute HCl. The solution was made basic with 10 M NaOH and finally extracted with chloroform. α-(*p*-Nitrophenyl)-β-(*N*-methylamino)-ethanol was obtained as light yellow crystals.

Acetylation of the *para* substituted α-phenyl-β-(*N*-methylamino)ethanols was achieved by using acetic anhydride.[9] No attempts were made to optimize yields which usually were in the 20–50 % range. Melting points and IR bands are given in Table 2.

Acknowledgements. We are indebted to Dr. Alois Steigel, University of Düsseldorf, for many stimulating discussions. Preparation of some of the β-(*N*-acylamino)ethanols by Mr. Leif Boström is gratefully acknowledged. We thank the Swedish Natural Science Research Council for financial support and Knut and Alice Wallenberg Foundation and The Swedish Council for Planning and Coordination of Research for financing of the superconducting NMR system.

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1,4,7-Trioxa-10-azacyclododecane and Some *N*-Substituted Derivatives; Synthesis and Cation Complexing

MARTIN J. CALVERLEY and JOHANNES DALE

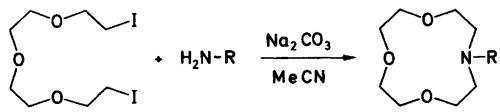
Kjemisk Institutt, Universitetet i Oslo, Oslo 3, Norway

Primary amines condense with 1,11-diiodo-3,6,9-trioxaundecane in acetonitrile solution containing dispersed Na_2CO_3 to give *N*-substituted derivatives of monoaza-12-crown-4, including several directly appended with an additional donor group. The unsubstituted azacrown and the *N*-methyl derivative were obtained from the *N*-benzyl derivative.

The alkali cation complexing properties were studied by ^{13}C NMR spectroscopy. It was found that the presence of an additional donor atom in the side-chain suppresses the formation of 2:1 in favour of 1:1 complexes.

Whereas monoaza-18-crown-6,[1-3] monoaza-15-crown-5,[3] and their variously *N*-substituted derivatives[1-4] have been known for several years, and new methods for preparing monoaza-crown ethers continue to appear,[5,6] only an *N*-aryl derivative of the 12-ring analogue has been reported.[7] Significantly, attempts to prepare non-aromatic monoaza-12-crown-4 derivatives by closing a C – O bond in the cyclization step by analogy with the reactions used for the synthesis of the larger-ring azacrowns have failed.[4,8,9] A recent report[10] on the preparation of a macrocyclic diazaoligoether *via* direct double *N*-alkylation of a diaminoether with a diiodoether promoted by alkali cations led us to consider the possibility that a corresponding C – N bond-forming reaction may generate the monoaza-12-crown-4 system. Accordingly, the condensation of primary amines with 1,11-diiodo-3,6,9-trioxaundecane (*1*) was investigated.

Heating of this diiodide (*1*), as the crude product prepared from the corresponding dichloride of tetraethylene glycol, with benzylamine in moderately dilute acetonitrile solution (0.07 M) containing dispersed Na_2CO_3 resulted in the complete consumption of the dihalide and the formation of one new compound detectable by gas chromatography. Distillation from co-polymeric material allowed the isolation of a colourless oil of narrow boiling range. This was readily shown to be *N*-benzyl-monoaza-12-crown-4 (*3a*). The isolated yield was 54 % and could be improved, as shown by GLC, by higher dilution of the reactants (>90 % in 0.02 M solution), but not by replacing the Na_2CO_3 with Li_2CO_3 (*cf.* Ref. 10). Several other derivatives of monoaza-12-crown-4 were similarly prepared from the corresponding primary amines, and details are presented in Table 1. The volatility of ammonia and methylamine precluded their use, but the *N*-benzyl derivative could be converted into the parent azacrown (*3*, R = H) by hydrogenolysis over 10 % Pd – C in 85 % yield and to the *N*-methyl derivative (*3*, $R = CH_3$) *via* the urethane (*3*, R = COOPh) by successive reaction in THF with phenyl chloroformate and $LiAlH_4$ in 74 % yield.



1 *2* *3*

Finally, coupling of the *N*-phenyl derivative with *p*-nitrophenyldiazonium chloride gave the azo-dye (*3*, $R = -C_6H_4-p-N_2-C_6H_4-p-NO_2$), m.p. 166 °C. This latter preparation complements those reported by Dix and Vögtle[11] of the corresponding *N*-aryl-monoaza derivatives of 21-crown-7, 18-crown-6 and 15-crown-5. However, whereas the position of the UV-absorption around 475 nm suffers a marked hypsochromic shift on the addition

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Table 1. Reaction of primary amines with 1,11-diiodo-3,6,9-trioxaundecane.[a]

	R in substrate (*2*) and product (*3*)	Initial conc. of *1* (M)	Reaction time (h)	Yield[b] of *3* (%)	b.p. (m.p.) (°C/mmHg)	*m/e* (M^+)
a	$-CH_2Ph$	0.07	18	54	140–143/0.05	265
b	$-CH_2CH_2OMe$	0.08	18	51	100–102/0.005	233
c	$-CH_2CH_2OH$	0.08	18	48	106–108/0.01	219
d	$-CH_2CO_2Et$[c]	0.09	48	30	118–122/0.01	261
e	$-CH_2CONH_2$[c]	0.06	48	24	(106–108)	232
f	$-Ph$	0.06	120	51	150–152/0.005	251

[a] Reactions were run under N_2 in MeCN under reflux using per eq. diiodide 1.05 eq. amine and in the presence of *ca.* 4 molar eq. anhydrous Na_2CO_3. [b] Isolated yield based on 1,11-dichloro-3,6,9-trioxaundecane taken (see Experimental). [c] Substrate used as hydrochloride.

of alkali metal salts to acetonitrile solutions of these crowns,[11] no such shift could be detected during experiments on the new azo-dye using either LiSCN or NaSCN.

An important feature of the present method is that azacrown ethers can be prepared with a side-chain providing secondary binding sites directly in place (Table 1). Such compounds are commonly prepared using an *N*-protecting group, removing it after cyclization,[12] and introducing the desired sidechain in a separate reaction.[10,12,13] The term "lariat ether" has been coined to describe this type of macrocyclic polyether, and some 18-crown-6 and 15-crown-5 lariats having either a carbon or nitrogen atom of the ring as a pivot point, have very recently been prepared and the effect of "axial solvation" of a cation on the *strength* of the complexes studied.[6,13,14]

An effect on the *stoichiometry* has been observed when two ligating side-chains are present in a 12-crown-4 system; the formation of a 2:1 sandwich complex with sodium, which is particularly strong for the tetraether,[15,16] is suppressed completely for the 4,10-bis(2-hydroxyethyl) derivative of 1,7-dioxa-4,10-diazacyclododecane.[12]

It was now of interest to examine whether a single ligating sidechain would be sufficient to suppress the formation of a sandwich complex with sodium and other cations. The complexing properties in methanol solution of some of the *N*-substituted 1,4,7-trioxa-10-azacyclododecanes were therefore studied by titration with solid alkali salts monitored by the upfield shift of the averaged ^{13}C NMR resonance lines (fast exchange). Assuming that the limiting chemical shift of a given ligand is the same in 1:1 as in 2:1 complexes of the same cation, the position of the curve bend would then give the *stoichiometry*, and the deviation from a sharp bend a qualitative idea of the *strength* when the complexation is not too strong.[12,16]

The *N*-methyl derivative served as a reference compound and gave, like 12-crown-4,[15,16] a weak 1:1 complex with Li and somewhat stronger 2:1 complexes (sandwich) with Na and K (Fig. 1).* The Na complex is the strongest of these, as was also observed[15] for 12-crown-4, although the replacement of one ether oxygen by a tertiary amino nitrogen has led to the expected general weakening.

The *N*-(2-methoxyethyl) derivative formed a 1:1 complex not only with Li, but also with Na (Fig. 2). The strength of the Li complex is practically unchanged when compared qualitatively with the *N*-methyl derivative, suggesting that the side-arm is not used in coordination to Li. In sharp contrast, the Na complex has become very much stronger even than the sandwich complex of the *N*-methyl derivative. With K (as well as with Rb) the titration curve (Fig. 2) is of a more complicated nature. After the addition of one equivalent of KSCN a constant chemical shift is reached quickly (Fig. 2), which means that the final complex must be quite strong. Its stoichiometry cannot however be 2:1 since the

* Strictly, the curves of Fig. 1 can also be interpreted on the basis of mixtures of weak complexes with various stoichiometries. However, the geometry of the 12-membered ring limits the realistic possibilities to a 1:1 complex for Li and to 1:1 and 2:1 complexes for Na and K. Since the curve for the stronger Na complex is clearly best interpreted on the basis of a dominating 2:1 stoichiometry, it seems unlikely that the larger K cation should not also bias a 2:1 stoichiometry, although its being weaker leads to a curve permitting a wider range of interpretations.

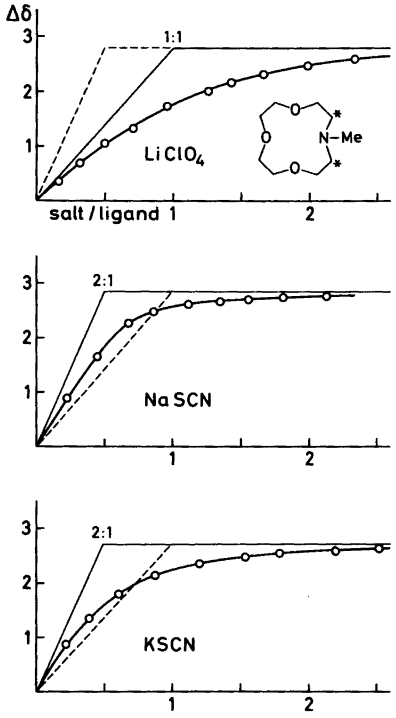

Fig. 1. ^{13}C NMR upfield chemical shift displacement ($\Delta\delta$) for the ring NCH_2 carbon of 10-methyl-1,4,7-trioxa-10-azacyclododecane (*3*, $R=CH_3$) in methanol solution on portionwise addition of solid $LiClO_4$, NaSCN and KSCN (molar ratio as abscissa). The sharp-bend curves are those expected for very strong complexing with limiting chemical shift fitted visually to the experimental curve.

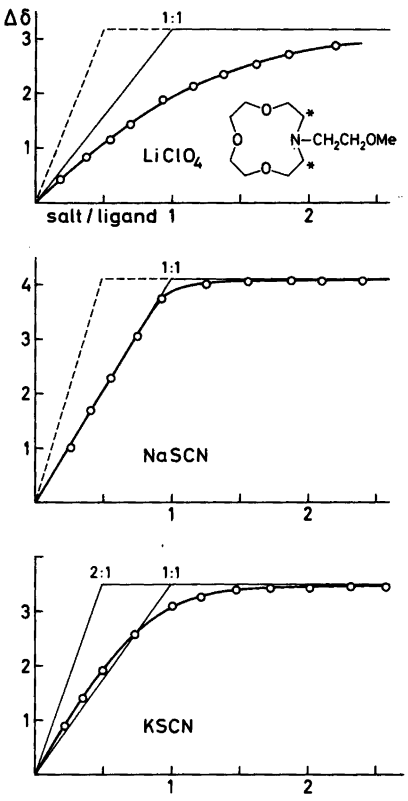

Fig. 2. ^{13}C NMR upfield chemical shift displacement ($\Delta\delta$) for the ring NCH_2 carbon of 10-(2-methoxyethyl)-1,4,7-trioxa-10-azacyclododecane (*3b*) in methanol solution on portionwise addition of solid $LiClO_4$, NaSCN and KSCN (molar ratio as abscissa). With RbSCN the titration curve resembles that of KSCN. The sharp-bend curves are those expected for very strong complexing with limiting chemical shift fitted visually to the experimental curve.

corresponding straight curve at low salt concentrations is not equally closely followed (Fig. 2). The most likely explanation is that the stoichiometry of the strong complex is 1:1 and that in the early phase of the titration a weaker 2:1 complex is present to some extent and only as long as there is an excess of ligand.

The *N*-ethoxycarbonylmethyl derivative also gave a relatively strong Na complex with 1:1 stoichiometry.

Since no seriously increased steric hindrance to sandwich complexation, involving two 12-membered rings, would be expected on passing from the *N*-methyl to the *N*-(2-methoxyethyl) derivative, we conclude that a fifth ligating oxygen atom, covalently bonded and correctly positioned 1,4 to a heteroatom of the ring, is more advantageous for Na and K cation complexing than the four ligating atoms of a second macrocycle. Of course, the loss of the second ring may in part be balanced by reestablishment of a contact ion-pair structure of the salt, since sandwiching of the cation requires its complete separation from the anion.[15]

A quantitative evaluation of the stability constants by computer analysis[12] of the titration curves was not considered justified since the results would be accurate only for the weaker 1:1 complexes among those studied here. Also, a comparison of

complexation constants is difficult when various stoichiometries are involved. We plan instead more elaborate ^{13}C NMR studies at temperatures low enough to freeze ligand exchange. The stoichiometries and the limiting chemical shifts of the complexed ligands, which must be guessed in the computer analysis, can thereby be obtained experimentally, as can the activation free energy for ligand exchange (by DNMR). The latter allows an independent estimate of the complexation constant to be made,[17] which is particularly useful in the case of strong complexing when the titration curve analysis fails.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. GLC analyses were performed on a Hewlett-Packard 5700 A Gas Chromatograph equipped with a flame ionization detector and a 2 m × 2 mm 10 % SP 2100 on Chromosorb W AW DMCS 80/100 column. TLC analyses were performed on Merck plates precoated with Kieselgel 60 F−254. Elemental analyses were carried out at Microanalytisches Laboratorium Ilse Beetz, 8640 Kronach, West Germany. IR spectra were recorded on a Jasco IRA-1 spectrophotometer. ^1H NMR spectra (normally in $CDCl_3$) were recorded on a Varian HA 100 instrument, and ^{13}C NMR spectra (normally in CH_3CN) on a JEOL JNM-FX60 instrument. Mass spectra were recorded on a Micromass 7070 F spectrometer either with electron impact (70 eV) or with chemical ionization using isobutane.

1,11-Dichloro-3,6,9-trioxaundecane. Thionyl chloride (195 g, 1.64 mol) was added to a mechanically stirred and ice-cooled solution of tetraethylene glycol (150 g, 0.77 mol) in dry pyridine (135 g, 1.71 mol) at a rate such that the temperature of the reaction mixture remained below 50 °C. Stirring was then continued at room temperature overnight (during which time the initially-formed precipitate had largely redissolved) before adding water (20 ml) and extracting with ether (5 × 100 ml), adding a further 20 ml of water before each subsequent extraction. The ether layers were combined, washed with water (100 ml), brine (100 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Distillation of the residue through a short Vigreux column gave the *dichloride* (148 g, 83 %) as a colourless liquid, b.p. 106−108 °C/0.1 mmHg. ^1H NMR ($CDCl_3$): δ 3.43−3.77 (complex sym. m, OCH_2CH_2Cl) and 3.56 (sharp s, OCH_2CH_2O).

1,11-Diiodo-3,6,9-trioxaundecane. 1. Powdered sodium iodide (100 g, 0.67 mol) was added to a solution of 1,11-dichloro-3,6,9-trioxaundecane (65.0 g, 0.28 mol) in acetone (150 ml), and the stirred mixture heated under reflux for 70 h. After cooling, the reaction mixture was filtered (washing with acetone), and the filtrate concentrated *in vacuo*, diluted with ethyl acetate (200 ml) and extracted with 20 % sodium thiosulphate solution (50 ml). The organic layer was washed with water (100 ml), brine (100 ml) and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave the *diiodide 1* (115.5 g) as a very pale yellow oil, which was used directly in the following preparations. ^1H NMR ($CDCl_3$): δ 3.19 (4H, t, *J* 7, OCH_2CH_2I), 3.58 (8H, s, OCH_2CH_2O) and 3.70 (4H, t, *J* 7, OCH_2CH_2I). GLC showed the presence of *ca.* 2 % of 1-chloro-11-iodo-3,6,9-trioxaundecane as the only significant impurity. Crystallization of the oil from ether solution gave the *diiodide 1* as needles, m.p. 92−93 °C initially colourless but soon becoming yellow on storage.

10-Benzyl-1,4,7-trioxa-10-azacyclododecane 3a. A stirred solution of 1,11-diiodo-3,6,9-trioxaundecane (30.0 g, 0.073 mol) and benzylamine (8.2 g, 0.077 mol) in dry acetonitrile (1 l) containing suspended powdered anhydrous sodium carbonate (30 g) was heated under reflux under an atmosphere of nitrogen for 18 h. The cooled solution was decanted from most of the solid and concentrated *in vacuo*. The residues from decantation and concentration were combined and partitioned between water (600 ml) and ether (300 ml). The aqueous layer was re-extracted with ether (2 × 200 ml), and the combined organic layers washed with water (200 ml), brine (200 ml) and dried (Na_2SO_4). After removing the ether *in vacuo*, the residue was distilled through a short path, collecting material volatile at 0.05 mmHg with a bath temperature up to 200 °C. The distillate was redistilled through a short Vigreux column to give the pure *aza-crown 3a* (10.5 g, 54 %) as a colourless oil, b.p. 140−143 °C/0.05 mmHg. Found: C 67.8; H 8.7; N 5.2. Calc. for $C_{15}H_{23}NO_3$: C 67.9; H 8.7; N 5.3. ^1H NMR ($CDCl_3$) δ 2.69 (4H, t, *J* 5, OCH_2CH_2N), 3.55 (4H, t, *J* 5, OCH_2CH_2N), 3.60 (10H, s, OCH_2CH_2O and $PhCH_2N$) and 7.02−7.30 (5H, m, Ar*H*). ^{13}C NMR (CH_3CN) δ 55.7 (N−*C* (ring)), 61.4 (N−*C*−Ph), 70.6, 71.0 and 71.8 (O−*C*), 127.6, 129.0 and 129.8 (tert. arom. *C*) and 141.1 (quatern. arom. *C*), MS, *m/e* (% rel. int.) 265 (M^+, 2) and 91 (100).

10-(2-Methoxyethyl)-1,4,7-trioxa-10-azacyclododecane 3b. A stirred solution of 1,11-diiodo-3,6,9-trioxaundecane (15.4 g, 0.038 mol) and 2-methoxyethylamine (2.9 g, 0.039 mol) in dry acetonitrile (0.5 l) containing suspended powdered anhydrous sodium carbonate (15 g) was heated under reflux under an atmosphere of nitrogen for 18 h. The cooled suspension was filtered and the filtrate concentrated *in vacuo* to give a semi-solid. This was

extracted with chloroform (3 × 50 ml), each time decanting the clear solution from the crystalline residue. After filtration, the combined extracts were concentrated *in vacuo* and the residue was distilled through a short path, collecting material volatile at 0.01 mm with a bath temperature up to 250 °C. (No distillation was observed before the bath temperature reached *ca.* 180 °C, although the main fraction subsequently distilled steadily at 110–115 °C.) The distillate was redistilled through a short Vigreux column to give the pure *aza-crown 3b* (4.5 g, 51 %), as a colourless oil, b.p. 100–102 °C/0.005 mmHg. Found: C 56.5; H 9.9; N 6.1. Calc. for $C_{11}H_{23}NO_4$: C 56.6; H 9.9; N 6.0. ^{1}H NMR ($CDCl_3$) δ 2.69 (2H, t, *J* 6, $CH_3OCH_2CH_2N$), 2.73 (4H, t, *J* 4.5, $CH_2OCH_2CH_2N$), 3.27 (3H, s, CH_3O), 3.43 (2H, t, *J* 6, $CH_3OCH_2CH_2N$), 3.58 (t*, *J* 4.5, $CH_2OCH_2CH_2N$) and 3.60 (s*, OCH_2CH_2O) (* these signals together integrate for 12 H). ^{13}C NMR (CH_3CN) δ 56.6 [N–*C* (ring)], 56.9 [N–*C* (side chain)], 58.9 (O–CH_3), 71.0, 71.0 and 71.9 [O–*C* (ring) (obscuring CH_3OC)]; with excess NaSCN: δ 65.6, 66.4 and 66.9 (O–*C* (ring)) and 69.2 (CH_3OC). MS, *m/e* (% rel. int.) 233 (M^+, 0.5) and 188 (100).

10-(2-Hydroxyethyl)-1,4,7-trioxa-10-azacyclododecane 3c. Ethanolamine (2.4 g, 0.039 mol) was used instead of 2-methoxyethylamine as substrate in the immediately preceding preparation. The first distillation commenced when the bath temperature was over 200 °C, the main fraction distilling at *ca.* 130 °C/0.1 mmHg. Redistillation gave the *aza-crown 3c* (3.9 g, 48 %) as a very pale yellow oil, b.p. 106–108 °C/0.01 mmHg. Found: C 54.5; H 9.5; N 6.1. Calc. for $C_{10}H_{21}NO_4$: C 54.8; H 9.7; N 6.4. IR ν_{max} 3300 (broad, OH). ^{1}H NMR (CD_3CN) δ 2.53 (2H, t, *J* 5.5, $HOCH_2CH_2N$), 2.60 (4H, t, *J* 4.5, $CH_2OCH_2CH_2N$), 3.36 (t*, *J* 5.5, $HOCH_2CH_2N$), 3.45 (t*, *J* 4.5, $CH_2OCH_2CH_2N$) and 3.52 (s*, OCH_2CH_2O) (* these signals together integrate for 15 H, and obscure $HOCH_2CH_2N$). ^{13}C NMR (CH_3CN) δ 55.8 (N–*C* (ring)), 57.5 [N–*C* (side chain)], 59.9 (O–*C* (side chain)), 69.9, 71.1 and 71.2 [O–*C* (ring)]. MS, *m/e* (% rel. int.) 219 (M^+, 1.6) and 188 (100); CIMS 220 $(M+1)^+$.

10-Ethoxycarbonylmethyl-1,4,7-trioxa-10-azacyclododecane 3d. Glycine ethyl ester hydrochloride (6.5 g, 0.047 mol) was used instead of 2-methoxyethylamine as substrate in the procedure for the preparation of the *aza-crown 3b*, but using 18.5 g (0.045 mol) diiodide *1* and 18 g sodium carbonate, and heating under reflux for 48 h. The first distillation commenced when the bath temperature was over 200 °C, the main fraction then distilling at 138–140 °C/0.05 mmHg. Considerable darkening of the residue occurred during this distillation. Redistillation gave the *aza-crown 3d* (3.5 g, 30 %) as a colourless oil, b.p. 118–122 °C/0.01 mmHg. IR ν_{max} 1725 (C=O). ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, *J* 7, OCH_2CH_3), 2.88 (4H, t, *J* 5, OCH_2CH_2N), 3.43 (2H, s, NCH_2CO_2), 3.56 (t*, *J* 5, OCH_2CH_2N), 3.60 (s*, OCH_2CH_2O) and 4.07 (2H, q, *J* 7, OCH_2CH_3) (* these signals together integrate for 12 H). ^{13}C NMR (CH_3CN) δ 14.6 (CO_2–C–*C*), 55.5 [N–*C* (ring)], 57.3 (CO_2–*C*–C), 60.7 [N–*C* (side chain)], 70.8, 70.8 and 71.5 [O–*C* (ring)] and 172.4 (O=*C*). MS, *m/e* (% rel. int.) 261 (M^+, 1.0) and 188 (100).

10-Carbamoylmethyl-1,4,7-trioxa-10-azacyclododecane 3e. The use of glycinamide hydrochloride (1.25 g, 0.011 mol) instead of glycine ester hydrochloride as substrate in the preceding preparation, except using 4.5 g (0.011 mol) diiodide *1*, 5 g sodium carbonate and 200 ml acetonitrile, gave a residue, after concentration of the chloroform solution, from which the *aza-crown 3e* (0.60 g, 24 %) was sublimed at 250 °C/0.01 mmHg as needles, m.p. 106–108 °C. IR ν_{max} (nujol mull) 3330, 3130 (NH_2) and 1680 (C=O). ^{1}H NMR (CD_3CN) δ 2.60 (4H, t, *J* 4.5, OCH_2CH_2N), 3.00 (2H, s, NCH_2CONH_2), 3.40 (4H, t, *J* 4.5, OCH_2CH_2N), 3.53 (8H, s, OCH_2CH_2O) and 5.77 (br, NH_2). ^{13}C NMR (CH_3CN) δ 56.4 [N–*C* (ring)], 59.8 [N–*C* (side chain)], 69.2, 70.7 and 71.5 [O–*C* (ring)]. MS, *m/e* (% rel. int.) 232 (M^+, 0.3) and 188 (100).

10-Phenyl-1,4,7-trioxa-10-azacyclododecane 3f. Aniline (1.9 g, 0.020 mol) was used instead of benzylamine as substrate in the procedure for the preparation of the *N*-benzyl derivative *3a*, but using 8.0 g (0.019 mol) diiodide *1*, 8 g sodium carbonate and 300 ml acetonitrile, and heating under reflux for 5 days. The extractions were performed using 1/4 of the described quantities. After removing the ether *in vacuo* the residue was distilled through a short path, collecting the *aza-crown 3f* (2.5 g, 51 %) at 150–152 °C/0.005 mmHg as a pale yellow viscous oil. Considerable darkening of the residue occurred during this distillation. ^{1}H NMR ($CDCl_3$) δ 3.47 (4H, t, *J* 5, OCH_2CH_2N), 3.54 (8H, s, OCH_2CH_2O), 3.76 (4H, t, *J* 5, OCH_2CH_2N), 6.42–6.66 (3H, unsymm. m, N–Ar–*o,p*–H) and 6.91–7.14 (2H, m, N–Ar–*m*–*H*). ^{13}C NMR (CH_3CN) δ 52.7 (N–*C* (ring)), 70.1, 70.6 and 72.0 (O–*C*), 113.2, 116.7 and 129.9 (tert. arom. *C*) and 149.7 (quatern. arom. *C*). MS, *m/e* (% rel. int.) 251 (M^+, 19) and 105 (100).

1,4,7-Trioxa-10-azacyclododecane. A solution of the *N*-benzyl-aza-crown *3a* (3.5 g, 0.013 mol) in acetic acid (10 ml) containing suspended 10% palladium on activated carbon catalyst (0.5 g) was stirred at 60 °C under 3 atmospheres of hydrogen for 15 h. The cooled mixture was filtered through a bed of celite, using ethanol for washing. The solution was concentrated *in vacuo* and the residue dissolved in water (10 ml). After basification with 30 % potassium carbonate solution (20 ml), the

solution was extracted with chloroform (4 × 25 ml) and the combined organic layers washed with 10 % potassium carbonate solution (10 ml), brine (20 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue distilled (air condenser) to give the unsubstituted *aza-crown* (*3*, R = H) (2.3 g, 85 %), b.p. 72 – 74 °C/0.01 mmHg, m.p. 59 – 60 °C. Sublimation at 60 °C/0.01 mmHg gave needles of unchanged m.p. Found: C 54.7; H 9.6; N 8.0. Calc. for $C_8H_{17}NO_3$: C 54.8; H 9.8; N 8.0. IR ν_{max} (nujol mull) 3300 (NH). ^{1}H NMR ($CDCl_3$) δ 2.70 (5 H, symm. m with t structure, "*J*" 5, OCH_2CH_2NH*) and 3.44 – 3.72 (12H, unsymm. m OCH_2) (* exchanges with D_2O, after which the m integrates for 4H). ^{13}C NMR (CH_3CN) δ 48.7 (N – *C*), 69.2, 69.8 and 71.1 (O – *C*). MS, *m/e* (% rel. int.) 175 (M^+, 0.9) and 57 (100); CIMS 176 $(M+1)^+$.

10-Methyl-1,4,7-trioxa-10-azacyclododecane. Redistilled phenyl chloroformate (3.0 g, 0.019 mol), was added to a stirred solution of the *N*-benzyl-aza-crown *3a* (4.0 g, 0.015 mol) in dry tetrahydrofuran (50 ml) containing suspended anhydrous potassium carbonate (2 g) and stirring continued overnight.

The reaction mixture was shaken well with 10 % potassium hydroxide solution (50 ml) and extracted with ethyl acetate (3 × 50 ml). The combined organic layers were washed with water (30 ml), brine (30 ml) and dried ($MgSO_4$). Removal of the solvents *in vacuo* gave an equimolar mixture of 10-phenoxy-carbonyl-1,4,7-trioxa-10-azacyclododecane (*3*, R = – COOPh) and benzyl chloride as an oil. IR ν_{max} 1720 (urethane C = O). ^{1}H NMR ($CDCl_3$) δ 3.40 – 3.98 (16H, m, crown ring protons), 4.45 (2H, s, $PhCH_2Cl$) and 6.88 – 7.20 (10H, m, Ar*H*). MS, *m/e* (% rel. int.) 202 (M^+ crown-OPh, 27) and 114 (100).

This mixture was dissolved in dry tetrahydrofuran (200 ml) and the stirred solution cooled to 0 °C. Lithium aluminium hydride (2.5 g, 0.066 mol) was added in one portion, and stirring was continued at room temperature for 18 h. Excess metal hydride was destroyed by the careful addition of a mixture of water (5 ml) and tetrahydrofuran (20 ml) to the ice-cooled suspension, which was then stirred for an additional 30 min before filtering on a sintered glass funnel, the residue being well washed with tetrahydrofuran. The filtrate was concentrated *in vacuo*, dissolved in water (50 ml) and extracted with ether (3 × 20 ml). The ether extracts (containing phenol) were discarded, and the aqueous layer concentrated *in vacuo*. After removal of the last traces of water by concentration of an absolute ethanolic solution, the residue was distilled through a short Vigreux column to give the *N*-methylated *aza-crown* (*3* R = CH_3) (2.1 g, 74 %) as a colourless oil, b.p. 65 – 67 °C/0.1 mmHg. Found: C 57.4; H 10.1; N 7.2. Calc. for $C_9H_{19}NO_3$: C 57.1; H 10.1; N 7.4. ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, s, NCH_3), 2.58 (4H, t, *J* 4.5, OCH_2CH_2N), 3.58 (4H, t, *J* 4.5, OCH_2CH_2N) and 3.60 (8H, s, OCH_2CH_2O). ^{13}C NMR (CH_3CN) δ 44.8 (N – CH_3), 58.3 (N – *C* (ring)), 70.2, 71.0 and 71.5 (O – *C*). MS, *m/e* (% rel. int.) 189 (M^+, 4) and 71 (100); CIMS 190 $(M+1)^+$.

10-[4-(4-Nitrophenylazo)-phenyl]-1,4,7-trioxa-10-azacyclododecane. 4-Nitroaniline (6.50 g, 0.047 mol) was dissolved with warming in a mixture of water (15 ml) and hydrochloric acid (36 %, 10.5 ml, 0.104 mol). The stirred solution was cooled in ice while a solution of potassium nitrite (4.1 g, 0.048 mol) in water (7 ml) was added slowly enough to keep the temperature below 5 °C. The resulting solution of 4-nitrophenyldiazonium chloride was diluted to 250 ml with iced water.

An aliquot (17 ml) of the diluted solution was withdrawn and the N-phenyl-aza-crown *3f* (0.80 g, 3.2 mmol) was added to it with stirring at 0 °C. A solution of potassium acetate (0.38 g, 3.9 mmol) in water (3 ml) was added and stirring continued for 30 min at 5 °C. The precipitated dye was filtered off, washed consecutively with water, 10 % acetic acid, water and ethanol, and dried in air. Recrystallization from toluene (50 ml) gave the *azo-dye* (*3*, R = $-C_6H_4-N_2-C_6H_4NO_2$) (0.65 g, 51 %) as orange-red needles, m.p. 166 °C. Found: C 60.8; H 6.0; N 13.7. Calc. for $C_{20}H_{24}N_4O_5$: C 60.0; H 6.0; N 14.0. ^{1}H NMR ($CDCl_3$) δ 3.54 (8H, s, OCH_2CH_2O), 3.61 (4H, t, *J* 5, OCH_2CH_2N), 3.83 (4H, t, *J* 5, OCH_2CH_2N), 6.67 (2H, d, *J* 9, $CH_2N-Ar-o-H$), 7.68 (2H, d, *J* 9, $CH_2N-Ar-m-H$), 7.71 (2H, d, *J* 9, $O_2N-Ar-m-H$) and 8.11 (2H, d, *J* 9, $O_2N-Ar-o-H$). MS, *m/e* (% rel. int.) 400 (M^+, 100). UV λ_{max} (CH_3CN) 479 nm (unchanged on the addition of either Li-, Na- or K-SCN).

Titration curves. The ligand solutions in CD_3OD (2 ml) were ~0.5 *M*. After each addition of solid alkali salt the ^{13}C NMR chemical shift (rel. to TMS) was measured at room temperature in a Bruker CXP-200 instrument operating at 50 MHz. The four signals for the ring carbons underwent the strongest upfield displacement; the one for the ring NCH_2 carbons was best resolved and selected for plotting the curves in Figs. 1 and 2.

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Silver Imidazolate-assisted Glycosidations. Part 6. Synthesis of 1,2-*trans*-Linked Disaccharides

PER J. GAREGG, ROLF JOHANSSON and BERTIL SAMUELSSON

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

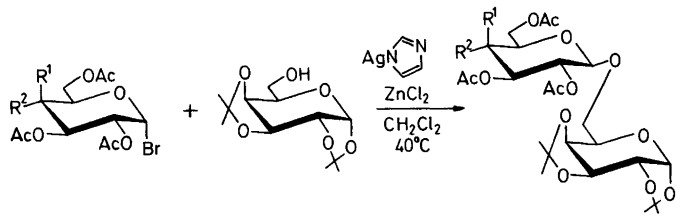
The use of silver imidazolate and zinc chloride as a promoting system for the synthesis of 1,2-*trans*-linked disaccharides in the D-glucopyranose and D-galactopyranose series is described.

Syntheses of 1,2-*trans*-linked di- and oligosaccharides [1,2] are generally based upon the original work of Koenigs and Knorr [3] or upon the orthoester glycosylation method.[4,5] In the former type of approach silver triflate has been shown to be a most efficient glycosidation promotor.[6–8]

In our continued exploration of the use of silver imidazolate–Lewis acid promoted glycosidation reactions [9] we now report on the use of silver imidazolate and zinc chloride as a useful promoting system for the synthesis of 1,2-*trans*-linked glycosides. In disaccharide syntheses the molar proportions of 2,3,4,6-tetra-*O*-acetyl-α-D-hexopyranosyl bromide–aglycone–zinc chloride–silver imidazolate was 1.5:1: ~6:0.75 in dichloromethane containing molecular sieves. Using this procedure four disaccharides, *3*, *4*, *5* and *6* were made. The yields of the β-1,6-linked disaccharides *3* and *4* were 90 and 87 %, respectively, those of *5* and *6* were 30 and 37 %, respectively, demonstrating the expected lowering of yield with lowering aglycone reactivity. In the reaction mixture containing *5* a by-product with higher mobility than that of *5*, presumably a product of acetal migration,[10] was observed on TLC with ^{13}C NMR parameters identical to those of 6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose.[10] In these reactions it is essential that not more than 0.75 mol equivalents of silver imidazolate is used, in order to avoid the formation of orthoesters.

EXPERIMENTAL

General methods were the same as those reported elsewhere.[11] Silver imidazolate was prepared as previously described. Commercial dry zinc chloride was fused by heating in a Pyrex tube until vigorous boiling ceased and the melt was poured into tetrachloromethane. The pellets of dry zinc chloride thus formed may be stored under carbon tetrachloride for up to two weeks. Before use, the pellets were transferred into a mortar under dichloromethane, crushed to a fine powder, weighed, and transformed rapidly into the reaction vessel. Twice the required amount of zinc chloride was weighed out to compensate for adhering solvent.



1 R^1=H R^2=OAc
2 R^1=OAc R^2=H

3 R^1=H R^2=OAc (90%)
4 R^1=OAc R^2=H (87%)

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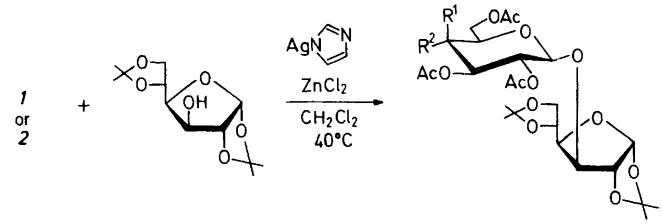

5 R^1=H R^2=OAc (30%)
6 R^1=OAc R^2=H (37%)

Glycosylation procedure. 6-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (3). A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (*1*) (616 mg, 1.5 mmol), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (260 mg, 1.0 mmol), silver imidazolate (131 mg, 0.75 mmol) and zinc chloride (about 820 mg, ~6 mmol) in dichloromethane (15 ml) containing 4 Å molecular sieves was stirred in the dark at 40 °C for 14 h. The mixture was filtered, the solids were washed with toluene (75 ml) and the combined filtrate and washings poured into saturated aqueous sodium carbonate (250 ml) and toluene (250 ml). The mixture was stirred for 30 min and then separated. The organic phase was washed with aqueous sodium hydrogencarbonate and then water, dried ($MgSO_4$), filtered, concentrated and subjected to silica gel column chromatography (toluene–ethyl acetate 2:1) to yield *3* (530 mg, 90 %), $[\alpha]_D^{22}$ −53° (*c* 1.0, chloroform) [lit. [3] $[\alpha]_D$ −55° (chloroform)], $\delta_{^{13}C}$ ($CDCl_3$, from internal TMS): 96.0 (C-1), 101.7 (C-1'), 108.4 and 109.1 [2 × *C* $(OR)_2$].

6-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (4). After a reaction time of 14 h and work-up including silica gel column chromatography (toluene–ethyl acetate 5:2), an 87% yield of *4* was obtained. $[\alpha]_D^{22}$ −44° (*c* 1.0, chloroform) [lit.[3] −47° (chloroform)], $\delta_{^{13}C}$ ($CDCl_3$): 96.4 (C-1), 101.4 (C-1'), 108.6 and 109.3 [2 × *C* $(OR)_2$].

3-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (5). After a reaction time of 72 h and work-up, including silica gel column chromatography (toluene –ethyl acetate 1:1) a 30 % yield of *5* was obtained, m.p. 131–133 °C (from diethyl ether–light petroleum), $[\alpha]_D^{22}$ −20° (*c* 1, chloroform) [lit.[5] m.p. 130–132 °C, $[\alpha]_D$ −21° (chloroform)]. $\delta_{^{13}C}$ ($CDCl_3$): 101.1 (C-1'), 105.0 (C-1), 108.8 and 112.2 [2 × *C*$(OR)_2$].

3-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (6). After a reaction time of 48 h and work-up [including silica gel column chromatography (toluene–ethyl acetate 2:1)], a 37% yield of *6* was obtained, m.p. 157 °C (from chloroform–light petroleum), $[\alpha]_D^{22}$ −9° (*c* 1, chloroform). Anal. $C_{26}H_{38}O_{15}$: C, H. $\delta_{^{13}C}$ ($CDCl_3$): 100.0 (C-1') 105.0 (C-1), 108.7 and 112.1 [2 × *C* $(OR)_2$].

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A Facile Preparation of 2′,3′-Unsaturated Nucleosides and Hexopyranosides from Acetylated Halohydrins by Reductive Elimination

BJÖRN CLASSON, PER J. GAREGG and BERTIL SAMUELSSON

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Reductive eliminations by treatment of acetylated halohydrins with zinc powder in ethanol containing acetic acid are described. The examples include a purine, a pyrimidine and a disaccharide derivative. The procedure allows facile access to 2,3-dideoxy-2-enoside derivatives.

Nucleosides containing unsaturation in the sugar moiety are found among naturally occurring antibiotics *e.g.* blasticidin S[1,2] and angustomycin.[3] Modified nucleosides are also of interest as potential enzyme inhibitors of viruses and malignant cells. Unsaturated sugar derivatives may further be used for synthetic manipulations.

Several routes to 2′,3′-unsaturated nucleosides have been described.[4–8] In one of these, 2′-deoxynucleosides are transformed *via* the 3′,5′-ditosylate into the 3′,5′-anhydrosugar with inverted configuration at C-3′. The latter, upon treatment with sodium hydroxide in hexamethylphosphoric triamide, gives 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-uracil and -thymine.[4] Another route, exemplified by the synthesis of a 2′,3′-unsaturated derivative from adenosine, proceeds *via* the intermediate 6-*N*-pivalamido-9-[3-deoxy-3-iodo-2-*O*-(4,4-dimethyl-3-pivaloxy-pent-2-enoyl)-5-*O*-pivalyl-β-D-xylofuranosyl]purine.[5,6] This route involves chromatographic separation of the desired intermediate from various other compounds formed simultaneously.[5,6] In two other methods, 2′(3′)-*O*-acyl-3′(2′)-deoxyhalonucleosides, readily obtained in a single reaction step from the parent ribonucleosides,[7] are subjected to reduction, either with chromium(II) acetate[7] and ethylene diamine or electrolytically.[8]

We now describe an improved, efficient reductive elimination of vicinal haloacetates producing unsaturation, applicable to nucleosides and hexopyranosides. In this method, zinc powder in ethanol containing acetic acid is used at room temperature. Three examples are given, comprising a purine and a pyrimidine nucleoside and a disaccharide derivative.

The crude 5′-*O*-substituted 2′(3′)-*O*-acetyl-3′(2′)-deoxyhalonucleosides *1*, *2* and *4* (Scheme 1) were synthesized as described by Moffatt and co-workers.[7] Attempted iodination of uridine according to procedure a, Scheme 1, failed, probably due to product decomposition. Treatment of *1*, *2* and *4* with zinc powder in ethanol containing acetic acid, followed by Zemplén deacylation afforded the product *3* and *5*, respectively, in the yields indicated.

9-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-adenine (*3*) and 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)uracil (*5*) have been tested for activity against *Herpes influenzae* virus. Compound *3* had general cell toxicity, while *5* was essentially inactive.

Ferrier and co-workers[9,10] have described the reaction of 3,4,6-tri-*O*-acetyl-D-glucal with alcohols in inert solvents in the presence of boron trifluoride to obtain 4,6-di-*O*-acetyl-α-D-*erythro*-hex-2-enopyranosides. The corresponding reaction starting from 3,4,6-tri-*O*-acetyl-D-galactal did not give the 4,6-di-*O*-acetyl-α-D-*threo*-hex-2-enopyranosides due to subsequent elimination reactions.[9,10] The use of tin(IV) chloride instead of boron trifluoride did, however, give the *threo*-isomers. Thus, the reaction of 3,4,6-tri-*O*-acetyl-D-galactal with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose in 1,2-dichloromethane in the presence of tin(IV) chloride afforded 6-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactofuranose in a 56 % yield.[11]

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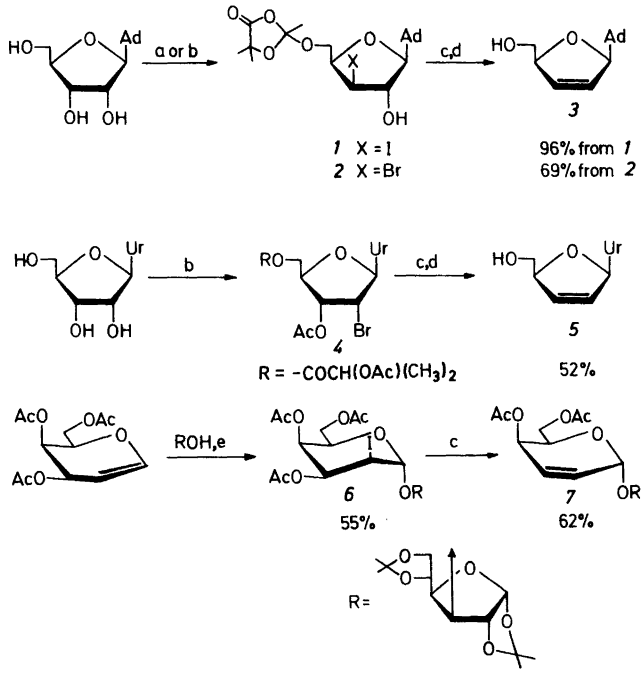

Scheme 1. a: $(CH_3)_2C(OAc)COCl$, NaI, CH_3CN; b: $(CH_3)_2C(OAc)COBr$, CH_3CN; c: Zn(powder), CH_3CO_2H, CH_3CH_2OH; d: $NaOCH_3$, CH_3OH; e: $ZnCl_2$ (or $HgCl_2$), I_2, silver imidazolate, CH_3CN.

In the present work, 1,2:5,6-di-*O*-isopropylidene-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-iodo-α-D-talopyranosyl)-α-D-glucofuranose (*6*) was obtained by the reaction of 3,4,6-tri-*O*-acetyl-D-galactal with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose in acetonitrile in the presence of iodine, zinc chloride and silver imidazolate as previously described.[1,2] Treatment of *6* with zinc dust in ethanol containing acetic acid gave the disaccharide *7* containing a 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyl residue in a 62 % yield. The reductive elimination from *6* can conceivably follow two paths, *cis*-elimination yielding *7* and also *trans*-diaxial elimination yielding 3,4,6-tri-*O*-acetyl-D-galactal. The latter was indeed observed as a by-product. When better leaving groups than alkoxyls are positioned at C-1, the *trans*-diaxial elimination is favoured.[13]

EXPERIMENTAL

General methods were the same as those previously reported.[14] *9-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)adenine*[4,6,7] (*3*). Zinc powder (2.0 g, 30 mmol) and acetic acid (0.37 g, 6.2 mmol) were added with stirring at room temperature to a solution of 9-[2-*O*-acetyl-3-deoxy-3-iodo-5-*O*-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-xylofuranosyl]adenine (*1*)[7] (1.7 g, 3.1 mmol) in ethanol (80 ml). After a reaction time of 15 min the mixture was filtered through Celite, concentrated to about 10 ml (at a bath temperature below 30 °C), diluted with ethyl acetate (100 ml) and transferred to a separating funnel. The solution was washed with aqueous sodium carbonate (3 × 20 ml) and then water (2 × 20 ml); dried ($MgSO_4$), filtered and concentrated to dryness. The residue was dissolved in 0.1 M sodium methoxide in methanol (40 ml) and allowed to stand for 10 min at room temperature. The solution was neutralized with acetic acid, concentrated to dryness and the product was purified by silica gel column chromatography (ethyl acetate – methanol – water 6:3:1) to yield *3* (695 mg, 96 %), $[\alpha]_D^{22}$ + 20° (*c* 0.25, methanol), m.p. 185 – 187 °C (from methanol) lit.[7] $[\alpha]_D$ + 23° (methanol), m.p. 194 – 195 °C], ^1H NMR shifts and coupling constants were in agreement with those published.[7]

1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil[4,7] (*5*). Zinc powder (2.74 g, 41.9 mmol) and acetic acid (1.0 g, 16.7 mmol) were added with stirring at room temperature to 5′-*O*-(2-acetoxyisobutyryl)-3′-*O*-acetyl-2′-bromo-2′-deoxyuridine

(*4*)[7] (2.0 g, 4.2 mmol) in ethanol (300 ml). After a reaction time of 10 min the mixture was filtered through Celite. Triethylamine (1.0 ml) was added to the filtrate which was concentrated to 10 ml; 0.4 M sodium methoxide in methanol (50 ml) was added. After 30 min at room temperature the solution was neutralized with acetic acid and concentrated to dryness. The solid residue was extracted with boiling ethyl acetate, the combined extracts were concentrated to dryness and the product subjected to flash chromatography on silica gel[15] (chloroform–ethanol 95:5) to yield *5* (455 mg, 52 %), $[\alpha]_D^{22}$ −15° (*c* 0.2, methanol), m.p. 156–158 °C (from methanol) [lit.[7] $[\alpha]_D$ −15° (methanol), m.p. 155–156 °C]. ^{1}H NMR shifts and coupling constants were in agreement with those published.[7]

3-O-(3,4,6-Tri-O-acetyl-2-deoxy-2-iodo-α-D-talopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (*6*) was prepared as described before[12] except that dry zinc chloride[16] was used instead of mercury (II) chloride in the glycosidation step.

3-O-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-threo-hex-2-enopyranosyl)-1,2:5,6-di-O-isopropylidene)-α-D-glucofuranose (*7*). Zinc powder (1.1 g, 16.8 mmol) was added to a solution of *6* (1.0 g, 1.52 mmol) in ethanol (50 ml) and then acetic acid (350 mg, 1.63 mmol) at room temperature with stirring. After a reaction time of 15 min, pyridine (1 ml) was added. The mixture was filtered through Celite, concentrated to about 5 ml, dissolved in toluene (200 ml) and transferred to a separating funnel. The solution was washed with aqueous sodium carbonate (4 × 75 ml), water (2 × 75 ml), dried ($MgSO_4$), filtered and concentrated to dryness. The product was purified by silica gel column chromatography (toluene–ethyl acetate 1:1 containing 0.5 % pyridine) to yield 7 (477 mg, 66 %). $[\alpha]_D^{22}$ −116° (*c* 1.0, chloroform). Anal. $C_{22}H_{32}O_{11}$: C, H. δ_{1H} (100 MHz, $CDCl_3$): 1.32 (s, 6 H, 2 CH_3), 1.41, 1.51 (2 s, each 3 H, 2 CH_3), 2.09, 2.12 (2 s, each 3 H, $COCH_3$), 4.00–4.37 (m, 8 H, H-3, H-4, H-5, H-6,6, H-5′, H-6′,6′), 4.62 (d, 1 H, H-2), 5.02 (dd, 1 H, H-4′), 5.35 (d, 1 H, H-1′), 5.89 (d, 1 H, H-1), 6.06 (d, 1 H, H-2′), 6.11 (d, 1 H, H-3′); $J_{1,2}$ 3.7 Hz, $J_{1',2'}$ 2.5 Hz, $J_{2',3'}$ ~0 Hz, $J_{3',4'}$ 5.2 Hz, $J_{4',5'}$ ~2 Hz, δ_{13C} (25 MHz, $CDCl_3$): 20.8, 20.9 (2 × $COCH_3$), 25.5, 26,6, 27.0, 27.1 (4 × CH_3), 62.9, 63.2, 67.3, 68.0, 72.8, 80.9, 81.4, 84.5 (C-2, C-3, C-4, C-5, C-6, C-4′, C-5′, C-6′), 95.0 (C-1′), 105.6 (C-1), 109.3, 112.1 [2 × C $(OR)_2$] 125.5, 130.1 (C-2′, C-3′), 170.4, 170.9 (2 × $COCH_3$).

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Short Communications

A Comparison of Induction of Microsomal Glutathione S-transferase Activity in the Liver of the Mouse and Rat by Dietary 2(3)-*tert*-Butyl-4-hydroxyanisole (BHA)*

RALF MORGENSTERN[a,**] and LENNART DOCK[b]

[a] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden and [b] Department of Forensic Medicine, Karolinska Institute, S-104 01 Stockholm 60, Sweden

Glutathione *S*-transferases (EC 2.5.1.18) (for a review, see Ref. 1) are a family of enzymes involved in the detoxification of numerous mutagenic, carcinogenic and pharmacologically active substances.[2] These enzymes, as well as others involved in drug metabolism, can be induced by different compounds, notably phenobarbital, 3-methylcholenthrene, *trans*-stilbene oxide[3,4] and 2(3)-*tert*-butyl-4-hydroxyanisole.[5,6] The glutathione *S*-transferase activity in rat liver microsomes is, however, not significantly affected by these inducers.[7,8] The highest induction of glutathione *S*-transferase activity reported to data is about 10-fold in mouse liver cytosol when the animals received BHA in their diet.[5] We therefore decided to investigate whether this species demonstrates a liver microsomal glutathione *S*-transferase activity which can be distinguished from its cytosolic counterpart by *N*-ethylmaleimide (MAlNEt)-activation[9] and also whether this *in vitro* activity can be elevated by treatment with BHA. A parallel study was made on the rat.

Experimental. Female NMRI mice (25–30 g) and male Sprague-Dawley rats (180–200 g) were used in these studies. Mice and rats were fed a diet containing 0.75 g BHA/kg for 2 weeks. Control animals received the same diet without BHA. Microsomes and cytosol were prepared according to Ref. 10 except that microsomes were washed twice in 0.15 M Tris-Cl, pH 8, in order to remove cytosolic contaminations. Glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate was measured as in Ref. 11. Activation of microsomal activity with 1 mM MalNEt was carried out as described previously.[7] Protein was determined by the method of Lowry *et al.*[12] with bovine serum albumin as the standard. All chemicals were obtained from common commercial sources and were of the highest purity available.

The results in Table 1 show that glutathione S-transferase activity is found in mouse liver microsomes. The activity towards CDNB is nearly

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**To whom correspondence should be sent.

Table 1. Glutathione *S*-transferase activity towards CDNB in rat and mouse liver.[a]

	Cytosol	Microsomes + MalNEt	
Rat			
Control	1170 ± 110[b]	140 ± 20	600 ± 58
BHA treated	1990 ± 350	167 ± 10[c]	667 ± 28
Mouse			
Control	2050 ± 270	147 ± 35	689 ± 140
BHA treated	15500 ± 3600	251 ± 66[d]	995 ± 230[d]

[a] Expressed as (nmol/min) (mg protein). [b] $\bar{x} \pm$ SD $n = 9$ (mice), 6 (rats) and 3 (rat microsomes + MalNEt). [c] Significantly different $p < 0.005$. [d] Significantly different $p < 0.001$; students *t*-test was used.

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the same as in rat. In addition, activation with MalNEt occurs to about the same extent in both species. Because this SH reagent stimulatable activity is located on the endoplasmic reticulum,[7] one can study the effect of inducers without (or allowing for) cytosolic interference. As can be seen in the table, mouse microsomal glutathione *S*-transferase activity is significantly increased by BHA both in untreated (171%) and MalNEt treated (144%) microsomes. The higher figure in untreated microsomes is probably due to some increase in cytosolic contamination. This is not unexpected, because of the large increase in the soluble activity (756%). In the parallel study with the rat, a small but significant increase (119%) is seen in the microsomal activity. However, MalNEt-treated microsomes reveal no significant difference in activity between microsomes from BHA-treated and control animals. Hence the small elevation might be due to increased cytosolic contamination. Both the mice and rat cytosol induction agrees with previous data.[5,6,13] In conclusion we have shown the presence of microsomal glutathione *S*-transferase activity in mouse liver and shown it to be inducible by BHA. This could be important concerning drug biotransformation in the mouse and the protective effect exerted by BHA in connection with carcinogenesis.

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Purification of Microsomal Glutathione *S*-Transferase *

RALF MORGENSTERN, CLAES GUTHENBERG and JOSEPH W. DEPIERRE

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The glutathione *S*-transferases play a major role in the detoxication and excretion of xenobiotics.[1] The individual members of this family of enzymes exhibit broad and overlapping substrate specificities towards a variety of mutagenic, carcinogenic and pharmacologically active substances.[2] These substrates resemble one another in that they are all hydrophobic and electrophilic. Many of the reactive intermediates formed by the metabolism of xenobiotics *via* the cytochrome P-450 system are hydrophobic and electrophilic and can thus serve as substrates for the glutathione *S*-transferases.[3,2]

Soluble glutathione *S*-transferases have been purified and characterized from a number of different sources.[3] Rat liver cytoplasm contains at least 7 such enzymes and the most throughly studied of the glutathione *S*-transferases are the three major cytosolic forms from this organ, *i.e.*, glutathione *S*-transferases A, B, and C.[1,4,5] Recently, rat liver microsomes have also been shown to demonstrate glutathione *S*-transferase activity, both in our laboratory and by others.[6,7]

In order to characterize this enzyme, compare it to the soluble proteins which catalyze similar reactions and investigate its role in drug metabolism, we have developed a simple procedure for purifying the mixrosomal glutathione *S*-transferase to near homogeneity in high yield.

Male Sprague-Dawley rats weighing 180–200 g were starved overnight and liver microsomes prepared as described previously,[8] except that the microsomes were washed twice with 0.15 M Tris-HCl, pH 8.0, in order to remove cytosolic contamination.[6] The microsomal fractions from 10 rats were pooled and resuspended in 35 ml 0.25 M sucrose.

20 ml 10 mM *N*-ethyl maleimide in 10 mM potassium phosphate buffer, pH 7.0, was then added dropwise with gentle stirring to the microsomal suspension at 4 °C over the course of 5 min. This treatment results in a 6–8-fold activation of the microsomal glutathione *S*-transferase,[9] thereby facilitating the purification and assuring that we isolate the endogenous microsomal enzyme and not cytosolic contaminants in the microsomal fraction. After an additional 2 min, 2 ml 0.1 M glutathione, adjusted to pH 7.0 with KOH, was also added to the incubation mixture, which was then gently stirred for 5 min. Glutathione reacts rapidly with remaining *N*-ethyl maleimide, thus terminating the activation, as well as preventing the inhibition of the microsomal glutathione *S*-transferase which occurs when microsomes are incubated with this sulfhydryl reagent for longer periods of time.

Subsequently, the microsomal transferase was solubilized by adding 20 ml 10% Triton X-100 dropwise to the mixture over the course of 10 min and stirring for an additional 20 min. The potassium phosphate concentration of the mixture was then adjusted to 10 mM by the addition of 0.57 ml 1 M potassium phosphate, pH 7.0. All subsequent operations were conducted in the coldroom at 4–8 °C.

The activated and solubilized microsomes were then loaded onto an hydroxyapatite column (4 × 35 cm) equilibrated with 10 mM potassium phosphate, pH 7.0 – 1 mM glutathione – 0.1 mM EDTA – 1% Triton X-100 – 20% glycerol (hereafter referred to as standard buffer). This column was eluted at a rate of 1 ml/min with 5 l of a linear gradient of 0.02–0.3 M potassium phosphate, pH 7.0, in standard buffer. 20 ml fractions were collected and the fractions containing glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene[5] (fractions 112–135 eluting between 135 and 165 mM potassium phosphate) were pooled to give a total volume of 480 ml. (At least 0.02% Triton X-100 is required in the assay mixture to obtain full activity of the solubilized microsomal glutathione *S*-transferase.) The potassium phosphate concentration in this pool was reduced by passage through a Sephadex G-25 fine column (9 × 30 cm) eluted with standard buffer.

The Sephadex G-25 pool, with a total volume of 500 ml, was subsequently applied to a carboxymethyl-Sepharose column (2 × 7 cm) at a rate of 0.3 ml/min. This column was then eluted with 200 ml of a linear gradient of 0–0.2 M KCl in standard buffer at the same rate. 1.8 ml fractions were collected and the top fractions and edge fractions (as shown in Fig. 1) were pooled individually. These pools can be stored at −20 °C for at least half a year without loss of activity.

Protein was determined by the method of Peterson[10] including the TCA-deoxycholate precipitation step in order to avoid interference by the detergent and glutathione present. Bovine serum albumin was used as the standard. SDS-polyacrylamide gel electrophoresis was carried out

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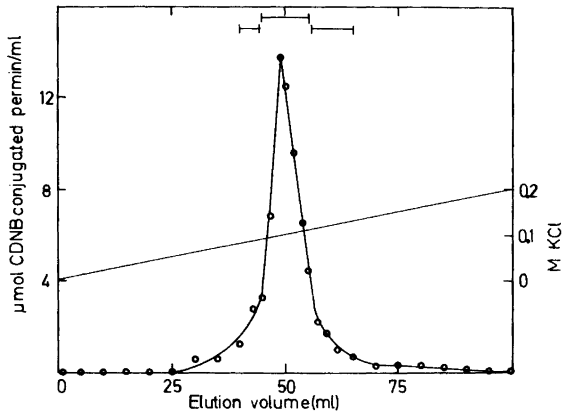

Fig. 1. Elution of the microsomal glutathione *S*-transferase from CM-Sepharose. The details of this purification step are present in the text. 5–25 µl aliquots of the different fractions were assayed for glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene. The fractions which were pooled and used for subsequent analysis are indicated by the bars at the top of the figure.

with 15% acrylamide in 1 mm thick slab gels with a discontinuous buffer system as described by Laemmli.[11]

After activation with *N*-ethyl maleimide and solubilization with Triton X-100 a 3-fold purification of the microsomal glutathione *S*-transferase could be achieved by chromatography on hydroxyapatite (Table 1). After desalting on a column of Sephadex G-25 fine, a further 11–12-fold purification was accomplished by chromatography on CM-Sepharose (Table 1). The glutathione *S*-transferase activity was eluted by a KCl gradient from the CM-Sepharose column as one symmetric peak (Fig. 1).

As also can be seen from Table 1, the specific activities of the peak fractions and the pooled side fractions were equal. In addition both of these preparations were found to be more than 95% homogeneous (as approximated by scanning the gels with filters open between 440–630 nm) and the molecular weight approximately 14 000 by SDS-polyacrylamide gel electrophoresis (not shown). Thus, the peak and side fractions could be pooled.

Since of 30–40-fold purification sufficed to yield a nearly homogeneous protein, it can be concluded that the microsomal glutathione *S*-transferase accounts for approximately 2.5–3% of the total

Table 1. Purification scheme for microsomal glutathione *S*-transferase.

Step	Volume (ml)	Activity/ml (µmol/min ml)	Total activity (µmol/min)	Protein (mg/ml)	Total protein (mg)	Specific activity (µmol/min mg)	Purification factor	Recovery %
Microsomes	35	2.8	98	23.8	833	0.12		
Activated solubilized	78	9.2	718	10.7	835	0.86		100
Hydroxy apatite pool	480	0.79	379	0.32	154	2.46	2.9	53
G-25 pool	500	0.60	300	0.26	130	2.31	2.7	42
CM-Sepharose	500	0.13	65	0.22	110	0.59		9
flow through	500	0.13	65	0.22	110	0.59		9
CM-Sepharose peak pool[a]	20	8.2	164	0.27	5.4	30.3	35	23
CM-Sepharose side pool[a]	26	3.7	96.2	0.12	3.1	30.9	36	13

[a] The pools are indicated in Fig. 1.

microsomal protein. The overall recovery of activity was about 37% (peak plus side fractions). This corresponded to a yield of 8.5 mg protein from 10 rats.

This preparation is highly suitable for investigations on the nature of the microsomal glutathione *S*-transferase. We are in the process of determining, among other things, the minimum molecular weight of this enzyme; the size and composition of the complexes it forms with Triton X-100; its amino acid composition, isoelectric point, pH optimum, substrate specificity, *etc.* We are also trying to isolate the unactivated form of the microsomal glutathione *S*-transferase in order to compare its properties to those of the *N*-ethyl maleimide-activated form and to study the process of activation. Of great interest is the possibility that the activity of the microsomal glutathione *S*-transferase could be regulated *in vivo* through a key sulfhydryl group.

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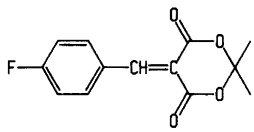
Mechanisms of the Electrohydrodimerization of Activated Olefins. V.* Substituted Benzylidene Meldrum's Acid Anion Radical Reactions in the Presence of Acetic Acid

PAUL MARGARETHA [a] and
VERNON D. PARKER [b]

[a] Institute for Organic and Biochemistry, University of Hamburg, Martin-Luther-King-Platz 6, 2000 Hamburg 13, West Germany and [b] Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

Following the pioneering work of Baizer,[2] electrohydrodimerization of activated olefins has been investigated intensively from both the synthetic and mechanistic viewpoints.[3-5] Primary mechanistic questions are centered around the order of events, *i.e.* whether the initially formed anion radical undergoes dimerization (i), reacts with substrate (ii) or is protonated (iii). Although pathway (i) has been favored by most investigators in this field,[4,5] reaction (ii) has recently been shown to take place when protons are effectively removed from the reaction media.[1b,1c] In the presence of a relatively strong proton donor (phenol) methyl cinnamate was observed to undergo exclusively reaction (iii) but further steps led to the reduced monomer rather than to electrohydrodimerization.[1d] We now report the unexpected results obtained during kinetic studies of the electrodimerization of 4-fluorobenzylidene Meldrums acid (*1*) in acetonitrile in the presence of acetic acid which show that the



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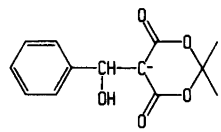

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anion radical does not undergo reaction (iii) even in the presence of the strong proton donor, HOAc. Compound *1* is of special interest in view of the unusual acid-base properties of the parent in the series, which is half converted to *2* at pH 5.4.[6-8]

Derivative linear sweep voltammetry (DLSV)[9] and derivative cyclic voltammetry (DCV)[10-12] kinetic data for the reactions of $1^{\cdot-}$ are summarized in Tables 1-3. The DLSV data can be analyzed using equations (1)-(3).[9] The equations refer to reaction (4) and the lower case letters a, b and x are

$$dE^p/d\log v = [\ln 10/(b+1)]RT/nF \quad (1)$$

$$dE^p/d\log C_A = [(\ln 10)(a+b-1)/(b+1)]RT/nF \quad (2)$$

$$dE^p/d\log C_x = [(\ln 10)x/(b+1)]RT/nF \quad (3)$$

$$A \pm e^- \rightleftarrows B \xrightarrow{X} \text{products} \quad (4)$$

* Parts I-IV, see Ref. 1.

Table 1. The effect of acetic acid concentration on the peak potential sweep rate dependence.[a]

[HOAc]/mM	0.0	4.4	8.8	22.0
$(dE^p/d\log v)$/(mV decade^{-1})	19.2	19.8	18.9	20.1

[a] In acetonitrile containing Bu_4NBF_4 (0.1 M) and H_2O (278 mM). Peak potentials were measured at a mercury electrode at 18 °C at 100, 200, 400 and 1000 mV s^{-1} with standard deviations of the order of ±0.1 mV. Correlation coefficients were 0.999 or greater at every [HOAc].

Table 2. The effect of the concentrations of the substrate and acetic acid on the linear sweep voltammetry peak potentials.[a]

v/V s^{-1}	$(dE^p/d\log C_A)$/(mV decade^{-1})	$(dE^p/d\log C_X)$/(mV decade^{-1})
0.200	−31.5(0.987)	−5.6(0.980)
0.400	−28.8(0.997)	−5.6(0.991)
1.000	−26.5(0.999)	−6.3(0.989)

[a] For measurement conditions see Table 1. Measurements were made at C_A = 0.25, 0.50, 1.00, 2.00 and 4.00 mM and C_X = 4.4, 8.8 and 22 mM. The numbers in parentheses refer to correlation coefficients.

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Table 3. Derivative cyclic voltammetry reaction order study of the hydrodimerization of *p*-fluorobenzylidene Meldrum's acid.[a]

C_A/mM	$[H_2O]$/mM	[HOAc]/mM	$v_{\frac{1}{2}}$/V s^{-1} [b]	$v_{\frac{1}{2}}/C_A$ [c]	$v_{\frac{1}{2}}/C_A^2$ [d]
0.25	0	0	25.6	102.4	410
0.50	0	0	99.5	199	398
1.00	0	0	309	309	309
0.25	278	22	25.5	102	408
0.50	278	22	66.3	133	265
1.00	278	22	154	154	154

[a] Conditions as in Table 1. [b] The voltage sweep rate necessary for the derivative peak ratio to equal 0.500 as described in Ref. 12. [c] This ratio is constant for second order kinetics.[12] [d] This ratio is constant for third order kinetics.[12]

the reaction orders in A, B and X, respectively, where X is a species in excess which reacts with B. In (1) v refers to the voltage sweep rate. At 18 °C, the theoretical value for $dE^p/d \log v$ is 19.3 mV/decade when b is 2 (eqn. 1). The observed value was found to be independent of [HOAc] under the conditions of the study (Table 1) and equal to 19.5 ± 0.5 mV/decade. On the other hand $dE^p/d \log C_A$ was observed to be dependent upon v (Table 2). Application of (2) with $b = 2$ results in values of a, the reaction order in substrate, equal to 0.63 (0.2 V/s), 0.49 (0.4 V/s) and 0.37 (1.0 V/s). The dependence of $dE^p/d \log C_A$ on v is expected when two competing mechanisms with different reaction orders in A are involved.[9] This is because of the concentration gradient of A near the electrode which depends upon v. The apparent reaction order in HOAc, x, was observed to be 0.30 ± 0.02 upon application of eqn. (3) to the data in Table 2.

The DCV reaction order analyses of the reactions of $1^{\cdot -}$ in acetonitrile (AN) or AN in the presence of HOAc and H_2O are summarized by the data in Table 3. The apparent reaction order $R_{A/B}$ which may have contributions from both B and A is given by eqn. (5) where $v_{\frac{1}{2}}$ is the value of v necessary for the

$$R_{A/B} = 1 + z(v_{\frac{1}{2}}/C_A^z = \text{constant}) \quad (5)$$

derivative peak ratio to equal 0.500. The last two columns in Table 3 show that $v_{\frac{1}{2}}/C_A^z$ is not constant when z is either 1 or 2, the values for $R_{A/B}$ equal to 2 and 3, respectively. The values of z most consistent with eqn. (5) were observed to be 1.75 and 1.3 in the presence and absence of proton donors, respectively. These values correspond to $R_{A/B}$ equal to 2.75 and 2.3.

It is clear from the data discussed to this point that either the mechanism is complex or that competing reactions are involved, both of which are second order in anion radical and one of which gives rise to a rate law including the substrate concentration. Apparent rate constants of complex mechanisms of ion radical reactions very often have low or inverse temperature coefficients.[13] The apparent activation energy for the reactions of electrode–generated intermediates can be obtained directly from eqn. (6) which does not involve

$$\ln v_{\frac{1}{2}}/T = (-E_a/R)(1/T) + c \quad (6)$$

the necessity of the explicit knowledge of rate constants.[14] Analysis of the reactions of $1^{\cdot -}$ at 290, 273 and 245 K resulted in $v_{\frac{1}{2}}/T$ equal to 0.46, 0.47 and 0.42, respectively. This gives an apparent E_a of about 0.

All of the data can be accommodated into a reaction scheme involving competing mechanisms (7)–(8) and (9) to generate the dimeric dianion A^-

$$A^{\cdot -} + A \rightleftarrows A^- - A^{\cdot} \quad (7)$$

$$A^- - A^{\cdot} + A^{\cdot -} \rightleftarrows A^- - A^- + A \quad (8)$$

$$2\,A^{\cdot -} \rightleftarrows A^- - A^- \quad (9)$$

$$A^- - A^- + HOAc \rightarrow A^- - A - H + AcO^- \quad (10)$$

$-A^-$ which then reacts with a proton donor such as acetic acid in (10). The very low temperature coefficient can be due to reversible reactions (7) and (9). The radical ion – substrate coupling mechanism observed during dimer forming reactions of 4-methoxybiphenyl cation radical has been observed to be accompanied by a near zero apparent activation energy.[15] The fractional orders in substrate and HOAc are accounted for by this scheme as well as is the integral second order in anion radical.

The most significant aspect of the results reported here is that the coupling reactions (7) and (9) occur without the interference of protonation of the anion radicals by acetic acid which only becomes involved after dimer-forming reactions. This must be a consequence of the very weakly basic nature of $1^{\cdot -}$

which has structural similarities to *2*. The work also points out that the reversibility of coupling reactions and the feasibility of competitive pathways during electrohydrodimerization contribute to the complexity of the overall mechanism. Further work is in progress in order to clarify the effect of substituents on the rates and mechanisms of the reactions of anion radicals of benzylidene Meldrum's acid.

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A Comment on Resonance Raman Spectroscopic Investigations of Axial Ligation of the Heme Iron Atom in Soybean Leghemoglobin *a*

R. S. ARMSTRONG, M. J. IRWIN, J. E. WELLINGTON and P. E. WRIGHT

Department of Inorganic Chemistry, University of Sydney, N.S.W. 2006, Australia

We wish to comment on a recent paper by Österlund and Sievers purporting to identify the axial ligands of the heme iron atom in soybean leghemoglobin *a* using resonance Raman spectroscopy.[1] These workers suggested coordination of the distal histidine to the iron in the low spin component of ferric leghemoglobin and its acetate complex as well as in a previously unreported low spin component of deoxyferrous leghemoglobin. However, our own studies show that their interpretation is incorrect and indicate that the low spin components observed by Österlund and Sievers[1] in fact arise from residual coordinated nicotinate in their leghemoglobin preparations.

Fig. 1(a) shows the 400 MHz ^{1}H NMR spectrum of ferric soybean leghemoglobin *a* prepared according to the method of Appleby *et al.*[2] The protein was oxidized with $Fe(CN)_6^{3-}$ and stripped of exogenous nicotinate[3] by chromatography on Sephadex G-15 at high pH (0.01 M Tris buffer, pH 9.2) before separation of leghemoglobin *a* by anion exchange chromatography using DE-52 cellulose eluted with acetate buffer (0.01 M, pH 5.2). Fig. 1(b)

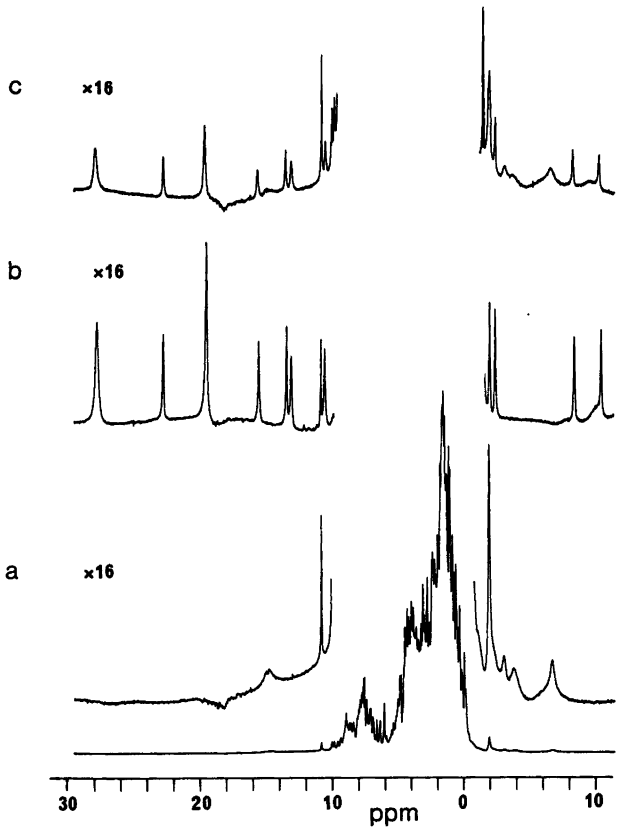

Fig. 1. 400 MHz ^{1}H NMR spectra at 25 °C and pH 6.2 of (a) soybean ferric leghemoglobin *a* stripped of nicotinate by the method of Appleby *et al.*,[2] (b) ferric leghemoglobin nicotinate obtained by addition of nicotinic acid to the sample in (a), and (c) ferric leghemoglobin *a* prepared by the method of Ellfolk[5] as modified by Sievers and Rönnberg.[6]

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shows the characteristic ^1H NMR spectrum of the nicotinate complex of ferric soybean leghemoglobin *a*. Resonances from the heme and nicotinate ligand are evident at fields lower than 10 ppm and higher than 2 ppm.[4] These resonances of the nicotinate complex are also present in spectra of ferric soybean leghemoglobin prepared according to the method of Ellfolk[5] as modified by Sievers and Rönnberg[6] [Fig. 1(c)]. In this preparation the high pH gel filtration which serves to strip off the nicotinate is omitted. The NMR spectrum indicates conclusively that residual nicotinate remains bound to the heme.

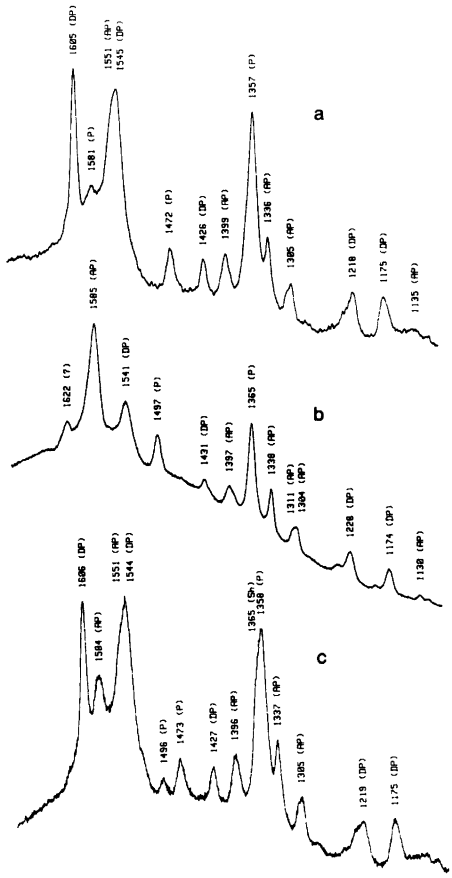

Fig. 2. Resonance Raman spectra of (a) deoxyleghemoglobin (pH 7.0) purified by the method of Appleby *et al.*,[2] (b) ferrous leghemoglobin nicotinate (pH 5.9) obtained by addition of nicotinic acid to the sample in (a), and (c) deoxyleghemoglobin (pH 7.0) purified by the method of Ellfolk[5] as modified by Sievers and Rönnberg.[6] Samples were 0.5 mM in heme and in 100 mM phosphate buffer. Spectral conditions were 514.5 nm laser irradiation; 5 cm^{-1} resolution; 0.5 cm^{-1} s^{-1} scan; 1s time constant. The spectrometer was calibrated with indene and band positions are accurate to ± 1 cm^{-1}.

The presence of this residual nicotinate in the leghemoglobin preparations used for resonance Raman studies by Österlund and Sievers[1] clearly gives rise to additional low spin bands in their spectra. Fig. 2 compares the resonance Raman spectra of samples of deoxyferrous leghemoglobin prepared by the two methods described above with that of ferrous leghemoglobin nicotinate. Additional Raman bands at 1584 and 1496 cm^{-1} due to residual nicotinate complex are evident (Fig. 2c) in the sample prepared by the methods of Ellfolk[5] and Sievers and Rönnberg.[6] At lower pH, more favourable for nicotinate binding, the Raman bands of the nicotinate complex are even more pronounced. The Raman spectra of deoxyferrous leghemoglobin prepared from ferric leghemoglobin stripped of nicotinate by the method of Appleby *et al.*[2] (Fig. 2a) indicate a pure high spin ferrous compound. The low spin bands seen by Österlund and Sievers at 1497, 1586 and 1628 cm^{-1} in the spectrum of deoxyferrous leghemoglobin were attributed by them to coordination of the imidazole of the distal histidine or residual nicotinate.[1] Our spectra clearly indicate that these bands arise from the nicotinate complex alone. The low spin Raman bands in spectra of ferric leghemoglobin and its complex with acetate[1] also arise from residual nicotinate.

The resonance Raman spectra of ferric leghemoglobin acetate measured by us[7] differ in some features from those obtained by Österlund and Sievers[1] with excitation at 514.5 nm. These workers report an anomalously polarized band at 1584 cm^{-1}, presumably arising from residual leghemoglobin nicotinate. Our spectra of leghemoglobin acetate[7] contain a polarized band at 1584

Table 1. Frequency[a] of the strong polarized Raman band near 1375 cm^{-1} in the spectra of ferric leghemoglobin complexes.

Complex	488 nm excitation	413 nm excitation
Imidazole	Not measured	1375
Nicotinate	1375	1374
CN^-	1375	1373
OH^-	1378	1377
H_2O	1375	1373
Formate	1375	1375
F^-	1373	1373
Acetate	1374	1373

[a] Frequencies are in cm^{-1} and are accurate to ± 1 cm^{-1}.

cm^{-1}. Österlund and Sievers also report bands at 1365 and 1300 cm^{-1} which are absent from Raman spectra of leghemoglobin acetate obtained by us with excitation at 413.8, 488.0 and 514.5 nm. The absence of the 1300 cm^{-1} band from our spectra is of particular importance since it has been suggested by Österlund and Sievers to be specific to a carboxylate ligand.[1] We have also measured the Raman spectrum of leghemoglobin formate[7] and find no evidence of a band in that region.

Table 1 lists the position of the strong polarized Raman band near 1375 cm^{-1} for a number of ferric leghemoglobin complexes. It is clearly not possible to distinguish the type of axial ligand from the position of this band.

Ferric soybean leghemoglobin *a* which has been completely stripped of nicotinate still exists as an equilibrium mixture of high and low spin states.[8,9] The rate of spin state exchange is unusually slow.[10] Although coordination of the distal histidine to the iron atom in the low spin form of ferric leghemoglobin has been suggested,[9-11] definitive evidence for this is still lacking. Our own resonance Raman studies (Ref. 7 and unpublished experiments) have so far failed to identify unequivocally the nature of the sixth ligand.

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The Reaction of Dimesityl Ketone with Grignard Reagents. The Role of Alkyl Radicals, Catalysis by Iron

TORKIL HOLM

Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark

After the appearance of several pieces of non-conclusive evidence,[1,2,3] Grignard reagents were shown to react with benzophenone by a rate-determining electron transfer step (SET) followed by fast combination or disproportionation of the alkyl and the magnesium ketyl radicals.[4,5] Information concerning the lifetime of the alkyl radical has been obtained by the use of various 5-hexenyl radical probes.[6]

2,2',4,4',6,6'-Hexamethylbenzophenone (DMK) has been reported[7] to have a SET step which is faster than the radical combination step so that high concentrations of pairs of ketyl radicals and magnesium bound alkyl radicals are allegedly present in the reaction mixture. Since alkyl radicals, either free or magnesium bound, are usually considered extremely reactive, a series of experiments has served to reinvestigate the problem.

Ashby and Goel[7] state that the formation of addition product with Grignard reagents is completely suppressed by the use of DMK, while the reduction is only slowed down. In the present work, however, it was found that conjugate addition to DMK was the normal reaction, when using secondary, tertiary, or benzylic Grignard reagents. With these there was an insignificant build-up only of the blue magnesium ketyl as measured either by the absorption at 579 nm or by bulk susceptibility measurements (see Experimental) of the spin concentration. NMR of the addition products (see Experimental) showed two one proton signals in the vinylic region, which were split by coupling to each other and to a high field and a low field methyl group, respectively. A 1,4- or a 1,6-adduct would comply with these data and the 1,6-adduct was preferred for steric reasons; Fig. 1.

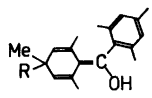

Fig. 1. Structure of products obtained by 1,6-addition of RMgX to DMK.

The use of primary Grignard reagents, and especially of ethylmagnesium bromide, apparently led to the build-up of high concentrations of magnesium ketyl, which accounted for more than half of the reaction product before work-up, according to the bulk susceptibility of the solution. When the gas pressure above the reaction mixture was measured it was found that ethane/ethene was formed simultaneously with the ketyl in a ratio of 3:2. Upon work-up the magnesium ketyl is known[8] to disproportionate to form one mol each of ketone and benzhydrol.

The experiments described thus far were carried out using DMK which was purified by recrystallization solely. When the ketone was distilled, however, no color and no spin was produced in the reaction with ethylmagnesium bromide, and reduction product was formed. It was found that the non-distilled DMK was contaminated with traces of iron, which is known to catalyze the formation of ketyl and benzopinacol in the reaction of benzophenone with Grignard reagents.[9] Because of the very low reactivity of DMK, even the smallest traces of iron, *e.g.* $10^{-7}-10^{-8}$ g ml^{-1} of $FeCl_3$, have a significant effect when using ethylmagnesium bromide. With secondary and tertiary Grignard reagents the addition reaction is rather fast and iron catalysis is less important. With these reagents 5 – 10% of ketyl is formed as a by-product even in the absence of iron, just like 5 – 10% of benzopinacol is formed when using unsubstituted benzophenone.[4] The important difference is that the hindered ketyl produced from DMK is rather stable in solution, while the unhindered benzophenone-ketyl dimerizes to form benzopinacol.

Unsuccessful attempts were made to reproduce the EPR spectra presented by Ashby and Goel as evidence for the existence of pairs of radical anion – radical cation.* Well-resolved spectra were obtained eith ethyl- and methylmagnesium bromide reacting with DMK. The spectra were almost identical and very unlike the spectrum obtained by the authors mentioned using methylmagnesium bromide. It was obvious, however, that various coordination equilibria exist in solutions of the ketyl in Grignard reagents. For example, a highly dilute solution of the ketyl absorps at λ_{max} 579 nm, but not at 640 nm. When a Grignard reagent is added to the solution the absorption at 640 nm appears as a shoulder. The band at 579 nm should therefore be assigned to the magnesium ketyl and not as claimed[7] to the radical ion pair, while the band at

* *Note added in proof.* In a recent report[13] the spectra published[7] are interpreted as being those of radical anion – organometal cation complexes, since they have an obvious doublet character.

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640 nm arises from a complex and is not typical for the ketyl as such. The presence of complexes may explain that the ESR spectra vary with concentration and also with the presence of different alkylmagnesium compounds in the solution.

In the reaction of DMK with Grignard reagents the rate of spin formation was found by Ashby and Goel to parallel, at least qualitatively, the oxidation potentials of the Grignard reagents as originally observed for the reaction of unsubstituted benzophenone.[5,11,12] Since the spin concentration and the color observed in the reactions of DMK with Grignard reagents concern only the small fraction of ketyl which escapes from the solvent cage during the reaction or is produced by a catalytic process, the rates are not useful for mechanistic interpretations and do not constitute "direct evidence for a single electron transfer in the reduction of ketones by Grignard reagents".[7] As mentioned above, however, such evidence has been obtained earlier for the reaction of unsubstituted benzophenone by correlation of the oxidation potentials of the Grignard reagents with the reaction rates. It seems a reasonable assumption that the substituted benzophenone reacts by an analogous mechanism.

The conclusion of the present experiments is that in the reaction studied the only observable species with an unpaired spin is the magnesium ketyl, while the lifetime of the alkyl radical is probably too short to be measured by available methods.

Experimental. NMR spectra were taken on a Bruker HX 90 and a Varian EM 360, UV-visible spectra on a Perkin Elmer 402 and ESR spectra on a JEOL ME 1X.

Materials. Dimethyl ketone (DMK) was prepared by acylation of mesitylene with mesitoyl chloride using aluminum chloride as a catalyst. A sample (A) was recrystallized from benzene – ethanol, m.p. 137.5 – 138.5 °C. The presence of iron(III) was demonstrated by the reaction with potassium thiocyanate in acidic solution. The ketone was distilled, b.p. 185 °C (2 Pa); this material (B) had m.p. 135 – 137 °C and gave a negative reaction for iron. Grignard reagents were prepared from sublimed magnesium (Dow Chemical Corp.); THF was distilled from lithium aluminum hydride. Alkyl halides were distilled.

Addition products. One mmol of DMK A was added to 10 ml of 0.5 M *t*-butylmagnesium or isopropylmagnesium bromide in THF and kept at room temperature for 2 h during which a blue color developed. For *t*-butyl the work-up procedure was as described.[4] For the isopropyl derivative, the excess Grignard reagent was destroyed by addition of excess of carbon dioxide followed by the addition of cold, saturated ammonium chloride solution. The organic phase was separated and the solvent removed at 0.25 Pa without heating. The *t*-butyldihydrobenzophenone obtained was crystalline, while the isopropyl derivative was an oil. Upon heating, isobutane and propane were evolved, respectively, and admission of air led to the regeneration of the ketone. Identical reactions were reported for unsubstituted benzophenone.[4] ^{1}H NMR ($CDCl_3$): The spectra were similar, with signals at δ 6.77 (2 H-arom,s), 5.40 (1 H-vinyl,m), 5.25 (1 H-vinyl,m) 2.06 – 2.26 (12H,Me,m), 1.11 (3H,Me,d), 0.98 (3H,Me,s), 0.85 (9H,*t*-Bu,s) or the last signal substituted with δ 0.85 (6H,i-Pr,d).

Bulk susceptibility measurements. DMK A (100 mg) were mixed with 1 ml of 1 M ethylmagnesium bromide in THF in an NMR sample tube. A sealed glass capillary with a mercury drop was mounted in the rubber stopper and the tube was suspended under a semimicroanalytical balance. The weight of the tube was measured every 30 min to 10^{-5} g both directly and after applying a magnetic field of approximately 10 000 gauss to the lower 2 cm of the tube. The difference between the two weighings was 3.49 mg at the time of mixing, but decreased to 2.50 mg after 12 h. By calibration weighings of solutions of dipicryldiphenyl hydrazyl in benzene, this was found to indicate a spin concentration of 0.17 M. The pressure in the tube increased to a final value of 2.8 atm after approximately 20 h. NMR spectroscopy of the gas revealed a ratio between ethane and ethene of 3:2. The weight of the gas escaping by releasing the pressure of the tube after 3 days was 20 mg including 11 mg of solvent vapour. Analogous experiments with DMK A were performed using isopropylmagnesium bromide and *t*-butylmagnesium bromide in THF. No gas pressure was observed and the spin concentrations were below 0.01 M.

When 100 mg of DMK B was reacted with 1 ml of 1 M iron-free ethylmagnesium bromide in THF the solution remained pale greenish yellow for 6 days. The addition of $10^{-7} - 10^{-8}$ g ml^{-1} of $FeCl_3$ to the reaction mixture caused the development of an intense blue color within 1 h. In the iron-free experiment no spin concentration was detected and a gas pressure of 2.8 atm developed after 40 h. The ratio ethene – ethane was 2:2.3. The product isolated after 6 days was the benzhydrol.

Visible spectroscopy. To a solution 0.5 M in THF of ethylmagnesium bromide was added DMK A to a final concentration of 0.0005 M and spectra were taken at room temperature every 5 min in a 10 mm cell. An intense blue color developed with absorption at λ_{max} 579 nm and 640 nm (shoulder). The absorbance at 579 nm after 12 h was $\cong$ 3.0. In an analogous experiment using isopropylmagnesium bromide the same spectrum appeared, but the absorbance at 579 nm reached a maximum of 0.22 after 1 h. Using 0.05 M DMK A and 0.5 M *t*-

butylmagnesium bromide the absorbance at 579 nm reached its final value, 0.95, after 80 min.

EPR. Mixtures of Grignard reagents 0.05 M and 0.05 M DMK A were observed at various reaction times. Well-resolved spectra were obtained with methyl- and ethylmagnesium bromide. The spectra were almost identical and very unlike the spectrum reported by Ashby and Goel[7] using methylmagnesium bromide. Quantitative EPR spectroscopy was not attempted.

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Stabilizing Effects by Mg^{2+} on Na,K-ATPase *

SVEN MÅRDH

Institute of Medical and Physiological Chemistry, Biomedical Center, University of Uppsala, Box 575, S-751 23 Uppsala, Sweden

Mg^{2+} is required for the activity of Na,K-ATPase.[1,2] It is required for the phosphorylation by ATP as well as by P_i.[3,4] When added before P_i, Mg^{2+} was reported to inhibit phosphorylation by P_i.[4] In contrast, addition of Mg^{2+} early, or late, in the phosphorylation by ATP had no detectable effect on the initial rate of phosphorylation.[3] Addition of a high concentration of a chelator of divalent cations, 1,2-cyclohexylenedinitrilotetraacetic acid, to the phosphoenzyme decreased the rate of dephosphorylation.[5] This result may indicate that Mg^{2+} is bound to the phosphoenzyme and that it plays a role in the dephosphorylation step. Recently, evidence for an Mg^{2+}-induced conformational change at the ATP-binding site of the ATPase was demonstrated with a photoreactive ATP-analogue.[6] No direct studies on binding of Mg^{2+} to Na,K-ATPase have been reported.

The present paper describes two different conditions where Mg^{2+} is important in stabilizing the enzyme activity. The first condition is protection against inactivation of the dephosphoenzyme at acidic pH. The second condition is protection against inactivation by *N*-ethyl-5-phenylisoxazolium-3′-sulfonate (Woodward's reagent K) which is a reagent modifying carboxyl groups in proteins.[7]

Experimental. Na,K-ATPase was prepared from pig kidney as described by Jørgensen.[8] The sodium salt of ATP and Tris salt of *p*-nitrophenylphosphate (pNPP) were obtained from Sigma. The ATP was converted to its Tris salt as previously described.[3] *N*-Ethyl-5-phenylisoxazolium-3′-sulfonate (Woodward's reagent K) was purchased from Aldrich Chem. Comp., Inc. ATPase assay and the technique of testing the stability of the enzyme at various pH were previously described.[9] *p*-Nitrophenylphosphatase (pNPPase) was assayed in 3 mM $MgCl_2$, 10 mM KCl and 3 mM pNPP in 20 mM imidazole–HAc buffer, pH 7.5. Incubation volume was 0.5 ml. The reaction was stopped by the addition of 25 μl of 50 % trichloroacetic acid. One ml of 0.5 M Tris base was then added and the absorbance at 410 nm was measured. Protein was measured according to the method of Lowry *et al.*.[10] Chemical modification with Woodward's reagent K was carried out at 20 °C. About 10 μg of enzyme protein in 175 μl of 15 mM 2-morpholinoethanesulfonic acid (MES) buffer, pH 5.5, was incubated with 25 μl of 5 mM Woodward's reagent K which was prepared immediately before use in 1 mM HCl. To the control 25 μl of 1 mM HCl without the reagent was added. Exactly 2 min after the addition of Woodward's reagent K, 2.0 ml of cold 30 mM histidine buffer, pH 7.2, was added. Samples were then assayed for Na,K-ATPase and pNPPase.

Results and discussion. Na,K-ATPase was incubated at various pH at 30 °C for 60 min. The pH was then adjusted to 7.5 and the enzyme activity was assayed. At low pH the Na,K-ATPase activity and the pNPPase activity were inactivated (Fig. 1). At pH 4 only a few per cent of the original activities remained. At pH 5 the remaining activity was dependent on whether Mg^{2+} was present or not. Addition of 7.5 mM Mg^{2+} during the incubation at pH 5 protected the enzyme against inactivation.

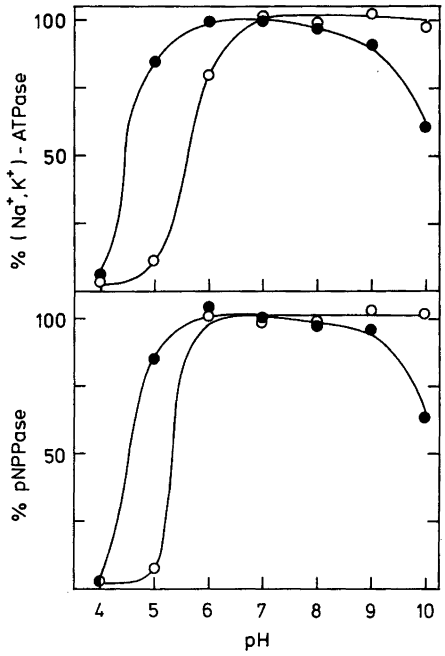

Fig. 1. Stability of Na,K-ATPase and pNPPase at various pH. $MgCl_2$ was omitted (○), or 7.5 mM $MgCl_2$ was included in the incubation mixture while the enzyme was treated at various pH (●). Final concentration of $MgCl_2$ in both the Na,K-ATPase assay and in the pNPPase assay was 3 mM. Relative activities are presented.

* Communication at the Meeting of the Swedish Biochemical Society in Stockholm, 26–27th November, 1981.

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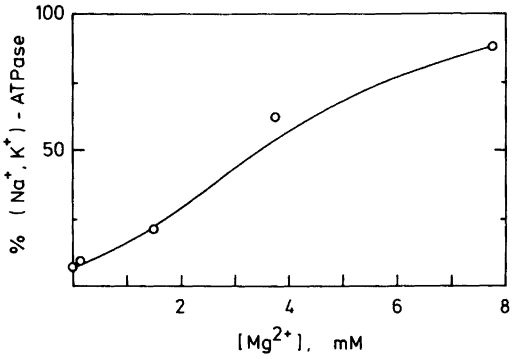

Fig. 2. Dependence of the stability of Na,K-ATPase at pH 5 on the concentration of Mg^{2+} at 30° and pH 5 for 60 min. The pH was readjusted to 7.5 before assay of Na,K-ATPase.

About 85 % of the Na,K-ATPase and pNPPase was retained when Mg^{2+} was present at pH 5 but only about 10 % in its absence. At alkaline pH the activities were stable in the absence of Mg^{2+}. The presence of Mg^{2+} made the activities more labile. In the presence of Mg^{2+} about 60 % was retained after incubation at pH 10. The activities appeared to be stable between pH 6 and 8.

The ability of Mg^{2+} to protect Na,K-ATPase and pNPPase was similar. Therefore the concentration dependence of Mg^{2+} as a protective agent was tested on the ATPase activity only (Fig. 2). Relatively high concentrations of Mg^{2+} were required. Half-maximal effect was observed at about 3.5 mM Mg^{2+}.

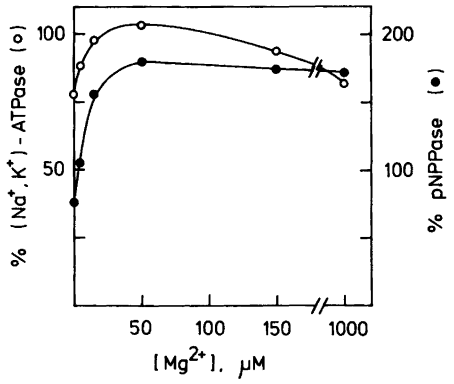

Fig. 3. Chemical modification of Na,K-ATPase by Woodward's reagent K. The enzyme was incubated with the reagent at various concentrations of Mg^{2+}. After stopping the reaction with ice-cold 30 mM histidine buffer, pH 7.2, Na,K-ATPase and pNPPase were assayed.

This concentration is at least ten times higher than the $K_{0.5}$ of Mg^{2+} in the Na,K-ATPase reaction.[11]

After chemical modification of the enzyme with Woodward's reagent K under standard conditions in the absence of Mg^{2+} about 75 % of the original Na,K-ATPase and pNPPase activities were retained (Fig. 3). In the presence of Mg^{2+} protection against inactivation by the reagent was observed. At 50 μm Mg^{2+} all activity assayed as Na,K-ATPase was retained. At a further increase of Mg^{2+} the activity decreased again. At 1 mM Mg^{2+} about 80 % of the original activity was retained. Similar to the effect on the Na,K-ATPase activity, Mg^{2+} protected the pNPPase half-maximally at 10 μM concentration (Fig. 3). The activity of pNPPase, however, was not only protected against inactivation. At 50 μM Mg^{2+} and higher concentrations, pNPPase reached activities which were about 180 % of the activity of enzyme not treated with the reagent. Previously, Woodward's reagent K has been used to modify carboxyl groups in proteins.[7] It is also known to act as a bifunctional cross-linking reagent.[7] A protection of Mg^{2+} against inactivation by this reagent might be explained by protection of some essential carboxyl groups at the active center of the enzyme *e.g.* the carboxyl group of Asp which is phosphorylated by ATP.[12] The increase of activity as observed for pNPPase is, however, more difficult to explain. Maybe the combination of Mg^{2+} and Woodward's reagent K resulted in the stabilization of a protein structure which has a high catalytic efficiency. Such a structure could be a functional dimer of α-subunits of the Na,K-ATPase with equally active (pNPPase) subunits in contrast to the native enzyme where these subunits might be out of phase.[13] Further experiments are necessary in order to find out the mechanism by which Mg^{2+} and the reagent modify the enzyme. Since the effect of Mg^{2+} was observed already at low concentrations, which were similar to the $K_{0.5}$ for Mg^{2+} in the Na,K-ATPase reaction, its observed effect probably involved binding to a site which normally binds the ion during hydrolysis of ATP. Contrarily, a high concentration of Mg^{2+} was required in order to stabilize the enzyme at slightly acidic pH. This stabilization might be the result of a general stabilization of the protein structure in the membrane.

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The Adsorption of Lactoperoxidase to Glass

E. HONKA, P. I. OHLSSON and K. G. PAUL

Department of Physiological Chemistry, University of Umeå, S-901 87 Umeå

Lactoperoxidase (LP, E.C.1.11.1.7) catalyzes the oxidation of thiocyanate by hydrogen peroxide to the antibacterial hypothiocyanite.[1-3] LP and thiocyanate are excreted in the saliva, and peroxide is generated by bacteria normally occurring in the mouth.[4] LP is irreversibly adsorbed to tooth enamel,[5] which may locally enhance the antibacterial effect. The mode of binding is incompletely known. To penetrate further the binding mechanisms, we have studied the adsorption of LP to a simpler, non-biological surface, glass.

Materials and methods. LP[6] (from milk) and horseradish peroxidase C[7] were isolated to give $A_{Soret}/A_{280} = 0.93$ and 3.40, respectively. Horse heart cytochrome *C* was purchased from Sigma. LP was assayed kinetically with 2 mM dicarboxidine [$\gamma\gamma'$-(diamino-3,3'-bifenylylenedioxy)dibutyric acid] as chromogen and 50 μM H_2O_2 as oxidant.[8,9] The activity is expressed as $\Delta A_{440} \times s^{-1}$. Horseradish peroxidase was determined spectrophotometrically from $\varepsilon_{mM} = 102\ cm^{-1}$ at 402 nm.[10] Pyrex® ballotini (diameter 0.2 mm, density 2.4 $g \times cm^{-3}$) were pretreated by submersion in 1 M NaOH or 1 M HCl for one hour at room temperature, washed with distilled water until the washing showed pH ~ 7, and dried overnight at 105 °C. During this treatment the solute cations will to some extent exchange with the surface of the glass but not penetrate very deeply. There will be only little difference in etching after the two treatments because of the short duration. Ballotini are manufactured by a melting procedure that gives a smooth surface, but some beads are broken or irregular in shape.

Beads were gently shaken for one hour with a peroxidase solution, and then the "residual" (not adsorbed) peroxidase was immediately assayed. "Bound LP" was obtained as the difference between residual activities and activities in controls with peroxidase in identical vials but without beads. The shaking itself did not inactivate LP.

Unless otherwise stated 50 mM sodium phosphate, pH 6.9, and 25 °C were used for all adsorptions, assays *etc.* Given values are based on triplicate determinations on 5–35 μl of the LP solutions.

Results. Alkali-treated glass adsorbed about twice as much LP as acid-treated glass did. Only alkali-treated beads were used in the sequel.

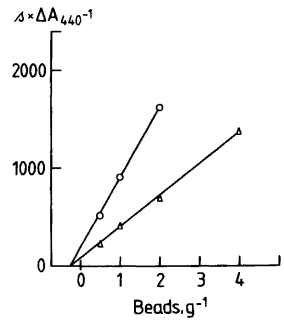

Fig. 1. A double inverse plot of peroxidase activity representing bound peroxidase (*cf.* Methods), and weight of beads when 1.5 ml of 1 μM LP in buffer were exposed to an increasing number of beads. The activity was determined on 10 (○) and 20 (△) μl samples.

Desorption by various media was tested on 1 g of beads, preequilibrated with 2.0 ml of 1 μM LP in buffer and subsequently washed with distilled water. Elution by sodium phosphate, pH 6.9, depended upon the ionic strength, 50 mM removing no LP and 300 mM about 25 % of the bound LP. Reinforcement of the weak buffer with 3.5 M ethanol had no effect whereas the presence of 1 M urea in this buffer eluted about 5 % of the LP. The enzyme is stable in both solutions.

When increasing amounts of beads were added to a constant amount of LP the residual activity decreased in such a manner that a plot of (bound LP)$^{-1}$ against (weight of beads)$^{-1}$ formed a straight line (Fig. 1).

The amount of LP bound per unit area of glass is obtained from Fig. 2. The extrapolation of the rectilinear slope to zero activity shows that 0.5 g of

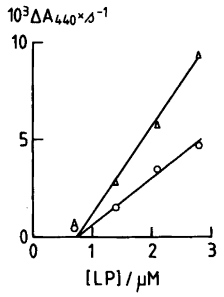

Fig. 2. Peroxidase activity remaining in solution when 0.5 g of beads is equilibrated with 1.5 ml of LP of various concentrations in buffer. The activity was determined on 8 (△) and 5 (○) μl samples.

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beads becomes saturated with LP when exposed to 1.5 ml of 0.7 μM LP, which gives a binding capacity of 2.1 nmol of LP per gramme of beads.

One gramme of beads adsorbed no horseradish peroxidase from 2 ml of a 1 μM solution in buffer, whereas under the same conditions Fe(III)cytochrome C was completely removed by the beads.

Discussion. Glass contains silicon-bound bridging and non-bridging oxygen atoms. The former can accept hydrogen bonds, and the latter carry a negative charge and act as a cation exchanger. Hydrolysis increases the ratio $Si-O^-/Si-O-Si$ and is favoured by alkali. LP is a basic protein containing 71 lysine+arginine residues.[11] Free electrophoresis showed pI = 6.9 in 0.1 M phosphate buffer and 8.0 in 0.1 M veronal; pI in phosphate varied with the ionic strength.[12] Prequisites for ionic bonds between LP and glass are thus at hand.

The effect of the ionic strength on the desorption of LP from glass, and the difference between alkali- and acid-treated beads, points at ionic bonds between LP and glass. There may also be a small contribution from hydrogen bonds.

The rectilinear relationship in Fig. 1 is compatible with a mechanism where all LP molecules are equally firmly and reversibly bound. The bonding capacity of 2.1 nmol of LP per gramme of beads corresponds to 1.3×10^{10} molecules per bead. The mean glass area available to one LP molecule, or to its projection on the glass, then becomes 970 Å^2, assuming a monolayer of LP molecules; the assumption is justified by Fig. 1. A spherical molecule of 78 400 dal with a partial specific volume of 0.725 ml $\times$ g^{-1} [11] and carrying a hydration mantle of 20 % (w/w) would require an area of 3100 Å^2 at closest packing. Sedimentation and diffusion analyses on LP gave the axial ratio of 5.6 for a prolate ellipsoid.[11] Such a conformation of this molecular weight and hydration requires a minimum area of 980 Å^2. This is in good agreement with the observed value of 970 Å^2 even with some allowance for the irregularities of the beads. The present result confirms the elongated form of the LP molecule and indicates the existence of positive charge(s) at one of its ends.

LP is very firmly bound to octyl-Sepharose® by what must be hydrophobic bonds.[6] The molecule is thus equipped for binding to negatively charged as well as to hydrophobic areas. Interestingly, its reactions with octyl-Sepharose®, Amberlite XE-64-N^+H_4 and glass equal those of a peripheral membrane protein, cytochrome *C*. Horseradish peroxidase is inert to the three adsorbants.

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Reaction of *N*-Acyl- and *N*-Sulfonylcarboxamides with Triethyl Orthoformate

SVEND TREPPENDAHL and
PALLE JAKOBSEN

Medicinsk-Kemisk Institut, University of Copenhagen, Rådmandsgade 71, DK-2200 Copenhagen N, Denmark

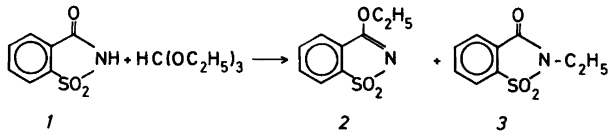
In connection with our investigation on the reaction of triethyl orthoformate with carboxamides[1] we became interested in the reaction between triethyl orthoformate and *N*-sulfonylcarboxamides, especially saccharine.

The reaction of secondary amides with triethyl orthoformate has not been reported in the literature. We have found that *N*-acetylacetamide, *N*-acetylbenzamide and *N*-benzoylbenzamide react very slowly compared with *N*-unsubstituted and *N*-monosubstituted carboxamides, and we were unable to isolate anything but starting material and esters. On the contrary phthalimide reacts easily with triethyl orthoformate under formation of *N*-diethoxymethylphthalimide *i.e.* a reaction product analogous to the product from *N*-monosubstituted sulfonamides.[2] An analogous product from the reaction between 2,3-dihydro-1,2-benzisothiazol-3-one-1,1-dioxide (saccharine) *1* and triethyl orthoformate could be expected, but instead a mixture of 3-ethoxy-1,2-benzisothiazol-1,1-dioxide *2* and 2-ethyl-2,3-dihydro-1,2-benzisothiazol-3-one-1,1-dioxide *3* was formed (Scheme 1). It is well known that *2* upon heating rearranges to *3*.[3] The reaction could therefore proceed through the 2-diethoxymethyl-2,3-dihydro-1,3-benzisothiazol-3-one-1,1-dioxide *4*, fragmentation to *2* and subsequent rearrangement to *3* (Scheme 2). Ethyl formate was detected together with ethanol in the reaction mixture.

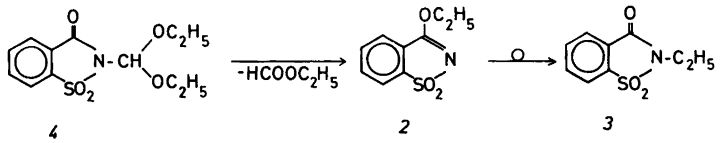
Another possible path for the reaction in which ethyl 2-sulfamidobenzoate is formed primarily and then cyclized to *2* could be ruled out because only ethyl *N*-(2-ethoxycarbonylbenzenesulfonyl)formimidate was formed when ethyl 2-sulfamidobenzoate was reacted with triethyl orthoformate (Scheme 3). If ethyl 2-sulfamidobenzoate was heated in an inert solvent like xylene no *2* was formed but only saccharine. Neither could *2* be prepared by ethylation of saccharine with ethyl benzoate, ethyl acetate or diethyl sulfate.

A mechanism in which both *2* and *3* are formed by direct ethylation, eventually by a carbenium ion formed in the presence of *p*-toluenesulfonic acid as catalyst is probably of minor importance since *2* and *3* were formed in the same overall yield without added catalyst. The product distribution was 1:5 instead of 1:2 with catalyst, presumably because of the prolonged reaction time.

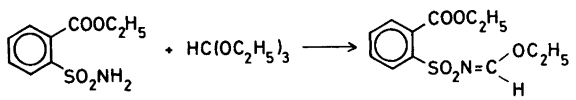
In order to find out whether *3* was formed from *2* or direct by fragmentation of *4*, we conducted the synthesis at lower temperature where the Chapman rearrangement of *2* to *3* proceeds slowly.[3] At 110 °C we found the rearrangement to proceed 10 % in



Scheme 1.



Scheme 2.



Scheme 3.

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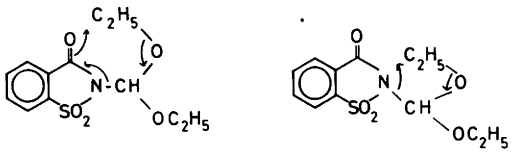

Scheme 4.

18 h and at that temperature both products *2* and *3* were formed in the usual ratio from the reaction of *1* with triethyl orthoformate.

It therefore seems plausible that the first formed *4* is mainly fragmented directly to *2* and *3*. This can proceed in two ways, namely, by a mechanism involving a six-membered ring and a four-membered ring (Scheme 4). We also investigated the reaction of two open chain *N*-sulfonylcarboxamides with triethyl orthoformate namely *N-p*-toluenesulfonylacetamide and *N-p*-toluenesulfonylbenzamide and found different reaction products in each case. For *N-p*-toluenesulfonylbenzamide only ethyl *N-p*-toluenesulfonylbenzimidate *7b* was formed presumably through the six-membered intermediate. For *N-p*-toluenesulfonylacetamide both *N*-ethyl-*N-p*-toluenesulfonylacetamide *6a* and ethyl *N-p*-toluenesulfonylacetimidate *7a* were formed in the ratio 2:1 (Scheme 5). Electronic factors must be the cause of this product distribution, the phenyl group decreasing the electron density on the nitrogen atom making *N*-diethoxymethyl-*N-p*-toluenesulfonylbenzamide to fragment through the six-membered intermediate.

Experimental. The experimental equipment was reported earlier.[1] Melting points are uncorrected.

2-Ethyl-2,3-dihydro-1,2-benzisothiazol-3-one-1,1-dioxide 3 *and 3-ethoxy-1,2-benzisothiazol-1,1-dioxide* 2. 2,3-Dihydro-1,2-benzisothiazol-3-one-1,1-dioxide (0.1 mol) was refluxed with triethyl orthoformate (0.3 mol) and *p*-toluenesulfonic acid (0.02 mol) so the formed ethanol and ethyl formate could distil from the reaction mixture. After collection of 11 ml the reaction was cooled and a mixture of *2* and *3* was filtered off in a yield of 79 %. Recrystallization from ethanol gave 68 % of *3* and recrystallization of the residue from toluene gave 32 % of *2*.

N-*Diethoxymethylphthalimide.* Phthalimide, triethyl orthoformate and *p*-toluenesulfonic acid were refluxed as described above. Yield 55 %, m.p. 73 °C. Anal. $C_{13}H_{15}NO_4$: C, H, N, ^{1}H NMR ($CDCl_3$): δ 1.32 (6 H, t), 3.25 (4 H, m), 6.15 (1 H, s), 7.77–7.92 (4 H, m). IR ($CHCl_3$, cm^{-1}): 1780 (s), 1730 (s), 1380 (m), 1360 (m).

Ethyl N-p-*toluenesulfonylbenzimidate* 7b. *N-p*-Toluenesulfonylbenzamide and triethyl orthoformate were refluxed as described above. Excess triethyl orthoformate evaporated and the residue distilled *in vacuo*. Yield 94 %, b.p. 210–215 °C/0.5 mmHg, m.p. 52 °C (toluene, light petroleum). Anal. $C_{16}H_{17}NO_3S$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.25 (3 H, t), 2.30 (3 H, s), 4.23 (2 H, q), 7.05–8.00 (9 H, m). IR ($CHCl_3$, cm^{-1}): 1615 (s), 1600 (s), 1580 (s), 1315 (s), 1295 (s), 1160 (s).

N-*Ethyl*-N-p-*toluenesulfonylacetamide* 6a and *ethyl* N-p-*toluenesulfonylacetimidate* 7a were prepared from *N-p*-toluenesulfonylacetamide and triethyl orthoformate as described above. The overall yield was 76 % distributed with 66 % of *N*-ethyl-*N-p*-toluenesulfonylacetamide and 33 % of ethyl *N-p*-toluenesulfonylacetimidate determined from the NMR spectrum of the distillate. B.p. of the mixture 138–140 °C/0.05 mmHg. Anal. $C_{11}H_{15}NO_3S$: C, H, N. IR ($CHCl_3$, cm^{-1}): 3000 (m), 1700 (s), 1605 (s), 1360 (s), 1320 (s), 1160 (s). ^{1}H NMR ($CDCl_3$): δ 1.26 (1 H, t), 1.28 (2 H, t), 2.32 (2 H, s), 2.43 (3 H, s), 2.48 (1 H, s), 3.85 (1.33 H, q), 4.12 (0.66 H, q), 7.06–7.95 (4 H, m). The assignments of the chemical shifts were made from the NMR spectra of the authentic compounds.

N-*Ethyl*-N-p-*toluenesulfonylacetamide* was prepared from *N*-ethyl-*p*-toluenesulfonamide and acetic anhydride. B.p. 123–127 °C/0.05 mmHg. ^{1}H NMR ($CDCl_3$): δ 1.32 (3 H, t), 2.32 (3 H, s), 2.45 (3 H, s), 3.92 (2 H, q), 7.15–7.97 (4 H, m). IR ($CHCl_3$, cm^{-1}): 1700 (s), 1360 (s), 1160 (s).

Ethyl N-p-*toluenesulfonylacetimidate* was prepared from *p*-toluenesulfonamide and triethyl orthoacetate. B.p. 146 °C/0.05 mmHg. ^{1}H NMR ($CDCl_3$): δ 1.36 (3 H, t), 2.41 (3 H, s), 2.46 (3 H, s), 4.15 (2 H, q), 7.20–8.00 (4 H, m). IR ($CHCl_3$, cm^{-1}): 1615 (s), 1320 (s), 1160 (s).

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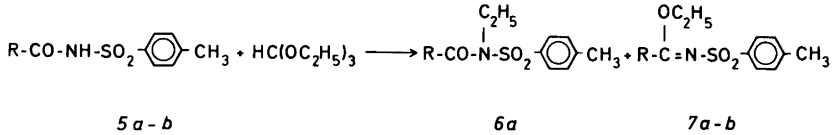


5a-b *6a* *7a-b*

Scheme 5. a: R = CH_3, b: R = C_6H_5

Formation of Methyl α-L-Arabinopyranoside on Alkaline Treatment of Methyl α-L-Arabinofuranoside

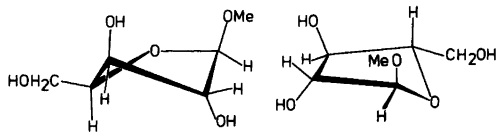
JAN JANSON [a] and BENGT LINDBERG [b]

[a] The Finnish Pulp and Paper Research Institute, P.O. Box 136, SF-00101 Helsinki 10, Finland and [b] Arrhenius Laboratory, University of Stockholm, S-10691 Stockholm, Sweden

On treatment of methyl α-L-arabinofuranoside with alkali, some methyl α-L-arabinopyranoside is formed. A possible mechanism for this reaction is proposed. Some aspects of the alkaline hydrolysis of glycosides are discussed.

Although alkyl glycosides are considered to be stable to alkali, they are hydrolysed and degraded under drastic conditions, *e.g.* strong alkali and 170 °C. An investigation of anomeric methyl aldopyranosides [1] demonstrated that the anomer in which OH-2 and the aglycone are *trans*-disposed was most reactive, indicating that the intermediate formation of the 1,2-anhydropyranose is of major importance. When OH-2 and the aglycone are equatorial in the most stable chair form (*e.g.* $^4C_1$ for methyl β-D-glucopyranoside) the glycoside has to assume the alternate conformation ($^1C_4$) before reaction can occur. The relative rates for alkaline hydrolysis of the *trans*-glycopyranosides therefore depend upon the free-energy differences between the two chair forms. Studies of other pyranosides [2] supported this assumption but demonstrated that other routes for the alkaline hydrolysis may also be significant.

Methyl aldofuranosides in which OH-2 and the aglycone are *trans*-disposed reacted much faster that the corresponding pyranosides.[3] The β-D-xylofuranoside and the β-D-glucofuranoside were considerably more reactive than the α-L-arabinofuranoside and the β-D-galactofuranoside. The conformations of the methyl aldofuranosides in aqueous solution have recently been investigated by Angyal,[4] by examination of their ^{1}H NMR spectra. He found that the β-D-xylo- (*1*) and β-D-glucofuranosides assume the $^3T_2$ conformation and that the α-L-arabino- (*2*) and β-D-galactofuranosides assume the E_0 conformation. As the energy differences between the different twist and envelope forms are small, this means that the furanoside will occupy a segment of the pseudorotational itinerary, centered around the conformation indicated.[5] Cyr and Perlin,[6] who studied the conformations of the methyl aldofuranosides by examining their ^{13}C NMR spectra, arrived at similar results.



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In the favoured $^3T_2$ conformation of methyl β-D-xylofuranoside (*1*) and methyl β-D-glucofuranoside, OH-2 and the aglycone are pseudoaxial. In methyl α-L-arabinofuranoside (*2*) and methyl β-D-galactofuranoside, in the favoured E_0 conformation, the aglycone takes up a pseudoaxial and OH-2 an isoclinal orientation. The favoured conformation of the two former furanosides is therefore closer to the transition state for the formation of a 1,2-anhydrofuranose, and the higher reactivity of these furanosides is thus comprehensible.

An unexpected result was the observation that some methyl β-D-xylopyranoside was formed on alkaline treatment of methyl β-D-xylofuranoside.[3] Unidentified neutral components were also formed in low yields on alkaline treatment of other furanosides. Although part of these products could have been impurities in the starting material which had become enriched, it seemed more likely that they were actually reaction products. This problem has now been reinvestigated for the alkaline hydrolysis of methyl α-L-arabinofuranoside (*2*).

The purity of the starting material *2* was checked by GLC of its triacetate. The isomeric methyl-L-arabinosides were well-separated from *2* on the columns used and it was concluded that the sample contained about 0.2% of the β-furanoside, 0.1% of the β-pyranoside and virtually no (<0.1%) α-pyranoside. After treatment with 2.65 M aqueous sodium hydroxide at 170 °C for 10 h, 20% of neutral material was recovered, most of which (93%) consisted of unchanged *2*. After partial removal of this by successive crystallisations, the remainder yielded raw crystals of the main neutral reaction product, methyl α-L-arabinopyranoside (*4*), in about 2% yield calculated on the amount of starting material. Recrystallisation gave pure *4* in 0.3% yield. The sample was indistinguishable from authentic material (optical rotation, m.p., mixed m.p., ^{1}H and ^{13}C NMR, GLC-MS of the triacetate). Considering that the α-pyranoside is also degraded during the reaction, at about one third of the rate of the furnoside, the actual amount of pyranoside formed should be greater than 2%.

In a series of experiments, the amounts of *2* and *4* after different reaction times were determined by GLC (Fig. 1). First order kinetics with respect to *2*

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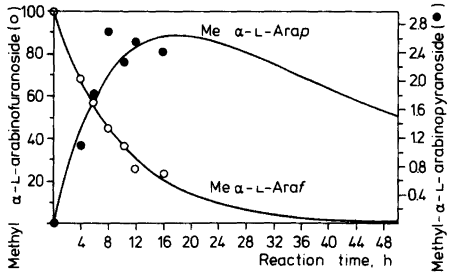
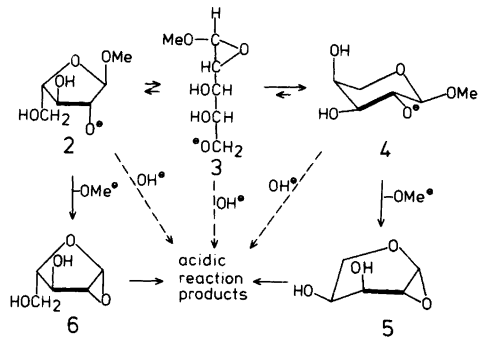


Fig. 1. Percentages of remaining starting material and of methyl α-L-arabinopyranoside on alkaline treatment of methyl α-L-arabinofuranoside. The curves are theoretically derived from calculated reaction constants.



Scheme 1.

and *4*, respectively, were assumed for the rate determining steps, namely:

$2 \xrightarrow{k_1} 4$

$4 \xrightarrow{k_2}$ degradation products

$2 \xrightarrow{k_3}$ degradation products

The value for k_2, $6{,}4 \times 10^{-6}$ s^{-1} was taken from Ref. 1, and those for k_1, 1.2×10^{-6} s^{-1} and k_3, 26 $\times 10^{-6}$ s^{-1}, were determined by regression analysis. A comparison between the observed points and the calculated yield curves (Fig. 1) shows that the agreement is not very good for *4*. This is most probably due both to uncertainties in the analyses and to deviations from the assumed pseudo-first order kinetics. The maximum yield of *4*, however, seems to be approximately 2.6%.

The most reasonable mechanism by which *4* is formed seems to be a nucleophilic attack of O^--2 upon C-1, but which cleavage of the linkage between C-1 and the ring oxygen, yielding the epoxide *3* (Scheme 1). This is rapidly opened during the reaction conditions, in part by intramolecular reaction with O^--5 and formation of pyranoside (*4*). The two consecutive inversions at C-1 result in an unaltered configuration. The previously observed formation of methyl β-D-xylopyranoside[3] from *1* should be quite analogous. Moreover, the formation of levoglucosan[3] from methyl β-D-glucofuranoside probably proceeds *via* methyl β-D-glucopyranoside.

Summing up, it can be assumed that *2* reacts through the pathways indicated in Scheme 1. The formation of *4* has now been demonstrated and that *5* is an intermediate was indicated by indirect evidence.[1,2] The assumption of the analogous 1,2-anhydro-β-L-arabinofuranoside (*6*) seems to be reasonable. Furthermore, a direct attack of a hydroxyl ion on C-1, with release of the aglycon, cannot be excluded. Any alkaline hydrolysis of the glycosidic links will result in formation of arabinose, the fast degradation of which into acids is well-known.

The alkaline hydrolysis of the methyl *trans*-glycosides is only 2.5 – 10 times faster than that of the *cis*-glycosides. One of the mechanisms by which the latter may react involves intermediate formation of an acyclic 1,2-epoxide with retention of the aglycone, as discussed above for the furanosides. This may account for the relatively high reactivity of these glycosides.

Experimental. Methyl α-L-arabinofuranoside was synthesised *via* its tribenzoate as described for the D-enantiomer by Wright and Khorana.[7] Both the benzoate and the free arabinoside were crystallised three times. Methyl α-L- and β-L-arabinopyranosides were prepared by a Koenigs-Knorr synthesis[8] and a Fischer synthesis,[9] respectively.

Quantitative GLC analyses of acetylated glycosides were performed by using packed columns of 3% SP-2340 on 100/200 Supelcoport, and 5% EGS on Chromosorb W. The mass spectrometer, Finnigan type 1020, was coupled to a GLC capillary column containing OV 225.

The alkaline treatments were carried out as described earlier.[2] However, in the kinetic series, performed on a 200 mg scale, pentaerythritol (300 mg) was added as an internal standard to the reaction mixture after the heating. The hydrolysates were deionised as described earlier,[2] and concentrated to dryness.

In the kinetic series, the dry residues were acetylated with acetic anhydride – pyridine, 1:1, before analysis, as were also small aliquots from the product of the preparative experiment.

In the preparative experiment, *2* (10 g) in 2.65 *M* aqueous sodium hydroxide (100 ml) was heated at 170 °C for 10 h. The solution was deionised and concentrated. The neutral product (2.0 g), which

according to GLC analysis contained unchanged *2* as the main component and approximately 6% *4*, was fractionated by crystallisations from ethyl acetate. Pure *4* (30 mg) was isolated and showed m.p. 131 – 134 °C and $[\alpha]_D^{22}$ +17°, in good agreement with published values.[9]

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Differential Activation Parameters of Rapid Atom Abstraction Reactions by Aryl Radicals. Entropy Effects in Diffusion Controlled Reactions

MATS TILSET and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

Differential activation parameters for bromine atom abstraction reactions of aryl radicals in *N,N*-dimethylformamide (DMF) were determined by competition kinetics. Using H atom abstraction from DMF as the standard reaction, rate constant ratios k_H/k_{Br} were evaluated over a 40 K temperature interval. For reactions involving CBr_4, $CHBr_3$ and CH_2Br_2 the relative k_H/k_{Br} changed from 1.0 to 12.6 to 679 and from 1.0 to 7.7 to 437 when the radicals were 4-nitrophenyl and α-naphthyl, respectively. The apparent activation energies at 273 K increased in the reaction series $CHBr_3 \leq CBr_4 < CH_2Br_2$ for the abstraction of Br by both aryl radicals. The relative entropies of activation decreased in the order $CBr_4 > CHBr_3 > CH_2Br_2$ in both reactions series. It was concluded that the reactions involving CBr_4 and $CHBr_3$ are diffusion-controlled and the order of magnitude rate constant differences are due to the more negative $\Delta S^{\neq}$ when $CHBr_3$ is the bromomethane.

In connection with our work on the selectivity of aryl radicals[1,2] in the $S_{RN}1$ reaction,[3] we have examined the reactions of two different aryl radicals, 4-nitrophenyl and α-naphthyl, with bromomethanes. The objective of this work was to determine how the activation parameters for the closely related series of reactions approaching the diffusion-controlled limit depend upon the nature of the reactants. We anticipated that the reactions of the radicals with symmetrical tetrabromomethane (1), in light of other recent work,[4] could be considered to be diffusion-controlled with zero or low entropy of activation. Successive substitution of the less symmetrical bromo compounds, tribromomethane and

$$Ar^{\cdot} + CBr_4 \xrightarrow{k_1} Ar\text{-}Br + {}^{\cdot}CBr_3 \qquad (1)$$

dibromomethane, for CBr_4 was then expected to be accompanied by higher Arrhenius activation energies and more negative entropies of activation. The results reported here show that the activation energies do not vary in the anticipated manner and that the entropies of activation of these rapid reactions play a dominating role in determining the relative rates of reaction.

The abstraction of halogen atoms by free radicals has been intensively investigated.[5] The mechanism of the reaction of phenyl radicals with alkyl iodides has been convincingly demonstrated by stereochemical arguments[6] and chemically induced dynamic nuclear polarization studies[7] to involve the direct abstraction reaction without complications from the possible competing reaction, displacement by backside attack on the carbon bonded to the iodine atom. The general conclusion[5] on the mechanism of the abstraction of halogen atoms from alkyl halides is that the reactions involve cleavage of the C–X bond and the formation of the new bond in a concerted or nearly concerted manner.

RESULTS AND DISCUSSION

Diazonium salts as aryl radical sources. Aryl radicals can be generated from diazonium ions (2) either in thermal,[8] photochemical[9] or electron

$$Ar\text{-}N_2^+ + Z \rightarrow Ar^{\cdot} + N_2 + Z^+ \qquad (2)$$

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transfer[10] processes. One electron reduction produces the aryldiazenyl radical which has a very short lifetime[11] before decomposing to the aryl radical and nitrogen (3). A variety of one electron

$$\text{Ar-N}_2^+ + e^- \rightarrow |\text{Ar-N}_2^{\cdot}| \rightarrow \text{Ar}^{\cdot} + \text{N}_2 \quad (3)$$

reductants can be used to affect reaction (3), including Zn,[12] Cu powder,[13] ferrocene,[14] nitrite,[15] Cu^{I},[16] and I^-.[17] Electron transfer from pyridine has also been implicated.[18] Cathodic reduction has been developed as a source of $Ar^{\cdot}$.[19]

We have selected reaction (4)[17] as the source of aryl radicals for reactivity studies for a number of

$$\text{Ar-N}_2^+ + I^- \rightarrow \text{Ar}^{\cdot} + \tfrac{1}{2}I_2 + \text{N}_2 \quad (4)$$

reasons. The reaction is conveniently carried out simply by the mixing of stable reactants. The reaction can be affected over a wide temperature range due to the low temperature coefficient of the electron transfer process. The yield of aryl radical is essentially quantitative.[17,20] An important consideration is that the initial reaction, *i.e.* the electron transfer, is slow enough in DMF so that it takes place after mixing is complete. The latter is a very important factor when determining relative rate constants by competition techniques since in cases where the generation of the intermediate is diffusion controlled the reaction rates can be determined by the rate of mixing.[21] Under the conditions used in this study, we observe that reaction is not complete 5 min after mixing at 0 °C, eliminating any possibility of complications due to incomplete mixing.

The kinetic procedure. The aryl diazonium salt (<0.009 mmol) dissolved in *N,N*-dimethylformamide (DMF, 2.0 ml) containing the appropriate bromomethane under an atmosphere of nitrogen was allowed to come to thermal equilibrium before injecting a 100 μl aliquot of a solution of Bu_4NI (0.01 mmol) in DMF. Mixing was achieved by the pressure injection of the Bu_4NI solution while momentarily bubbling N_2 through the solution. No difference could be detected in the product ratios when the Bu_4NI solution was added dropwise. The temperature was held constant by large capacity water baths at either room temperature, 0 °C in the presence of ice or at about 40 °C by a thermostated heater. In all cases, the temperature was constant within 0.2 °C. The solution was allowed to stand for 30 min before mixing with a dilute aqueous $Na_2S_2O_3$ solution (2.0 ml) and extracting with pentane (2.0 ml). The extraction procedure was tested on known mixtures.

The pentane solutions were treated with anhydrous $MgSO_4$ to remove traces of water before GLC analysis. The GLC response factors were determined in order to convert area ratios to mol ratios of the products. The relative rate constants were calculated from eqn. (5) where k_H refers to the rate constant for abstraction of hydrogen atoms

$$k_H/k_{Br} = (A_H/A_{Br})(r_H/r_{Br})(n_{BM}/n_{DMF}) \quad (5)$$

from DMF, k_{Br} to the rate constant for abstraction of bromine atoms from the bromomethane, the A values to the appropriate product GLC areas, the r values to the response factors, and the n values to the number of mol of bromomethane and DMF present in the reaction mixtures. Eqn. (5) is valid under conditions where n_{BM} and n_{DMF} remain effectively unchanged during the reaction. The maximum change in n_{BM} occurred when the bromomethane was CBr_4 and this was always less than 5 %.

Measurement precision. The limitation in the precision of the rate constant ratios was found to be in the reproducibility of duplicate runs. Variations of the order of ±5 % in k_H/k_{Br} were observed. Since the error (r) divided by k_H/k_{Br} is small compared to unity, relationship (6) holds. The error in the differential activation energy (ΔE_a) is then expressed in (7) when the data refer to two temperatures.[22] A 5 % error in k_H/k_{Br} for a temperature interval of 273 to 313 K then corresponds to an error of ±0.2 kcal/mol in ΔE_a. The differential entropy of activation is given by (8) and the error in $\Delta\Delta S^{\neq}$ is given by (9) derived from (6) and (7).

$$\ln(k_H/k_{Br} \pm r) = \ln k_H/k_{Br} \pm r(k_{Br}/k_H) \quad (6)$$

$$\text{Error in } \Delta E_a = \pm[RT_1T_2/(T_2 - T_1)]r(k_{Br}/k_H) \quad (7)$$

$$\Delta\Delta S_T^{\neq} = R \ln(k_H/k_{Br}) + \Delta E_a/T \quad (8)$$

$$\text{Error in } \Delta\Delta S_T^{\neq} = \pm(R(r) + (\text{Error in } \Delta E_a)/T) \quad (9)$$

Thus a 5 % error in k_H/k_{Br} over the temperature interval from 273 to 313 K results in an error in the differential entropy of activation at 273 K of 0.8 cal/K mol.

Relative rate constants and differential activation parameters for the reactions of aryl radicals with DMF and bromomethanes. The relative rates of reactions (10) and (11) were determined by the

$$Ar^{\cdot} + S\text{-}H(DMF) \xrightarrow{k_H} Ar\text{-}H + S^{\cdot} \quad (10)$$

$$Ar^{\cdot} + CH_mBr_n \xrightarrow{k_{Br}} Ar\text{-}Br + \dot{C}H_mBr_{n-1} \quad (11)$$

procedure outlined above and k_H/k_{Br} were calculated using eqn. (5). The differential activation energies, ΔE_a, corresponding to the differences in E_a between reactions (10) and (11), were obtained from eqn. (12) while the differential entropies of activation at 273 K were calculated using eqn. (8).

$$\ln k_H/k_{Br} = -(\Delta E_a/R)/T + \ln A_H/A_{Br} \quad (12)$$

Rate constant data for 4-nitrophenyl and α-naphthyl radicals are summarized in Tables 1 and 2, respectively. The corresponding differential activation parameters are given in Table 3. A series of reactions between 4-nitrophenyl and $CHBr_3$ were carried out at 0 °C in which n_{DMF}/n_{CHBr_3} varied from about 20 to 80 and k_H/k_{Br} was observed to be within 5 % of the value given in Table 1.

Similar trends in kinetic parameters were observed for the two radicals. In going through the series of reactions of CH_mBr_n at 273 K, the relative k_H/k_{Br} changed from 1.0 to 12.6 to 679 when 4-nitrophenyl was the reactant and from 1.0 to 7.7 to 437 when α-naphthyl was the reactant as m changed from 0 to 1 to 2 (Tables 1 and 2). In both cases ΔE_a, defined as in (12), increased in going from $n=4$ to 3 and then decreased substantially when $n=2$. In both series $\Delta\Delta S^{\neq}_{273}$, defined as in (8) decreased as n decreased.

The extent of reaction (10) relative to that of (11) was observed to be significantly greater for the reactions of 4-nitrophenyl radical than for those of α-naphthyl radical, with relative proportions ranging from 1.4 to 2.3 with the different bromomethanes. When either $CHBr_3$ or CH_2Br_2 was the reactant, the differential activation parameters for the reactions of the two aryl radicals were nearly the same, Table 3. However, the parameters appear to differ

*For each reactant pair, several experiments were necessary to determine the optimum n_{DMF}/n_{BM}. No concentration dependence of k_H/k_{Br} could be detected in any case.

Table 1. Competition kinetic data for the reactions of 4-nitrophenyl radical with bromomethanes.[a]

Bromomethane	n_{DMF}/n_{BM}[b]	t/°C	k_H/k_{Br}[c]
CBr_4	452	0.0	1.56×10^{-3}
CBr_4	452	20.2	1.95×10^{-3}
CBr_4	452	39.3	2.46×10^{-3}
$CHBr_3$	23.8	0.0	1.97×10^{-2}
$CHBr_3$	23.8	20.4	2.60×10^{-2}
$CHBr_3$	23.8	39.8	3.24×10^{-2}
CH_2Br_2	9.51	0.0	1.06
CH_2Br_2	9.51	20.4	1.18
CH_2Br_2	9.51	39.8	1.25

[a] The radical was generated from $ArN_2^+BF_4^-$ (0.0086 mmol) and Bu_4NI (0.01 mmol). [b] The mol ratio of DMF and bromomethane. [c] Calculated from the relative amounts of nitrobenzene and 4-bromonitrobenzene found by GLC analysis as described in the text.

Table 2. Competition kinetic data for the reactions of α-naphthyl radical with bromomethanes.[a]

Bromomethane	n_{DMF}/n_{BM}	t/°C	k_H/k_{Br}
CBr_4	452	0.0	1.11×10^{-3}
CBr_4	452	21.1	1.32×10^{-3}
CBr_4	452	39.4	1.51×10^{-3}
$CHBr_3$	23.8	0.0	8.51×10^{-3}
$CHBr_3$	23.8	20.9	11.4×10^{-3}
$CHBr_3$	23.8	39.5	13.5×10^{-3}
CH_2Br_2	9.51	0.0	0.485
CH_2Br_2	9.51	20.9	0.530
CH_2Br_2	9.51	39.4	0.595

[a] For conditions see Table 1.

Table 3. Differential activation parameters for the reactions of aryl radicals with bromomethanes and DMF.[a]

Bromomethane	ΔE_a[b]	$\Delta\Delta S^{\neq}$[c]
4-Nitrophenyl radical		
CBr_4	2.0	−6
$CHBr_3$	2.1	0
CH_2Br_2	0.7	3
α-Naphthyl radical		
CBr_4	1.3	−9
$CHBr_3$	2.0	−2
CH_2Br_2	0.9	2

[a] Differential activation parameters obtained by correlation of the rate constant data in Tables 1 and 2. [b] The difference in activation energy for H abstraction from DMF and Br abstraction from the bromomethane in kcal/mol. [c] The difference in entropy of activation for the H abstraction from DMF and Br abstraction from the bromomethane at 273 K in units of cal/K mol.

for reactions of the two radicals with CBr_4 but the difference is not large.

Conclusions. The most unexpected feature of the data is that both 4-nitrophenyl and α-naphthyl radicals react with CBr_4 about an order of magnitude more rapidly than with $CHBr_3$ but in both cases the apparent activation energy appears to be lower for the reactions with $CHBr_3$. Thus, if the reactions of the aryl radicals with CBr_4 are to be considered as diffusion controlled as expected from earlier work,[4] the corresponding reactions with $CHBr_3$ must be as well. The order of magnitude difference in rate constants for the reactions of either radical with CBr_4 and $CHBr_3$ is a consequence of the more negative entropies of activation for the reactions with $CHBr_3$. There does not appear to be any reason to believe that the entropies of activation for the reactions of the radicals with CBr_4 should be zero and it is conceivable that these quantities are also negative.

It has recently been pointed out that activation energies calculated for diffusion-controlled second order bimolecular reactions using eqn. (13) and assuming $\Delta S^{\neq}$ equal to zero results in E_a very much

$$E_a = -RT(\ln k_{diff} - \ln ek/h - \ln T) - T\Delta S^{\neq} \qquad (13)$$

greater than the activation energy of diffusion for typical organic molecules.[23] For example, application of (13) assuming k_{diff} equal to 10^{10} M^{-1} s^{-1} and $\Delta S^{\neq}$ equal to zero results in E_a equal to 4.4 kcal/mol which is of the order of twice the value for the activation energies of diffusion for aromatic compounds in DMF. The activation energies for the diffusion of several aromatic compounds in aprotic solvents have recently been determined and those in DMF were found to be equal to 2.4 ± 0.2 kcal/mol.[24] Thus, diffusion-controlled reactions of aromatic compounds in DMF with rate constants in the usual range,[21] from 10^9 to 10^{10} M^{-1} s^{-1}, are predicted by eqn. (13) to have $\Delta S^{\neq}_{298}$ of the order of -7 to -11 cal/K mol. Thus, if the equations, such as (13), based on transition state theory apply to diffusion-controlled reactions entropy effects are of great importance in determining the rate constants.

Orientational constraints and rotational diffusion[25,26] are factors which would be expected to show their influence in the entropy of activation. The bromomethanes should represent a case for which the spherical model with varying degrees of target surface, depending upon the number of Br in the molecule, would be expected to apply. However, the effect of the entropy of activation differences, 6 to 7 cal/K mol, on the relative rates of the reactions of the radicals with CBr_4 and $CHBr_3$ correspond to relative values ranging from 21 to 34 which suggests that differences in target surface on the two bromomethanes can only account for a fraction of the differences in activation entropies.

We find it somewhat surprising that the extent of reaction (10) relative to (11) is greater for 4-nitrophenyl than for α-naphthyl radical. The strongly electron withdrawing nitro group would be expected to significantly reduce the electron density in the ring which in turn should make the 4-nitrophenyl radical relatively electrophilic. Attack on the electron-rich halogen atoms as compared to attack on a hydrogen atom should be more favorable the more electrophilic the radical. A related series of experiments has shown this to be the case when different 4-substituted nitrophenyl radical reactions are compared.[27] The comparison between 4-nitrophenyl and α-naphthyl may involve factors other than relative nucleophilicity since the structures differ considerably. The effect of electron withdrawing groups on the nucleophilicity of radicals has been discussed in terms of frontier molecular orbital theory.[28]

EXPERIMENTAL

Reagent grade DMF was passed through a column of neutral alumina before use. The diazonium fluoroborates were prepared by a standard procedure[29] and stored at −5 °C. The bromomethanes were reagent grade and used without further purification.

GLC analyses were carried out using a 5 % OV-17 column in a Perkin Elmer 3920B gas chromatograph equipped with a Hewlett Packard 3380S integrator. The analysis procedure was described in an earlier section.

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Characterization of Cerebral Cysteine Sulfinic Acid Decarboxylase. Molecular Parameters and Inhibition Studies

AIMO A HEINÄMÄKI, ANJA K. PERÄMAA and R. SAKARI PIHA

Department of Biochemistry, University of Oulu, SF-90570 Oulu 57, Finland

The molecular properties of cysteine sulfinic acid decarboxylase (EC 4.1.1.29) from calf brain were examined in a crude enzyme preparation. The Stokes radius (42 Å) was determined by gel filtration and the sedimentation coefficient, $s_{20,w}$ (6.1×10^{-13} s) by density gradient centrifugation. Calculation of the molecular weight and frictional ratio gave values of 73 000 and 1.52, respectively.

β-Mercaptopropionic acid and an arginine reagent, phenylglyoxal, were observed to be potent inhibitors of the enzyme. Pyridoxal phosphate, a cofactor of the decarboxylase, was observed to protect the enzyme against phenylglyoxal inhibition. This result indicates that an arginine residue may be located at the active site of cysteine sulfinic acid decarboxylase. The components of taurine metabolism gave little or no inhibition of the decarboxylase. Glutaric acid, malic acid and *C*-allylglycine, all widely known as potent inhibitors of glutamic acid decarboxylase, only slightly inhibited cysteine sulfinic acid decarboxylase.

The molecular properties of cerebral cysteine sulfinic acid decarboxylase (CSAD) are poorly known. In our previous studies taurine-synthesizing CSAD from calf brain was purified to a high degree,[1,2] but because of the low yield and reduced stability of the pure preparation any extensive examination of molecular properties of cerebral CSAD would have proved laborious. In the present study a crude enzyme preparation was used to achieve some molecular data on CSAD from calf brain. The Stokes radius of the enzyme was determined by gel filtration and sedimentation coefficient by density gradient centrifugation. Both of these methods are applicable to proteins in crude extracts if the proteins do not interact with other components in the mixture.[3,4] Subsequently estimates were obtained for the molecular weight and frictional ratio (f/f_o) of the enzyme.

The second part of the work was aimed at examining the effects of some putative inhibitors on the activity of cerebral CSAD. The compounds to be tested were certain components of taurine metabolism,[5] some inhibitors of glutamic acid decarboxylase (GAD)[6] and an arginine reagent, phenylglyoxal.[7] A crude enzyme preparation was also used in these assays.

MATERIALS AND METHODS

Materials. DL-[1-^{14}C]cysteine sulfinic acid (sp. act. 11 mCi/mmol) was obtained from the Centre d'Energie Atomique (Seclay, France), L-cysteine sulfinic acid, L-cysteic acid, aldolase (rabbit muscle), alcohol dehydrogenase (yeast), catalase (bovine liver) and ribonuclease (bovine pancreas) were obtained from Sigma (St. Louis, U.S.A.), pyridoxal-5-phosphate (PLP), taurine, L-cysteine, L-methionine, isethionic acid (2-hydroxyethanesulfonic acid), DL-*C*-allylglycine, β-mercaptopropionic acid, malic acid, glutaric acid and phenylglyoxal from Fluka AG (Buchs, Switzerland) bovine serum albumin, egg albumin, α-chymotrypsinogen (bovine pancreas) and 2-aminoethylisothiouronium bromide (AET) from Calbiochem (Los Angeles, U.S.A.), cytochrome *c* (horse heart) from Boehringer Mannheim GmbH (West Germany), Lumagel from Lumac Systems AG (Basel, Switzerland), Sephadex G-200 from Pharmacia (Uppsala, Sweden) and fresh brains of male and female calves from a local slaughter house (Oulu, Finland).

Decarboxylase assay. CSAD activity was measured by collecting the $^{14}CO_2$ from [1-^{14}C]cysteine

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sulfinic acid under anaerobic conditions.[2] The reaction mixture contained 200 mM sodium phosphate (final concentration) at pH 7.2, 10 mM L-cysteine sulfinic acid, 4.6 kBq of DL-[1-^{14}C]cysteine sulfinic acid, 0.1 mM PLP, 1.0 mM AET, 0.1 % (w/v) Triton x-100 and 0.5 ml of enzyme solution containing 0.1 – 2 mg protein from the various enzyme preparations. Total volume of the reaction mixture was 1 ml. The exact performance of the assay proceeded as follows: Purified nitrogen gas was passed through the incubation tube for 1 min, after which the incubation tube containing the reaction mixture was capped with a rubber stopper and a disposable centre well (Kontes Glass Co., Vineland, NJ) containing 150 µl of 40 % KOH to trap the $^{14}CO_2$ produced by the decarboxylation. After incubation at 37 °C for 60 min the reaction was terminated by injecting 0.5 ml 5 N H_2SO_4 through the stopper, whereupon the tubes were allowed to incubate for an additional 30 min. The alkali in the centre well was neutralized with an equivalent amount of HCl. The centre wells were washed on the outside with distilled water to remove any radioactive contamination and then sunk into counting vials (Mill-6, Lumac) containing 5 ml of Lumagel. The vials were shaken well and any chemilumiscence was reduced by allowing them to stand at room temperature for 1 h before counting in a scintillator (1210 Ultrobeta, Wallac LKB). A blank tube was included, containing buffer instead of enzyme.

Decarboxylase preparation. Crude brain decarboxylase was prepared by a three-step method consisting of homogenization of the whole calf brain, acid precipitation and ammonium sulphate fractionation, as described previously.[2]

Gel filtration. The chromatography column Pharcia was packed with Sephadex G-200 according to the manufacturer's instructions, and the gel equilibrated with 0.2 M sodium phosphate buffer, pH 7.2, which contained 0.1 mM PLP and 1.0 mM AET. The column was calibrated a series of well-characterized globular protein standards with the following molecular weights (MW) and Stokes radii (a):[3,18] ribonuclease (MW = 13 700, a = 16.4 Å); chymotrypsinogen (MW = 25 000, a = 20.9 Å); egg albumin (MW = 43 000, a = 30.5 Å); serum albumin (MW = 67 000, a = 35.5 Å); aldolase (MW = 158 000, a = 48.1 Å) and catalase (MW = 210 000, a = 52.5 Å).

Inhibition. The chemicals to be tested were present in the reaction mixture at 10 mM concentrations and the substrate at a concentration of 1.0 mM (K_m = 1.2 mM).

Density gradient centrifugation. Linear sucrose gradients were produced using an Ultrograd Gradient Mixer (LKB 11300). One mixing chamber containing 32 % (weight per volume) of sucrose in 0.05 M sodium phosphate buffer, pH 7.2, with 0.1 mM PLP and 1.0 mM AET and the other 16 % sucrose (weight per volume) in the same buffer. Two cellulose nitrate centrifuge tubes were filled simultaneously with an identical sucrose gradient using a 2-channel MultiPerpex Pump (LKB 2115).[8] The linearity of the sucrose gradients was determined by adding Amido Black to the sucrose solution of lower concentration. Following centrifugation and collection of the fractions the absorbance at 400 nm was found to increase in a linear fashion with the fraction number. The gradients were stored in a 4 °C cold room for 5 to 20 h before use.

The protein samples were dissolved individually in 0.05 M sodium phosphate buffer, pH 7.2, with 0.1 mM PLP and 1.0 mM AET, after which 0.2 ml of protein solution containing 5 mg protein was layered on the surface of the 16 % sucrose gradient. The protein standards and the extracts were centrifuged in separate tubes.

Centrifugation was performed for 17 h at 39 000 g at 4 °C in the swinging bucket SW-39 rotor of a Spinco Model L ultra centrifuge (Beckmans Instruments).

The sucrose gradient was then unloaded by means of a self-made system consisting of a needle and adjustable stopcock. A hole was punched in the bottom of the centrifuge tube and 0.25 ml fractions were collected manually. The protein content and decarboxylase activity of the fractions were determined.

Calculation of the molecular parameters. The molecular weight (M) frictional ratio (f/f_o) of the decarboxylase were determined from the classical eqns. (I) and (II).

$$M = 6\pi\eta Nas/(1 - v\rho) \quad \text{(I)}$$

$$f/f_o = a/\left(\frac{3vM}{4\pi N}\right)^{1/3} \quad \text{(II)}$$

where η = viscosity of the medium, N = Avogadro's number, a = Stokes radius, s = sedimentation coefficient, v = partial specific volume, ρ = density of the medium, M = molecular weight and f/f_o = frictional ratio. A partial specific volume of 0.725 cm^3/g was used in the calculation, as this value is representative

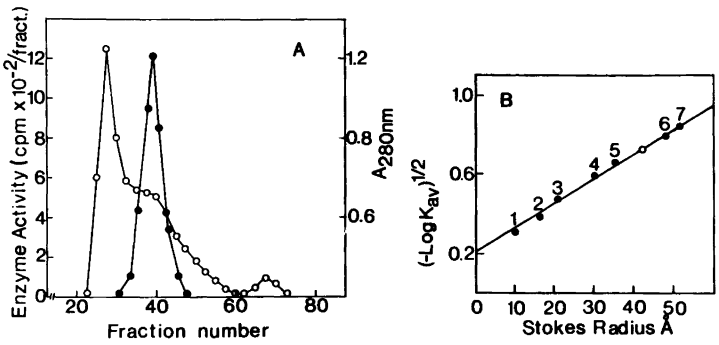

Fig. 1. A. Distribution of decarboxylase activity (●) and protein (○) on a Sephadex G-200 column (26 × 57 cm). Each sample applied to the column contained 100 mg of protein from the crude enzyme preparation. Fractions of 5 ml were collected; B. The gel filtration data were plotted according to the correlation of Laurent and Killander.[11] The standard proteins (●) used are: 1. cytochrome *c*, 2. ribonuclease, 3. chymotrypsinogen, 4. egg albumin, 5. serum albumin, 6. aldolase and 7. catalase. CSAD (○). For details, see Materials and Methods.

of most proteins in sucrose density gradient centrifugation studies.[4] The values of De Duve *et al.*[9] were used for the density and viscosity of the sucrose solutions as functions of concentration and temperature.

Protein estimation. Protein content was determined by the method of Lowry *et al.*[10] Bovine serum albumin was used as the standard.

RESULTS

Stokes radius. The apparent Stokes radius was measured by gel filtration using a calibrated Sephadex G-200 column.[3] The apparent Stokes radius of CSAD was estimated to be 42 Å when the gel filtration data were plotted according to Laurent and Killander[11] (Fig. 1).

Sedimentation coefficient. The sedimentation coefficient $s_{20,w}$ determined by sucrose density gradient centrifugation according to Martin and Ames,[4] gave a value of 6.1×10^{-13} s (Fig. 2).

Molecular weight and frictional ratio (f/f_o). The Stokes radius and the sedimentation coefficient obtained by gel filtration and density gradient centrifugation, together with the assumed partial specific volume of 0.725, were used to calculate the molecular weight and frictional ratio (f/f_o) of cerebral CSAD by means of eqns. (I) and (II), respectively. The calculations gave a molecular

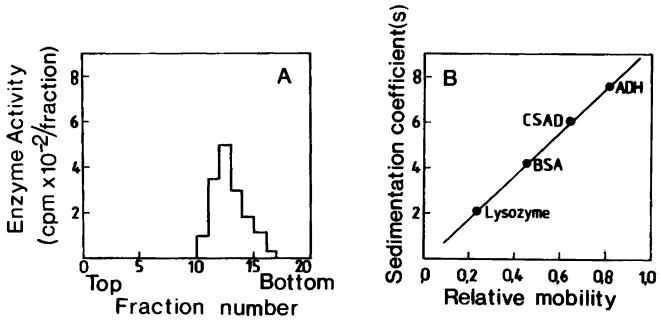

Fig. 2. Determination of the sedimentation cofficient of CSAD by density gradient centrifugation: A. Decarboxylase activity in the fractions of the sucrose gradient; B. Relative mobility of alcohol dehydrogenase (ADH),[4] bovine serum albumin (BSA),[3] lysozyme[4] and CSAD in the sucrose gradients during centrifugation. For details see Materials and Methods.

Table 1. Inhibition of cysteine sulfinic acid decarboxylase.

Compound (10 mM)	Inhibition (%) (mean of 4 experiments)
Components of taurine metabolism	
L-Methionine	0
L-Cysteine	7
L-Cysteic acid	32
Isethionic acid	0
Taurine	0
GAD inhibitors	
Glutaric acid	14
Malic acid	20
C-Allylglycine	15
β-Mercaptopropionic acid	95
Argine reagent	
Phenylglyoxal	93

weight of 73 000 and a frictional ratio of 1.52.

Inhibition of CSAD. Methionine, taurine and isethionic acid produced no inhibition of cerebral CSAD under the conditions mentioned in Materials and Methods. Cysteine, cysteic acid, glutaric acid, malic acid and *C*-allylglycine caused slight inhibition, whereas β-mercaptopropionic acid and phenylglyoxal were more potent inhibitors. The results of the inhibition studies are summarized in Table 1.

DISCUSSION

A combination of the gel filtration and centrifugation techniques was used here to estimate the molecular weight of cerebral CSAD, from the Stokes radius and sedimentation coefficient, together with a third parameter the partial specific volume, which varies over quite a narrow range for most proteins.[4] The sedimentation coefficient, Stokes radius and molecular weight of the calf brain enzyme are approximately equal to those for the rat liver enzyme.[12] The frictional ratio was calculated once the values of the above parameters had been established. This shows CSAD from calf brain to be an asymmetric and/or hydrated molecule.

The most potent inhibitor was β-mercaptopropionic acid (95 % inhibition at a concentration of 10 mM), which is widely used as an inhibitor of GAD. It is the mercapto group in this inhibitor which is thought to be important in its binding to the pyridoxal-phosphate enzyme complex,[6] and it is plausible that the inhibition mechanism may be the same for CSAD and GAD, since both are pyridoxal-phosphate enzymes.[6,13] Glutaric acid, malic acid and *C*-allylglycine also inhibited CSAD activity to a limited extent, whereas they have all been observed to be potent inhibitors of GAD.[6] The components of taurine metabolism, cysteine and cysteic acid, gave only a slight inhibition of CSAD. The most interesting result is the inhibition of the decarboxylase activity by phenylglyoxal, which is frequently used to detect essential arginine residues at the active sites of enzymes.[14-17] This compound reacts fairly specifically with arginine to form a stable complex.[7] Pyridoxal phosphate was observed to protect the enzyme against phenylglyoxal inhibition. The results indicate that the arginine residue may be located at the active site of cerebral CSAD.

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Philodendron scandens Koch et Sello subsp. *oxycardium* (Schott) Bunting, A New Source of Allergenic Alkyl Resorcinols

TORSTEN REFFSTRUP,[a] OLE HAMMERSHØY,[b] PER M. BOLL[a,*] and HENNING SCHMIDT[b]

[a] Department of Chemistry, Odense University, DK-5230 Odense M, Denmark and [b] Department of Dermatology and Venerology, Odense University Hospital, DK-5000 Odense C, Denmark

The allergenic activity of *Philodendron scandens* Koch et Sello subsp. *oxycardium* (Schott) Bunting (Araceae) has been investigated. Patch testing on volunteers and chromatographic separation of extracts of the plant led after structural determination of the pure active component to proposal of *1* as the allergenic constituent. A possible biosynthetic precursor (*3*) to *1* was isolated and identified. From the results of various extraction procedures the allergenic principles are believed to be associated with the cuticle.

Recently *Philodendron scandens* Koch et Sello subsp. *oxycardium* (Scott) Bunting (Araceae), indigenous to South America, but now widespread in the Western world, has been reported to cause allergenic contact dermatitis among gardeners working with the plant.[1] As occupational allergic contact dermatitis is a problem of growing importance in the greenhouse industry *P. scandens* subsp. *oxycardium* was phytochemically examined in order to reveal the structures of the involved allergens.

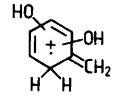
RESULTS

In a pilot experiment fresh leaves and stems were exhaustively extracted with methanol and the extract was partioned between water and chloroform. Patch testing on volunteers occupationally sensitized towards the plant showed the allergenic activity to be exclusively associated with the chloroform extract. Gel filtration, recombination of fractions according to their TLC characteristics followed by patch testing allowed selection of an allergenic fraction which upon chromatography on silica gel afforded a partial separation into components allowing registration of some spectral information. Mass spectrometry and gas chromatography-mass spectrometry of the more olar constituents revealed a compound (*1*) showing two remarkably stable fragments at *m/e* 123 and 124. These fragments had the elemental compositions $C_7H_7O_2$ and $C_7H_8O_2$, respectively, and were accompanied by a molecular ion at *m/e* 342 of elemental composition $C_{23}H_{34}O_2$. Acetylation of crude *1* produced a diacetate; thus *1* is assumed to be a diol.

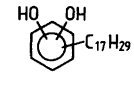
An accompanying compound *2* showed by GLC-MS the somewhat similar characteristics of the two intense fragments at *m/e* 107 and 108 of elemental compositions C_7H_7O and C_7H_8O, respectively, and a molecular ion of *m/e* 326 with the composition $C_{23}H_{34}O$ was detected in another fraction of the partly separated fraction. In a "direct inlet" spectrum of *2* the peak at *m/e* 326 was still detectable, but additionally an ion at *m/e* 370 ($C_{24}H_{34}O_3$) appeared. Apparently *2* could be an artifact obtained on the GLC-column on decarboxylation of a carboxylic acid, which then has to the genuine metabolite. To test this hypothesis the assumed carboxylic acid was esterified to give a methyl ester *4*. The IR spectrum of *4* indicated clearly the presence of an aromatic carboxylic ester. Since decarboxylation of aromatic carboxylic acids is highly facilitated by electron-donating *ortho* substituents *4* is assumed

The former data indicated also the type of compounds present since the very prominent peaks at

a



b



c

*To whom correspondence should be addressed.

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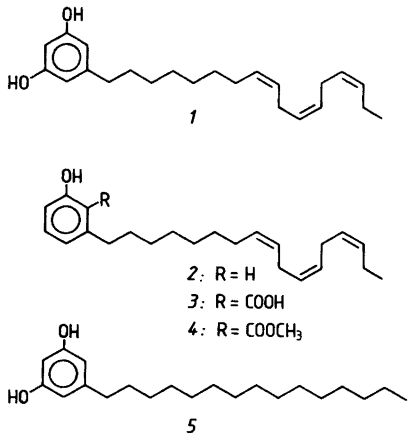
m/e 123 and 124 are indicative of fragment of the types[2] *a* and *b* and since all the oxygen present in the molecule is associated with the aromatic ring, *1* is assigned the partial structure *c*, although the presence of more than one alkyl substituent cannot excluded.

To give more easily separable extracts a new batch of plant material was extracted differently. The coarsely cut stems and leaves of *P. scandens* were exhaustively extracted with light petroleum and chromatography on silica gel with gradient elution (ether in light peroleum gave the spectroscopically pure compounds *1* and *3*. The 270 MHz ^{1}H NMR spectrum of *1* allowed assignment of all the signals in the aliphatic side chain on the basis of selective decoupling experiments. The configuration of the double bonds is considered all-*cis* since all the couplings of the vinylic signals are smaller than 10 Hz. The assignments (*cf.* Experimental) gave



basis for constructing the side chain as given for *1*. The spectrum showed additionally resonances corresponding to the aromatic protons of an alkyl substituted resorcinol with spin–spin couplings of about 2 Hz. A computer simulation of the aromatic part of the spectrum confirmed this assignment.

The presence of the resorcinol group in *1* was further supported by its UV spectrum which is similar to those quoted for the alkyl resorcinol grevillol[3] and a heptadecatetraenylresorcinol[4] and also by the ^{13}C NMR spectrum which showed 4 resonances attributable to a symmetrically substituted aromatic system. The chemical shifts of these carbons and their multiplicity are also in accord with the proposed structure. Compound *1* is thus 5-heptadeca-8(*Z*),11(*Z*),14(*Z*)-trienylresorcinol.

The 270 MHz ^{1}H NMR spectrum of the carboxylic acid *3* showed three aromatic protons resonating at δ 7.21 (1H, d of t) and 6.70 (2H, two dd). All protons show *ortho* couplings and the substitution pattern of the ring has to be 1,2,3. It is further found that the chemical shifts of the α-methylene group are shifted downfield by 0.45 ppm in comparison with the value recorded for the corresponding protons in *1*. Therefore, the carboxyl group must be *ortho* to the alkenyl substituent. As the hydrogens *para* and *ortho* to a carboxyl group are deshielded, the proton resonating at δ 7.21 is either *ortho* or *para* to the carboxyl group. Since this proton is a douplet of triplets, both with large *ortho* couplings, it has to be *ortho* to two protons and, therefore, *para* to the carboxyl group. The two highfield aromatic protons are thus assigned to be *meta* to the carboxyl group. The low field resonance of the phenolic proton of *4* (δ 10.9) is in agreement with this assignment and it also explains the ease of decarboxylation of *3*. The spectral information thus gives evidence for a substitution pattern as given in *3*.

Apart from the deshielding of the protons of the α-methylene group the pattern of the side chain of *3* is identical to that found for the resorcinol. Coupling patterns, number of protons and decoupling experiments give the conclusion that the side chains of *1* and *3* are identical. Compound *3* is thus 6-heptadeca-8(*Z*),11(*Z*),14(*Z*)-trienyl-2-hydroxybenzoic acid. The resorcinol (*1*) and the salicylic acid (*3*) would appear to be derived from a common C_{24} polyketide precursor.

The allergic activity of the total plant extracts can be completely destroyed upon treatment in ethanol for 24 h with traces of sodium hydroxide under stirred and aerated conditions. Patch testing on pure *1* as well as on the untreated total extract produced a vigorous response. Since phenols are known to be destroyed by oxygen and bases, the above experiment indicates that the allergic activity of *P. scandens* is mainly associated with *1*.

Among the various type of phenols, catechols[5] and hydroquinones[6] have frequently been found responsible for allergic reactions caused by plants. Since resorcinols only infrequently[7] cause allergic contact dermatitis a fresh extract of *P. scandens* was investigated by GLC-MS for the presence of other phenols. The method applied to the trimethylsilylated extract revealed, in addition to *1*, trace amounts of a compound $C_{21}H_{32}O_2$ believed to be

pentadecylresorcinol (*5*); but no traces of catechols, detectable by their characteristic loss of $SiMe_4$[8] could be observed.

Application of the very sensitive electron paramagnetic (EPR) technique to a freshly prepared extract of the plant showed clearly that hydroquinones and catechols are not present.[9] This information held together with the association of the main allergic activity with pure *1* gives support for our conclusion that *1* is the principal allergenic constituent of *P. scandens*.

DISCUSSION

The natural occurrence of 5-alkyl- and 5-alkenylresorcinols has been previously reported in the plant families Anacardiaceae (cashew nut shell oil),[10,11] Ginkgoaceae *(Ginkgo biloba)*,[12] Gramineae,[13] Proteaceae,[14,15] and most recently, Myrcinaceae.[16] Further, the isolation and characterization of two 5-alkylresorcinols from *Azotobacter vinelandii*[17] and a 5-heptatetraenylresorcinol from the brown alga *Cystophora torulosa*[4] have been reported.

6-Alkenylsalicylic acids have not been reported to cause allergic contact dermatitis, although they are reported to be antiinflammatory.[18] Such compounds with a C_{15}-side chain have been found in the cashew nut shell oil[7] and in *Ginkgo biloba*.[19] Salicylic acids with C_{17}-side chains and various degrees of unsaturation have been detected from *Pentaspodon officinalis* and *P. motlei*.[20]

In view of the frequent occurrence of allergic contact dermatitis initiated by phenolic compounds, it is of interest to add new plant sources of allergenic phenols in order to predict and prevent cross reactions for persons already sensitized. The present paper shows *Philodendron scandens* subsp. *oxycardium* (Araceae) as a hitherto unrecognized source of long chain alkenylresorcinols and the compounds *1* and *3* are new with respect to structure and origin and they have been isolated and characterized. An investigation of nine other species of *Philodendron* has proved four more species to contain resorcinols.[21]

It has also been found that the total amount of compounds *1* and *3* is extracted just by letting the coarsely cut stems and leaves stand in contact with light petroleum. Further extraction with more polar and water soluble solvents gave virtually no additional amounts of *1* and *3*. This make us conclude that the allergenic principle is a constituent of the cuticle, which is very appropriate if the resorcinols are considered a defense weapon.

EXPERIMENTAL

Philodendron scandens subsp. *oxycardium* was grown in a nursery. Fresh leaves and stems were coarsely cut and extracted with methanol in a Soxleth extractor for the preliminary experiments. A more efficient extraction was effected by using the sequence: light petroleum, ethyl acetate and then methanol on fresh plant material. The light petroleum fraction was an extract mainly of the cuticle and almost all of the allergenic principle was present in this fraction. Separations were carried out by column chromatography on Sephadex LH20 in methanol and on Silica Woelm 62-100 with chloroform – ethyl acetate or ether – light petroleum by solvent gradient elution. In all cases fractions of each 10 ml were collected.

TLC was performed on high performance silica coated plates (Merck). The plates were monitored by UV or by spraying with 1 % vanillin in conc. H_2SO_4 and then heated. Mass spectra were recorded on a Varian Matt 311 or a Varian CH7 instrument both equipped with inlet for gas chromatography – mass spectrometry. NMR spectra were obtained on Jeol Fx60 Q and Bruker Hx270 instruments. IR and UV spectra were recorded on Perkin Elmer 580 and Cary (Varian) 219 instruments, respectively.

5-Heptadecatri-8(Z),11(Z),14(Z)-enylresorcinol (1). The light petroleum extract (1.04 g) from 504 g of *P. scandens* was chromatographed on 200 g of silica gel with a gradient of ether in light petroleum and gave 25 mg (0.005 %) of *1* (10-ml fractions Nos. 146–156). UV (EtOH): λ_{max} 216 nm (sh), 278 nm (log ε 3.28); upon addition of NaOH the spectrum shifted to λ_{max} 236 nm and 290 nm (log ε 3.21). ^{1}H NMR (CD_3OD): δ 0.96 (3H, t, *J* 8 Hz, CH_2CH_3), 1.30 (8H, m, $(CH_2)_4$), 1.54 (2H, m, $ArCH_2CH_2$), 2.05 (4H, m, $CH_2CH=$), 2.41 (2H, t, *J* 7 Hz, $ArCH_2$), 2.75 (4H, m, $=CHCH_2CH=$), 5.33 (6H, m, $CH=CH$), 6.07 (1H, dd, *J* 2 and 1.2 Hz, ArH), 6.10 (2H, d, *J* 2 Hz, ArH). ^{13}C NMR (CD_3OD): 156.7 (s), 146.0 (s), 132.0, 130.4, 128.3, 127.7, 127.2, 108.0 (d), 100.1 (d), 35.8, 31.1, 29.7, 29.2, 27.3, 25.7, 20.6, 14.3. IR (film): 3380, 3040, 2930, 1600 cm^{-1}. MS: Found: M^+ 342.2548. Calc. for $C_{23}H_{34}O_2$: 342.2558, *m/e* (rel. int.) 342 (12), 177 (6), 163 (19), 149 (14), 137 (13), 136 (8), 135(8), 125 (8), 124 (100), 123(47), 121 (7), 109 (6), 108 (10).

6-Heptadecatri-8(Z),11(Z),14(Z)-enyl-2-hydroxybenzoic acid (3). The above chromatographic procedure also furnished 81 mg (0.016 %) of pure *3* (10-ml fractions Nos. 40–57). ^{1}H NMR (CD_3OD):

δ 0.95 (3H, t, J 8 Hz, CH_2CH_3), 1.33 (8H, m, $(CH_2)_4$), 1.56 (2H, m, $ArCH_2CH_2$), 2.06 (4H, m, $CH_2CH=$), 2.79 (2H, t, J 6 Hz, $ArCH_2$), 2.87 (4H, m, $=CHCH_2CH=$), 5.32 (6H, m, $CH=CH$), 6.71 (2H, two dd, J 7 Hz, ArH), 7.22 (2H, d of t, J 7 and 1 Hz) (the aromatic couplings have been calculated using the computational procedure). IR (film): 3600 to 2300 (broad), 3020, 2940, 1650, 1600 cm^{-1}. MS: Found: M^+ 370.2481. Calc. for $C_{24}H_{34}O_3$: 370.2508, *m/e* (rel. int.) 371 (9), 370 (35), 175 (6), 173 (8), 164 (5), 163 (7), 162 (9), 161 (14), 160 (6), 159 (8), 152 (18), 151 (22), 149 (14), 148 (13), 147 (27), 146 (10), 145 (6), 136 (10), 135 (20), 134 (25), 133 (19), 123 (7), 122 (14), 121 (24), 120 (10), 119 (9), 109 (21), 108 (85), 107 (51), 106 (6), 105 (27), 96 (9), 95 (78), 64 (26), 93 (45), 92 (7), 91 (28), 83 (6), 82 (8), 81 (43), 80 (39), 79 (100), 78 (14), 77 (24), 69 (19).

Methyl 6-heptadecatri-8(Z),11(Z),14(Z)-2-hydroxybenzoate (4). Compound *3* (16 mg, 0.043 mmol) was treated with diazomethane in ether for 5 min. Upon evaporation *4* was obtained quantitatively. ^1H NMR ($CDCl_3$): δ 3.95 (3H, s, CO_2CH_3), 11.09 (1H, s, OH). IR (film): 3450, 3020, 2970, 1665, 1610 cm^{-1}. MS: m/e (rel. int.) 385 (10), 384 (34), 283 (6), 201 (6), 187 (5), 173 (11), 166 (24), 165 (24), 163 (7), 162 (8), 161 (19), 160 (7), 159 (9), 149 (14), 148 (13), 147 (30), 146 (9), 145 (7), 136 (10), 135 (26), 134 (24), 133 (19), 123 (6), 122 (14), 121 (27), 120 (10), 119 (11), 109 (18), 108 (71), 107 (46), 106 (7), 105 (24), 95 (80), 94 (23), 93 (46), 92 (6), 91 (27), 82 (6), 81 (39), 80 (33), 79 (100), 78(13), 77 (16).

Trimethylsilylation of extracts of P. scandens. The light petroleum extract of leaves and stems corresponding to approximately 1 mg of material was evaporated in a vial. Upon dissolution in hexane (2 ml) 1 drop of distilled pyridine and 0.01 ml of *N,N*-bis(trimethylsilyl)acetamide were added. The vial was stoppered loosely and heated for 30 min at 60 °C. The reaction mixture was cooled and used directly for GC-Ms.

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Activation Energies for Diffusion in Aprotic Solvents. Application to the Estimation of Kinetic Parameters for Diffusion Controlled Bimolecular Reactions

BJØRG AALSTAD and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The activation energies for the diffusion of a number of aromatic compounds were determined in acetonitrile (AN), *N*,*N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) by chronoamperometry. Although the diffusion coefficients in each solvent varied by about a factor of 2 for the series of compounds studied, the activation energies varied very little. The mean values of $(E_a)_{diff}$ were found to be 2.00 ± 0.06 (AN), 2.43 ± 0.22 (DMF) and 3.19 ± 0.19 (DMSO) kcal/mol (1kcal = 4.184 kJ). Use of $(E_a)_{diff}$ and encounter controlled second order rate constants estimated from the Smoluchowski equation resulted in apparent entropies of activation at 298 K ranging from -6 to -9 cal/K mol. Using $(E_a)_{diff}$ along with $\Delta S^{\dagger} = 4/3\ \Delta S_c$ in an equation resulting from transition state theory resulted in second order rate constants in close agreement with those from the Smoluchowski equation. The cratic entropy for the reversible encounter ΔS_c was estimated for each solvent. It was concluded that reliable encounter controlled second order rate constants can be estimated by this approach.

Activation energies for the diffusion of aromatic compounds in acetonitrile (AN) and *N*,*N*-dimethylformamide (DMF) have recently been determined by the measurement of the temperature dependence of the peak currents during linear sweep voltammetry.[1] Estimates of diffusion controlled bimolecular rate constants were made by assuming zero entropy of activation and applying transition state theory equations. The rate constants estimated in this manner were from 20 to 30 times larger than those estimated using the Smoluchowski equation (1).[2] It was concluded that if zero entropy of activation is to be assumed and eqn. (2) used to estimate the diffusion controlled rate constant that the activation energy for diffusion of a single species must be multiplied by some factor in order to give k_{diff} in reasonable agreement with that obtained from (1). Values of k_{diff} calculated using eqn. (2) with $2(E_a)_{diff}$ were of the order of 3–4 times smaller than those estimated from eqn. (1). Since experimental tests of eqn. (1) have indicated that k_{diff} is over-estimated by a factor of 2–3,[3] it appeared that using $2(E_a)_{diff}$ in eqn. (2) gave reasonable estimates of k_{diff}.

$$k_{diff} = 4\pi\kappa a f N_o / 1000(D_A + D_B) \quad (1)$$

$$\ln k_{diff} = (\ln ek/h + \ln T) + (E_a)_{diff}/RT + \Delta S^{\dagger}/R \quad (2)$$

In this paper we show that reasonably good agreement between k_{diff} calculated from (1) and (2) is obtained using $(E_a)_{diff}$ in eqn. (2) if $\Delta S^{\dagger}$ for the formation of the encounter complex is taken into account. Diffusion activation energies are reported for a number of aromatic compounds in AN, DMF and dimethylsulfoxide (DMSO).

RESULTS AND DISCUSSION

The measurement of activation energies for diffusion. The initial measurements involved the temperature dependence of the linear sweep voltammetry (LSV) peak currents for reversible electrode processes (3).[1] The peak current is given by (4) where n is the number of electrons per mol transferred,

$$A \pm ne^- \rightleftarrows A^{\mp n} \quad (3)$$

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$$i_p = 0.4463nFAC_A(nF/RT)^{\frac{1}{2}}v^{\frac{1}{2}}D_A^{\frac{1}{2}} \quad (4)$$

F the Faraday, A the electrode area, C_A the substrate concentration, and D_A the diffusion coefficient. At a given sweep rate (v) and C_A (substrate concentration) at a particular electrode (4) at a particular electrode (4) reduces to (5) where c is a constant. Thus, the temperature dependence of D_A can be obtained by correlating $(i_p)^2T$ as a function of $1/T$.

$$D_A = c(i_p)^2T \quad (5)$$

Values of $(E_a)_{diff}$ obtained in this manner had standard deviations as great as $\pm 20\,\%$. We therefore abandoned the LSV measurements.

We find that chronoamperometry[4,5] measurements result in $(E_a)_{diff}$ values which are more reproducible. The method is based upon the Cottrell equation (6) which describes the current-time response of the reversible charge transfer (3) to a potential step from a potential where reaction does not take place to the potential region where (3) is diffusion controlled. In practice the latter can be accomplished by stepping about 200 – 300 mV beyond the LSV peak potential. Since D_A is the only factor on the right hand side of (6) that depends upon the temperature, D_A is given by (7) where c' is a constant.

$$i(t) = nFAD_A^{\frac{1}{2}}C_A/\pi^{\frac{1}{2}}t^{\frac{1}{2}} \quad (6)$$

$$D_A = c'(it^{\frac{1}{2}})^2 \quad (7)$$

The term in parentheses, $it^{\frac{1}{2}}$, is conveniently measured. In this study we have measured the current at 1.0 *ms* intervals beginning 5 *ms* after the potential step until 10 *ms* after the step.

Measurement precision. The 6 ($it^{\frac{1}{2}}$) values obtained from each current-time curve typically had standard deviations of the order of $\pm 0.2\,\%$. Each relative D_A value, eqn. (7), involved three sets of 10 current-time curves. The standard deviation in the mean ($it^{\frac{1}{2}}$) value for the 30 curves was in general less than $\pm 0.5\,\%$.

Table 1. Relative diffusion coefficients and activation energies for diffusion of aromatic compounds in DMF.[a]

Compound	$10^5D/cm^2s^{-1}$ [b]	$(E_a)_{diff}$ [c]
4-Cyanopyridine	1.14	2.55(0.01)
Nitrobenzene	1.08	2.38(0.16)
Anthracene	0.891	2.46(0.21)
Phenazine	0.758	2.38(0.04)
Nitromesitylene	0.727	2.01(0.06)
Fluoren-9-one	0.671	2.75(0.01)
9-Phenylanthracene	0.521	2.50(0.03)

[a] Determined by chronoamperometry. [b] Values at 298 K. [c] Based on measurements at 0, 20 and 40 °C in solvent containing Bu_4NBF_4 (0.1 M). Measurements with nitrobenzene were made on both mercury and platinum electrodes, mercury electrodes were used for 9-phenylanthracene and anthracene, and platinum electrodes were used for all other compounds. The numbers in parentheses refer to the standard deviations in two or more measurements.

Activation energies for diffusion of aromatic compounds in DMF, AN and DMSO. The data obtained from measurements made at 0° and about 20 and 40 °C are summarized in Table 1 (DMF), Table 2 (AN) and Table 3 (DMSO). The temperatures were known in all cases to ±0.2 °C. The numbers in parentheses refer to the standard deviations, usually with 3 or more determinations. More detail of a particular determination is given in Table 4 for measurements during the reduction of nitromesitylene in DMSO. In this case $(E)_{diff}$ was observed to be equal to 3.29 kcal/mol with a standard deviation of ±0.09. The coefficients for the Arrhenius correlations were in all cases greater than 0.999.

The diffusion coefficients listed in Tables 1 – 3 are from relative values obtained from relative i^2

Table 2. Relative diffusion coefficients and activation energies for diffusion of aromatic compounds in AN.[a]

Compound	Process	$10^5D/cm^2s^{-1}$ [b]	$(E_a)_{diff}$/kcal mol^{-1}
Nitrobenzene	Reduction	1.983	1.99(0.01)
Nitromesitylene	Reduction	1.607	2.04(0.06)
Fluoren-9-one	Reduction	1.581	1.95(0.08)
Thianthrene	Oxidation	1.415	1.95(0.13)
4,4'-Dimethoxybiphenyl	Oxidation	1.386	2.10(0.10)
Tris-*p*-tolylamine	Oxidation	0.936	1.98(0.15)

[a] For conditions see Table 1. Measurements were made on platinum electrodes. [b] At 298 K.

Table 3. Relative diffusion coefficients and activation energies for diffusion of aromatic compounds in DMSO.[a]

Compound	$10^5 D/cm^2 s^{-1}$ [b]	$(E_a)_{diff}$/kcal mol^{-1}
Nitrobenzene	0.471	3.02(0.22)
Nitromesitylene	0.367	3.29(0.09)
Phenazine	0.315	3.39(0.05)
Fluoren-9-one	0.279	3.44(0.02)

[a] For conditions see Table 1. Measurements were made on platinum electrodes. [b] At 298 K.

values according to eqn. (6). The values reported were obtained using the value reported[6] for nitrobenzene in DMF at 298 K, *i.e.* 1.08×10^{-5} cm^2/s.

Comparison of diffusion parameters in AN, DMF and DMSO. The diffusion coefficients measuremed in each solvent encompassed about a 2-fold variation. However, it is interesting to note that in DMF and AN there does not appear to be any relationship between D and $(E_a)_{diff}$. In fact, in AN $(E_a)_{diff}$ appears to be independent of the particular compound upon which measurements were made. The mean value was 2.00 kcal/mol with a standard deviation of only ± 0.06 kcal/mol. The variations were greater in DMF and the mean value was 2.43 ± 0.22 kcal/mol. On the other hand. decreases in D were accompanied by increases in $(E_a)_{diff}$ in DMSO. In this case a reasonably good correlation of ln D *vs.* $(E_a)_{diff}$ was obtained as would be expected from the Arrhenius-like equation (8).

$$D = (\text{constant}) \exp(-E_a)_{diff}/RT \quad (8)$$

Measurements were made on nitrobenzene (NB), nitromesitylene (NM) and fluoren-9-one (FL) in all three solvents. The relative viscosities of the solvents are 1.00:2.33:5.75 (AN – DMF – DMSO). According to the Stokes-Einstein relationship (9), in which k is Boltzmann's constant and r is the radius of the

$$D = kT/6\pi\eta r \quad (9)$$

molecule, D^{-1} should be linearly related to the viscosity η. The relative D^{-1} for FL were 1.00:2.36:5.67 (AN – DMF – DMSO) in almost exact agreement with the prediction based on eqn. (9). For NB and NM the correspondence was not as good. The ratios of relative D^{-1} were 1.00:1.73:4.21 and 1.00:2.21:4.38, respectively.

The activation energy for diffusion and the rate constants for bimolecular diffusion controlled reactions. It has been proposed that during the process of diffusion the motion of one molecule past another demands a free energy lying between those required by the two molecules in their separate viscous flows.[7] This served as the basis for proposing that if a diffusion controlled reaction has zero entropy of activation, eqn. (2) could be used to estimate k_{diff} by taking $(E_a)_{diff}$ to be the average activation energy for the two species reacting in the bimolecular reaction.[1]

Benson[8] has treated the formation of collision pairs as an equilibrium (10) and from calculated mean life times and rates of formation, estimated

$$A + B \rightleftarrows AB \quad (10)$$

equilibrium concentrations of AB. The conclusion was drawn that the entropy of the process is given by eqn. (11) and is equal to about -2.5 cal/K mol when r_{AB} is 4 Å and that this is an underestimation

$$\Delta S = R \ln(8r_{AB}{}^3 N/1000) \quad (11)$$

of the entropy of formation of chemically reacting pairs. Another approach to estimate ΔS for reaction (10) involves assuming that ΔH for the formation of the encounter pair is zero and that the cratic entropy change (ΔS_c) for bringing the pair together is given by eqn. (12) where [M] is the molarity

$$\Delta S_c = -R \ln[M] \quad (12)$$

of the solvent.[9] The values of ΔS_c for the solvents used in this study are -5.86, -5.08 and -5.26 cal/K mol for AN, DMF and DMSO, respectively.

Some observed values of activation parameters for reactions believed to be diffusion controlled are

Table 4. Activation energy determinations for the diffusion of nitromesitylene in DMSO.[a]

Run	$(E_a)_{diff}$/kcal mol^{-1}	r[b]
1	3.16	−1.0000
2	3.34	−1.0000
3	3.33	−0.9998
4	3.23	−0.9998
5	3.21	−1.0000
6	3.39	−0.9999
7	3.36	−0.9990

[a] For conditions see Table 1. Measurements were made on platinum electrodes. [b] The linear regression correlation coefficient for data obtained at 0, 20, and 40 °C.

Table 5. Activation parameters for some diffusion controlled reactions.

Reaction	Solvent	E_a/kcal mol^{-1}	$\Delta S^{\dagger}$/cal K^{-1} mol^{-1}
2 I→I_2[a]	CCl_4	3.2	−5
β-Naphthylamine + CCl_4	cyclohexene	2.5	−7
(Fluoresence quenching)[b]	iso-octane	1.6	−8
tert-Butyl radical	AN	2.03±0.2	−7.24
Self termination[c]			
Triplet energy transfer	AN	1.72±0.3	−7.91
from indeno[2,1-*a*]indene[d]			

[a] Data from Ref. 10 cited in Ref. 8. [b] Data from Ref. 11 cited in Ref. 8. [c] Data from Ref. 12 based on kinetic data reported in Ref. 13. [d] Data from Ref. 12.

gathered in Table 5. In all cases, $\Delta S^{\dagger}$ was found to be more negative than −5 cal/K mol and also more negative than ΔS for reaction (10) calculated either by eqn. (11) or (12). The most pertinent data regarding diffusion controlled reactions in the solvents used in this study are those reported by Saltiel *et. al.*[12] and by Schuh and Fischer.[13] The self termination reaction of *tert*-butyl radical was observed to be diffusion controlled in a number of aprotic solvents.[13] Activation entropies were observed to vary in the range, −3.65 to −7.24 cal/K mol.[12] In general, for the aprotic solvents $\Delta S^{\dagger}$ was observed to be roughly equal to 4/3 ΔS_c. It was concluded that the activation entropy for an encounter of hydrocarbon solutes in nonhydroxylic solvents reflects more ordering in the solvent than is required for the fully formed encounter complex.[12]

It is interesting to compare $\Delta S^{\dagger}$ for the two reactions reported in acetonitrile (Table 5) with those calculated from eqn. (2) using the $(E_a)_{diff}$ data in Table 2 and k_{diff} calculated using eqn. (1). The k_{diff} and $\Delta S^{\dagger}$ calculated in this manner are summarized in columns 3 and 4 of Table 6. In AN, $\Delta S^{\dagger}$ varied from −7.28 to −8.80 cal/K mol a mean value of −7.86±0.54 cal/K mol. The values of $\Delta S^{\dagger}$ determined for the self termination of *tert*-butyl

Table 6. Second order rate constants and entropies of activation for diffusion controlled reactions.

Compound	Solvent	$10^{-9}k_{diff}$/ $M^{-1}s^{-1}$[a]	$\Delta S^{\dagger}_{298}$/ cal K^{-1} mol^{-1}[b]	$10^{-9}k_{diff}$/ $M^{-1}s^{-1}$[c]
4-Cyanopyridine	DMF	8.64	−6.50	7.54
Nitrobenzene	DMF	8.19	−7.18	10.1
Anthracene	DMF	6.75	−7.29	8.78
Phenazine	DMF	5.75	−7.88	10.1
Nitromesitylene	DMF	5.51	−9.21	18.8
Fluoren-9-one	DMF	5.09	−6.88	5.38
9-Phenylanthracene	DMF	3.95	−8.22	8.21
Nitrobenzene	AN	15.0	−7.28	11.5
Nitromesitylene	AN	12.2	−7.53	10.6
Fluoren-9-one	AN	12.0	−7.86	12.3
Thianthrene	AN	10.7	−8.09	12.3
4,4′-Dimethoxybiphenyl	AN	10.5	−7.62	9.54
Tris-*p*-tolylamine	AN	7.09	−8.80	11.7
Nitrobenzene	DMSO	3.57	−6.68	3.02
Nitromesitylene	DMSO	2.78	−6.27	1.92
Phenazine	DMSO	2.39	−6.24	1.62
Fluoren-9-one	DMSO	2.11	−6.32	1.48

[a] Calculated from eqn. (1) assuming $a = 5$ Å and κ and f are unity. $D_A + D_B$ was taken to be twice D for the compound. [b] Calculated from eqn. (2) using k_{diff} from the previous column. [c] Calculated from eqn. (2) using $\Delta S^{\dagger} = 4/3\ \Delta S_c$.

and the triplet quenching of indeno[2,1-*a*]-indene in AN were −7.24 and −7.91 cal/K mol, respectively.

The discussion in the previous paragraph suggests that eqn. (2) will give reliable estimates of k_{diff} if proper account is taken of $\Delta S^{\dagger}$. The original suggestion[1] of assuming that $\Delta S^{\dagger}$ is zero obviously gives erroneous results. Available data[12] suggests that a best estimate of $\Delta S^{\dagger}$ for the diffusion controlled reaction is obtained from 4/3 ΔS_c. The k_{diff} in the last column of Table 6 were calculated from eqn. (2) using the pertinent $(E_a)_{diff}$ and ΔS_c assuming that $\Delta S^{\dagger} = 4/3\ \Delta S_c$. In AN this resulted in $k_{diff} = 1.13\ (\pm 0.11) \times 10^{10}\ M^{-1} s^{-1}$ at 298 K. The value reported for the self termination of *tert*-butyl was $6.7 \times 10^9\ M^{-1} s^{-1}$ (at 293 K)[13] and for the triplet quenching of indeno[2,1-*a*]indene was $1.62 \times 10^{10}\ M^{-1} s^{-1}$ (at 303 K).[12]

In conclusion, we can compare the assumptions involved in the use of eqns. (1) and (2). For (1) κ and f are assumed to be unity and a must be estimated. The assumptions were made for the k_{diff} in column 3 of Table 6 and a was arbitrarily taken to be 5 Å. When using eqn. (2) the only assumption necessary is the value of $\Delta S^{\dagger}$ and good experimental data are available for this purpose.

EXPERIMENTAL

The solvents containing the supporting electrolyte, Bu_4NBF_4 (0.1 M) were passed through a column of neutral alumina before dissolving the substrate (2.0 mM). The chronoamperometric experiments were conducted using potential steps of 600 mV with the LSV peak potential for the compound centered in this interval. The apparatus for data acquisition was the same as that used in other recent work from this laboratory.[14]

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The Crystal and Molecular Structure of a Dimeric Form of 5-*keto*-D-Fructose; D-*threo*-Hexo-2,5-diulose Dimer

LARS K. HANSEN, ASBJØRN HORDVIK and ROLF HOVE

Department of Chemistry, Institute of Mathematical and Physical Sciences, University of Tromsø, Box 953, N-9001 Tromsø, Norway

Crystals of the 5-*keto*-D-fructose dimer $C_{12}H_{20}O_{12}$ are orthorhombic, space group $P2_12_12_1$, with unit cell dimensions $a=12.094(3)$ Å, $b=21.700(6)$ Å, $c=5.329(3)$ Å and $Z=4$.

X-Ray data were collected on a Siemens AED diffractometer using Mo$K\alpha$ radiation and the five-value scan technique. The structure was solved by direct methods (MULTAN) and refined by full matrix least squares.

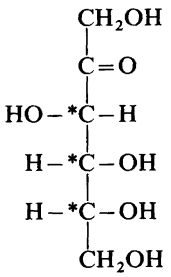
The dimer consists of α-D-fructose in the furanose form and α-L-sorbose in the pyranose form; the latter appears as a result of the dimerization. The two dimeric partners are interconnected through ether bridges from C(5) of sorbose to C(4) and C(5), respectively, of fructose, thus forming a dioxolane ring. The latter, as well as the furanosyl ring, has the envelope conformation.

Average values for C–C, C–O(hydroxyl) and C–O(ether) bond lengths are found to be 1.525(2), 1.419(2) and 1.422(2) Å, respectively.

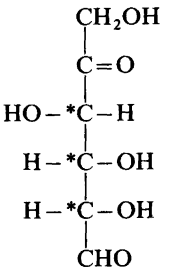
All the hydroxyl groups and one of the four ether oxygens participate in hydrogen bonding. Three hydrogen bonds are intramolecular.

The present compound (5KF) is the first dicarbonyl sugar to be isolated from natural material. It was discovered by Weidehagen and Bernsee[1] in 1960 in connection with studies on the metabolic product of acetic acid bacteria utilizing sucrose; the same product was later obtained directly from fructose using the same bacteria.

Weidehagen and Bernsee assumed that the fructose molecule had been dehydrogenated in the 6-position and transferred to 6-*aldo*-D-fructose which they report to have isolated in crystalline form. The specific rotation of the metabolic product was found to be almost equal to that of fructose itself, and this fact was used as an argument for the proposed structure; D(–)-fructose and 6-*aldo*-D-fructose have namely the same number of asymmetric carbons with almost equal configurations.



D(–)-fructose



6-*aldo*-D-fructose

Although the proposed structure later was found to be incorrect (see below), it is interesting to note that Weidehagen and Bernsee at that point had an IR-spectrum which they thought indicated the presence of a pyranose ring as well as a furanose ring in the molecule.

The title compound was also isolated (in 1960) by Terada *et al.*[2] in the course of their studies on the utilization of fructose by ketogenic bacteria. However, their conclusion was that fructose had been dehydrogenated in the 5-position.

The presence of two carbonyl groups was shown by the hydroxylamine hydrochloride titration method. Furthermore, reaction with periodate yielded twice as much glycolic and glyoxylic acid as did fructose itself and these results, together with the fact that the compound was stable to bromine

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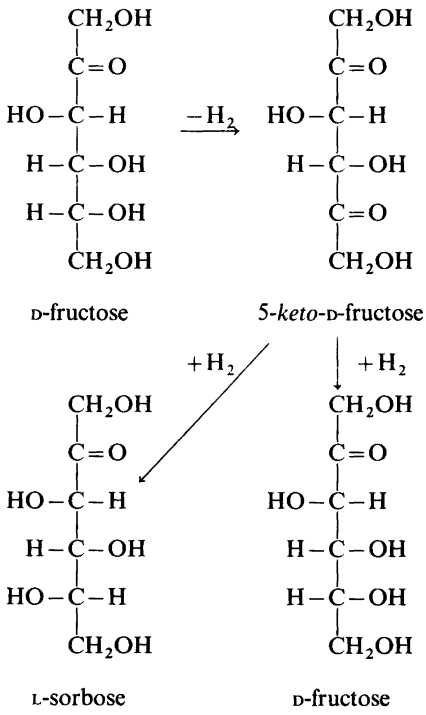
oxidation, suggested the presence of two $CH_2OH-CO-$ radicals in the molecule. In addition, stepwise reduction with borohydride gave ketoses with R_F values of fructose and sorbose, and not those of tagatose and psicose. One should note in this connection that hydrogenation of 5-*keto*-D-fructose at positions 2 or 5 gives the same products, namely D-fructose or L-sorbose.



Whiting and Coggings[3] found that bromine oxidation of L-sorbose, D-glucitol or D-fructose at 50 °C in the presence of freshly precipitated strontium carbonate yielded a dicarbonyl compound which they identified and named 2,5-D-*threo*-diketohexose.

The IR spectrum of this compound was identical to those of the metabolic products mentioned above, and mixed melting point determinations showed no depressions. It was therefore concluded that the three compounds were, in fact, identical.

Unequivocal proof that the mentioned dicarbonylhexose is indeed 5-*keto*-D-fructose has been given by Englard *et al.*[4] through enzymatic degradation of 5-tritiated D-fructose, obtained by stereospecific enzymatic reduction of the dicarbonylhexose.

A molecular structure proposal by Englard *et al.* is shown in Fig. 1. It is based on previously presented IR and NMR spectra which show that the molecule does not contain free carbonyl groups.[4] Furthermore, molecular weight determination by a freezing point depression method had indicated that the molecule was a monomer.[2] The proposed structure, *cf.* Fig. 1, is seen to contain two boat-shaped pyranose rings and one boat-shaped 1,4-dioxane ring, and may in addition have two-fold symmetry.

More recent IR, NMR and MS studies carried out by Jantzen[5] indicated that 5-*keto*-D-fructose probably occurs in a dimeric form, and a series of possible dimeric structures might be thought of.

We found a challenging task in this structural problem which we now have solved. Preliminary results from the structure study have been published,[6] and a more detailed description of the structure is given here.

STRUCTURE ANALYSIS

Crystals of the title compound (*5KF*) were generously supplied by Jantzen.[5] The crystals are colourless needles elongated in the *c*-axis direction.

Crystal data. $C_{12}H_{20}O_{12}$; F.W. 356.28.
Space group: $P2_12_12_1$; $a=12.094(3)$ Å, $b=21.700(6)$ Å, $c=5.329(3)$ Å;
$V=1398.54$ Å^3; $D_c=1.692$ g/cm^3, D_m(flotation)$=1.68$ g/cm^3;
$Z=4$; $\mu=1.66$ cm^{-1} (Mo$K\alpha$).

A crystal of approximate dimensions $0.20\times 0.15\times 0.40$ mm was used for the X-ray analysis which was carried out on a papertape controlled Siemens AED diffractometer using Mo$K\alpha$ radiation ($\lambda=0.71069$ Å).

The unit cell dimensions were determined from the 2θ values of 17 high order reflections in the range $26°<2\theta<42°$ measured at room temperature,

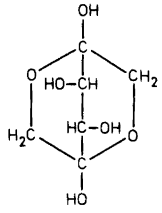


Fig. 1. The structure of the title compound as proposed by Englard *et al.*[4]

Table 1. Fractional atomic coordinates and temperature parameters U_{ij} (Å^2) for oxygen and carbon, and U (Å^2) for hydrogen. The expressions used are $\exp[-2\pi^2(h^2a^{*2}U_{11}+\cdots 2hka^*b^*U_{12}+\cdots)]$ and $\exp[-8\pi^2U(\sin^2\theta/\lambda^2)]$, respectively. Standard deviations in parentheses.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{23}	U_{13}
O(1)	.2269(3)	.08296(19)	.4186(8)	.0232(13)	.0333(15)	.0454(18)	−.0088(12)	.0109(15)	−.0074(16)
O(2)	.3278(3)	.10699(17)	.8721(7)	.0221(14)	.0370(15)	.0251(16)	.0047(13)	−.0047(13)	−.0015(13)
O(3)	.4475(3)	−.00254(15)	.9442(8)	.0349(16)	.0277(14)	.0357(17)	−.0150(13)	.0091(14)	−.0067(16)
O(4)	.65642(29)	.04563(17)	1.0215(8)	.0238(14)	.0331(15)	.0392(18)	−.0018(12)	.0184(14)	−.0019(13)
O(5)	.67359(26)	.17659(14)	.9496(7)	.0189(10)	.0255(21)	.0208(12)	−.0050(10)	−.0046(11)	.0048(12)
O(6)	.44250(26)	.13458(15)	.5382(7)	.0245(12)	.0239(12)	.0287(15)	−.0064(11)	.0069(13)	−.0042(13)
O(11)	.7032(3)	.29329(17)	.7346(8)	.0311(16)	.0256(14)	.0445(18)	.0061(12)	.0057(14)	.0029(15)
O(12)	.86477(25)	.16227(14)	.9522(7)	.0236(12)	.0278(12)	.0171(12)	.0075(11)	−.0032(11)	−.0046(11)
O(13)	.68143(25)	.11438(14)	.6059(6)	.0172(10)	.0214(11)	.0218(13)	−.0042(10)	−.0050(10)	.0024(11)
O(14)	.95350(29)	.17111(17)	.4332(8)	.0224(13)	.0434(15)	.0248(14)	−.0055(12)	.0039(14)	.0017(13)
O(15)	.8715(3)	.05518(16)	.8852(8)	.0252(15)	.0241(13)	.0359(17)	.0025(12)	.0063(12)	.0015(14)
O(16)	1.0931(3)	.16673(19)	.8339(8)	.0347(15)	.0372(16)	.0394(19)	−.0107(13)	.0079(16)	−.0127(15)
C(1)	.3253(3)	.05028(20)	.4823(9)	.0251(20)	.0268(19)	.0258(23)	−.0043(18)	−.0017(18)	.0013(19)
C(2)	.3935(3)	.08492(18)	.6743(8)	.0158(16)	.0222(17)	.0212(20)	−.0011(14)	.0009(17)	.0028(17)
C(3)	.4863(3)	.04471(18)	.7834(9)	.0212(18)	.0182(17)	.0245(21)	−.0017(14)	.0045(16)	.0045(17)
C(4)	.5659(3)	.08214(18)	.9441(9)	.0180(17)	.0236(17)	.0203(18)	.0025(15)	.0000(17)	.0017(18)
C(5)	.6097(3)	.13612(18)	.7939(8)	.0205(16)	.0178(16)	.0196(17)	−.0035(15)	−.0009(16)	.0017(15)
C(6)	.5133(3)	.17252(20)	.6845(10)	.0236(19)	.0204(18)	.0311(23)	−.0015(16)	.0027(18)	.0000(19)
C(11)	.7929(3)	.25966(18)	.8365(10)	.0201(19)	.0197(19)	.0197(19)	−.0028(15)	−.0011(18)	.0031(19)
C(12)	.7728(3)	.19047(17)	.8263(7)	.0158(16)	.0188(17)	.0188(17)	.0029(14)	.0007(14)	−.0003(16)
C(13)	.7654(3)	.15906(17)	.5705(8)	.0179(16)	.0168(16)	.0168(16)	.0007(14)	.0029(16)	−.0027(17)
C(14)	.8764(3)	.12755(19)	.5353(8)	.0192(16)	.0238(18)	.0238(18)	−.0034(15)	−.0014(15)	−.0007(16)
C(15)	.9108(3)	.11348(19)	.8058(8)	.0195(17)	.0208(17)	.0208(17)	.0025(15)	−.0031(16)	−.0002(16)
C(16)	1.0356(4)	.11001(21)	.8631(10)	.0246(19)	.0246(18)	.0246(18)	.0048(17)	−.0006(19)	−.0062(18)

Atom	x	y	z	U	Atom	x	y	z	U
H(O1)	.243(3)	.1155(17)	.352(7)	.027(12)	H(C3)	.533(3)	.0244(18)	.640(9)	.015(9)
H(O2)	.277(3)	.1186(17)	.799(7)	.036(14)	H(C4)	.530(4)	.0973(21)	1.090(11)	.022(10)
H(O3)	.406(3)	−.0206(15)	.889(7)	.013(11)	H(1C6)	.476(3)	.1931(18)	.831(8)	.009(8)
H(O4)	.638(3)	.0204(19)	1.110(10)	.028(12)	H(2C6)	.540(4)	.2037(21)	.565(10)	.028(10)
H(O11)	.660(4)	.2957(20)	.841(10)	.073(20)	H(1C11)	.814(3)	.2701(16)	1.018(8)	.010(9)
H(O14)	.921(6)	.1720(28)	.270(14)	.074(17)	H(2C11)	.857(4)	.2689(21)	.743(10)	.032(11)
H(O15)	.809(4)	.0536(19)	.903(10)	.047(15)	H(C13)	.746(3)	.1865(18)	.442(8)	.007(8)
H(O16)	1.081(3)	.1800(20)	.705(8)	.031(13)	H(C14)	.870(3)	.0873(13)	.424(6)	.010(6)
H(1C1)	.300(3)	.0079(16)	.555(8)	.006(8)	H(1C16)	1.062(4)	.0731(24)	.747(11)	.040(12)
H(2C1)	.373(4)	.0402(20)	.340(10)	.028(11)	H(2C16)	1.038(4)	.0995(21)	1.057(11)	.037(11)

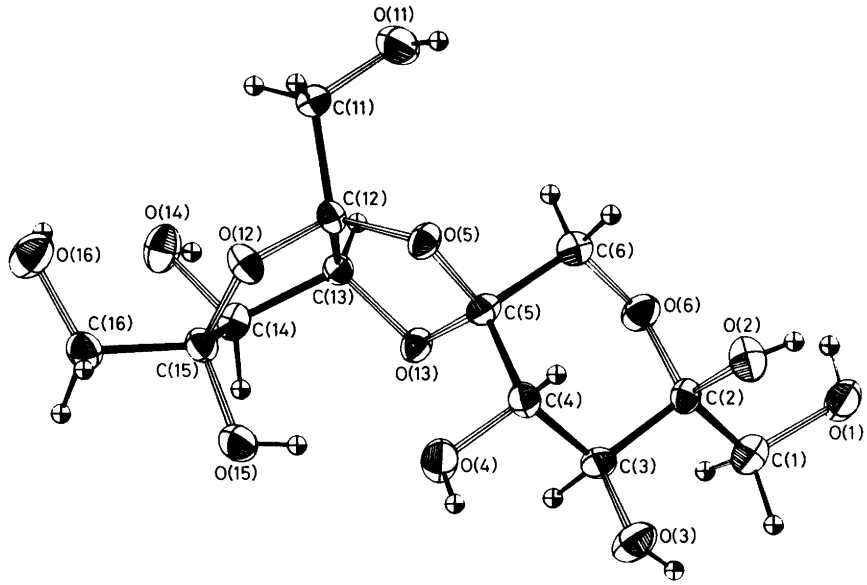


Fig. 2. ORTEP[12] drawing of the molecular structure of the title compound with numbering of atoms.

Table 2. Bond lengths and angles in the pyranose part of the title compound (I) as compared with those in α-L-sorbose (II) and β-D-fructose (III). Standard deviations in parentheses.

Atoms			Bond lengths (Å)			Bond angles (°)		
i	*j*	*k*	(I) *l*(*ij*)	(II) *l*(*ij*)	(III) *l*(*ij*)	(I) ∠(*ijk*)	(II) ∠(*ijk*)	(III) ∠(*ijk*)
O(1)	C(1)	C(2)	1.427(5)		1.422(4)	111.6(3)		110.4(2)
C(1)	C(2)	C(3)	1.514(5)	1.515(6)	1.520(4)	111.8(3)	111.9(3)	112.3(2)
C(1)	C(2)	O(2)				111.7(3)	111.1(3)	110.8(2)
C(1)	C(2)	O(6)				104.9(3)	106.9(3)	104.6(2)
O(2)	C(2)	O(6)	1.404(4)	1.415(5)	1.411(4)	111.0(3)	110.2(3)	111.2(2)
O(2)	C(2)	C(3)				108.8(3)	107.2(3)	106.8(2)
C(3)	C(2)	O(6)	1.536(5)	1.527(5)	1.540(4)	108.5(3)	109.5(3)	111.2(2)
C(2)	C(3)	C(4)				111.8(3)	112.1(3)	111.2(2)
C(2)	C(3)	O(3)				113.5(3)	109.9(3)	111.1(2)
O(3)	C(3)	C(4)	1.416(4)	1.429(5)	1.425(4)	104.8(3)	108.9(3)	108.8(2)
C(3)	C(4)	C(5)	1.523(5)	1.511(5)	1.518(4)	109.7(3)	110.3(3)	109.3(2)
C(3)	C(4)	O(4)				110.8(3)	108.9(3)	110.2(2)
O(4)	C(4)	C(5)	1.413(4)	1.427(5)	1.415(4)	108.5(3)	110.4(3)	109.6(2)
C(4)	C(5)	C(6)	1.514(5)	1.515(5)	1.524(4)	109.6(3)	110.7(3)	111.1(2)
C(4)	C(5)	O(5)				110.9(3)	108.5(3)	
O(13)	C(5)	C(4)	1.407(4)		1.423(4)	109.4(3)		110.8(2)
O(5)	C(5)	O(13)	1.434(4)	1.426(5)		106.5(3)		
O(5)	C(5)	C(6)				108.4(2)	109.2(3)	
C(6)	C(5)	O(13)	1.523(5)	1.513(6)	1.494(4)	111.9(3)		107.3(2)
O(6)	C(6)	C(5)	1.421(4)	1.440(5)	1.436(4)	111.8(3)	111.8(3)	111.0(2)
C(5)	O(5)	C(12)				108.8(3)		
C(2)	O(6)	C(6)	1.428(4)	1.420(5)	1.413(3)	114.1(3)	114.2(3)	114.6(2)

$t = 22$ °C. A least squares procedure gave the values quoted above.

The intensities of the reflections were measured at $t = 22$ °C by means of the five-value scan technique.[7] 1443 out of 2174 independent reflections in the range $0° < \theta < 27°$, and for which $I > 2\sigma(I)$, were accepted as observed.

Lp corrections were carried out in the usual way, but absorption corrections were considered unnecessary.

The structure was solved by direct methods (MULTAN)[8] and refined by full matrix least squares (see Ref. 9) to an R of 0.04. All the hydrogen atoms were found from a difference map.

Final atomic coordinates and temperature parameters are listed in Table 1. The final structure factor list is available on request.

The scattering factors for oxygen and carbon were taken from the *International Tables*.[10] For hydrogen, the scattering factor curve given by Stewart *et al.*[11] was used.

The calculations mentioned above were carried out on a UNIVAC 1110 computer at the University of Bergen. The programs, with few exceptions, originate from the Weizmann Institute of Science, Rehovoth, Israel.

Table 3. Bond lengths and angles in the furanose part of the title compound. Standard deviations in parentheses.

Atoms			Bonds (Å)	Angles (°)
i	*j*	*k*	*l*(*ij*)	∠(*ijk*)
O(11)	C(11)	C(12)	1.416(5)	111.9(3)
C(11)	C(12)	C(13)	1.523(5)	118.8(3)
C(11)	C(12)	O(12)		106.3(3)
O(5)	C(12)	C(11)	1.401(4)	109.4(3)
O(12)	C(12)	O(5)	1.436(4)	110.7(3)
O(12)	C(12)	C(13)		105.8(3)
C(13)	C(12)	O(5)	1.527(5)	105.8(3)
C(12)	C(13)	C(14)		105.1(3)
C(12)	C(13)	O(13)		103.2(3)
O(13)	C(13)	C(14)	1.417(4)	110.0(3)
C(13)	C(14)	C(15)	1.518(4)	102.3(3)
C(13)	C(14)	O(14)		108.9(3)
O(14)	C(14)	C(15)	1.435(4)	108.2(3)
C(14)	C(15)	C(16)	1.531(5)	117.5(3)
C(14)	C(15)	O(15)		111.6(3)
C(14)	C(15)	O(12)		105.1(3)
O(15)	C(15)	O(12)	1.416(4)	111.6(3)
O(15)	C(15)	C(16)		103.1(3)
C(16)	C(15)	O(12)	1.541(5)	108.0(3)
O(16)	C(16)	C(15)	1.422(5)	114.5(3)
C(15)	O(12)	C(12)	1.428(4)	111.3(3)
C(13)	O(13)	C(5)		107.9(2)

DISCUSSION

The molecular structure of *5KF* as found in the present study is shown in Fig. 2. Bond lengths and angles are given in Tables 2 and 3, and a stereoscopic view of the molecule is given in Fig. 3. It is realized that standard deviations in molecular dimensions may be overestimated when based on the standard deviations in positional parameters from the least squares refinement.[13]

Figs. 2 and 3 show that the molecule is a dimeric form of *5KF*. It contains a pyranose part, numbered

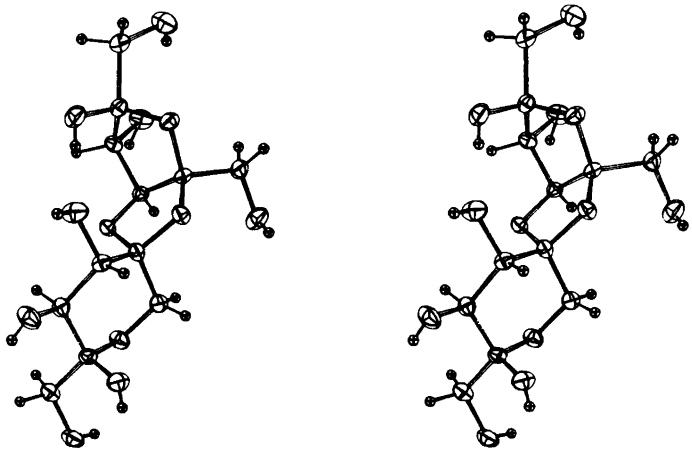


Fig. 3. A stereoscopic view of the molecule.

1–6, and a furanose part, numbered 11–16; a dioxolane part occurs as a result of dimerization.

The pyranose part occurs as α-L-sorbose with ring conformation *1C*, *cf.* Fig. 2. This structure differs from that of β-D-fructopyranose with respect to the orientation of the hydroxyl group in 5-position which is equatorial in the former and axial in the latter.

Hydrogenation of the title compound at position 5 may, as mentioned above, give either D-fructose or L-sorbose, and the same products may therefore result from dimerization at that position.

One notes from Fig. 2 that C(5) is also bonded to O(13) of the furanose part, and this means that the molecule contains a β-D-fructopyranose configuration as well as the α-L-sorbopyranose configuration.

The present study gives, in fact, the first complete structure description of α-L-sorbose; the crystal structure of α-L-sorbose itself is disordered, with unreasonable values for the length of the C(1)–O(1) bond.[14]

A comparison of bond lengths and angles in the pyranose part of the present molecule with those in α-L-sorbose[14] and β-D-fructose[15] are given in Table 2. The dimensions of the three pyranosyl rings are seen to agree closely. Some differences in C–C–O(hydroxyl) angles occur, but they are probably due to molecular packing in the crystal; C–C–O(hydroxyl) angles in β-D-fructose as found in the pure sugar and in its $CaCl_2$ and $CaBr_2$ complexes show similar differences.[15–17]

The equations for three planes through atoms of the pyranosyl ring have been calculated; *A* through C(2), O(6) and C(6), *B* through C(2), C(3), C(5) and C(6), and *C* through C(3), C(4) and C(5). The deviations of atoms from least squares plane *B* are less than 0.002 Å.

The angles between these planes have been calculated and are compared with equivalent angles for α-L-sorbose and β-D-fructose in Table 4; the latter values are based on the atomic coordinates given in Refs. 14 and 15, respectively.

Table 4. A comparison of pyranose ring configurations. See the text for further explanation.

	Angle (°)		
	A/B	*B/C*	*A/C*
Pyranose part of *5KF*	54.1	49.9	5.2
α-L-Sorbose	52.5	47.4	5.2
β-D-Fructose	51.3	48.6	2.8

One notes from Table 4 that the differences in angles are small and the best agreement occurs between α-L-sorbose and the pyranose part of *5KF*. It may therefore be concluded that the dimerization has not affected the configuration of the pyranose part to any noticeable degree.

The furanose part occurs as α-D-fructose, *cf.* Fig. 2 and Table 3. A structure study of fructose in this anomeric form has so far not been carried out.

The furanosyl ring of the present molecule has very nearly the envelope conformation E_3. This means that four of the ring atoms lie in a plane; the ring carbons are numbered 2–5 in this convention, and subscript 3 points to the atom which lies out of the plane;[18] this numbering is opposite to that on Fig. 2.

The deviations from the least aquares plane through O(12), C(12), C(13), and C(15) are −0.015, 0.023, −0.015, and 0.015 Å, respectively, reckoned in the same order; C(14) lies −0.496 Å from the plane.

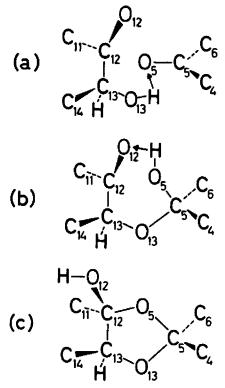
Corresponding plane deviations for the atoms of the furanosyl ring in the β-D-fructose moiety of sucrose are, 0.045, −0.028, 0.025, and −0.042 Å, respectively, for O(2), C(2), C(4), and C(5), with C(3) −0.542 Å out of the least squares plane.[19–21]

Looked upon in this way the furanosyl ring in sucrose might be described as being roughly E_3. It is realized, however, that the latter ring is referred to as being twisted (*T*), which means that C(3) and C(4) lie on opposite sides of the plane defined by C(2), O(2) and C(5);[18] the actual deviations are −0.372 and 0.208 Å, respectively. Similar furanosyl ring conformations occur in the β-D-fructose moieties of planteose and raffinose;[18,22] in the latter C(4) shows the greatest plane deviation. In 1-kestose where there are two β-D-fructose moieties the furanosyl ring is almost exactly E_3 in one of them and nearly so in the other,[23] and it might be added that in a carbohydrate derivative[24] where the furanosyl ring is condensed with other rings it is found to be very nearly planar.

This illustrates to which extent the furanosyl ring is able to adjust its structure in order to minimize intramolecular strain and/or crystal energy.

The dioxolane ring which comprises the atoms C(5), O(5), C(12), C(13) and O(13), is a result of the dimerization. A possible route for the actual dimerization reaction is given below, (a)–(c) (Scheme 1.).

The first step (a) is a hemiketal formation in which



Scheme 1.

the hydroxyl group on C(13) and the keto group on C(5) participate. One should note that due to the symmetry of *5KF*, hemiketal formations between O(13)–H and C(2)–O(2), O(14)–H and C(2)–O(2), and O(14)–H and C(5)–O(5) would be identical to that mentioned above. By this reaction the right hand side molecule, *cf.* Fig. 2, becomes L-sorbose, and the pyranosyl ring closure gives the α-anomer.

The second step (b) too is a hemiketal formation between the O(5)–H hydroxyl group and the C(12)–O(12) keto group. During this reaction the left hand side molecule remains as D-fructose, and subsequent furanosyl ring closure gives the α-anomer.

The deviations from the least squares plane through C(5), O(5), C(12), and C(13) are, −0.012, 0.019, −0.019, and 0.011 Å, respectively, reckoned in the same order; O(13) lies −.386 Å from the plane. This shows that the dioxolane ring of the present compound has an envelope conformation in agreement with the result from structure studies of other compounds containing dioxolane rings. One should note, however, that twist forms have also been reported.[24-26]

The dihedral angle of the O–C–C–O sequence in a free dioxolane ring, corresponding to minimum strain, is assumed to be about 25°.[26] In the present compound this angle is found to be 18.9°.

A comparison of the average values for C–C, C–O(hydroxyl) and C–O(ether) bond lengths in *5KF* dimer with those found in planteose dihydrate,[18] raffinose pentahydrate,[22] l-kestose[23] and sucrose (by X-ray as well as neutron diffrac-

Table 5. A comparison of average C–C, C–O(hydroxyl), and C–O(ether) bond lengths in planteose dihydrate, raffinose pentahydrate, 1-kestose and sucrose with those in *5KF* dimer.

	Ref.	Average bond lengths (Å) C–C	C–O(hydroxyl)	C–O(ether)
Planteose dihydrate	18	1.515(1)	1.422(1)	1.428(1)
Raffinose pentahydrate	22	1.524(2)	1.421(2)	1.429(2)
l-Kestose	23	1.524(1)	1.425(2)	1.428(2)
Sucrose (X-ray)	21	1.523(1)	1.423(1)	1.429(1)
Sucrose (neutron)	20	1.526(1)	1.418(1)	1.425(1)
5KF Dimer	This	1.525(2)	1.419(2)	1.422(2)

Table 6. The hydrogen bonding scheme in crystals of *5KF* dimer.

Atoms I	II	Position of atom II	Distances (Å) $O_I\cdots O_{II}$	$H_I\cdots O_{II}$
O(1)	→O(11)	$x-\frac{1}{2}, \frac{1}{2}-y, 1-z$	2.821(4)	2.09(4)
O(2)	→O(1)	x, y, z	2.757(4)	2.25(4)
O(3)	→O(1)	$\frac{1}{2}-x, -y, \frac{1}{2}+z$	2.741(4)	2.11(3)
O(4)	→O(15)	$3/2-x, -y, \frac{1}{2}+z$	2.942(4)	2.20(4)
O(11)	→O(16)	$x-\frac{1}{2}, \frac{1}{2}-y, 2-z$	2.795(4)	2.08(5)
O(14)	→O(12)	$x, y, z-1$	2.786(4)	1.84(5)
O(15)	→O(4)	x, y, z	2.708(4)	1.96(4)
O(16)	→O(14)	x, y, z	2.724(4)	2.12(3)

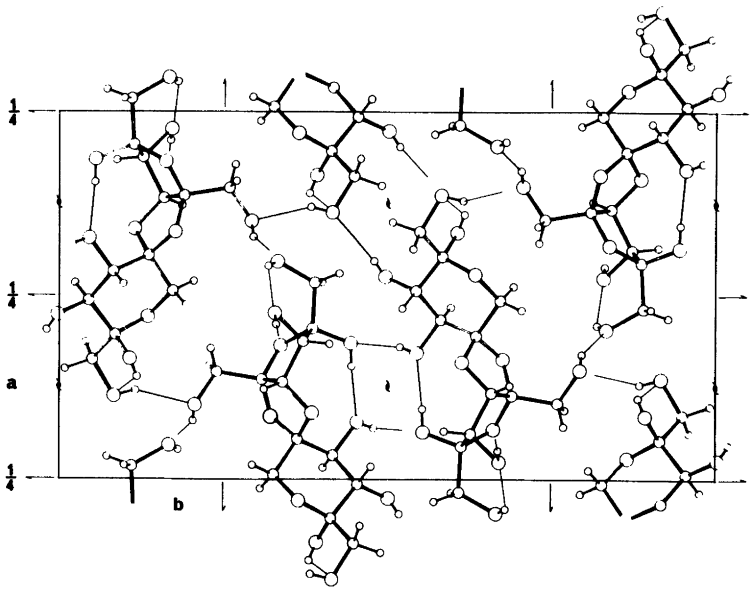


Fig. 4. A *c*-axis projection of the crystal structure showing O···H contacts corresponding to hydrogen bonding.

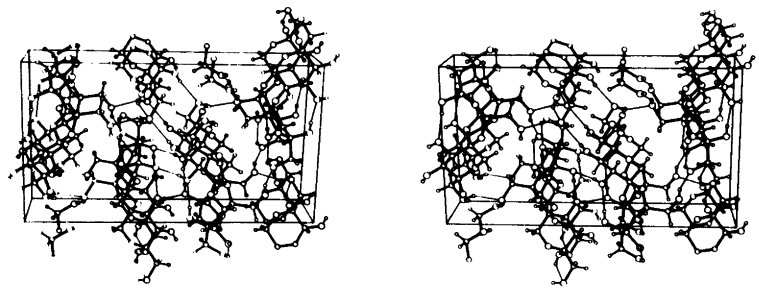


Fig. 5. A stereoview of the crystal structure.

tion),[20,21] is given in Table 5. One notes that the values for *5KF* dimer are in best agreement with the neutron diffraction values for sucrose.

The hydrogen bonding scheme in crystal of *5KF* dimer is given in Table 6 and shown in Fig. 4. Each hydroxyl oxygen donates one hydrogen bond, and apart from O(2) and O(3) each of them also accepts one; O(1) accepts two. There is one rather strong intramolecular hydrogen bond between O(15) and O(4), *cf.* Figs. 3 and 4.

A stereoscopic view of the crystal structure is given in Fig. 5. One notes that the hydrogen bonds form helixes around the respective screw axes.

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The Selectivity of Aryl Radicals in Reactions with Halide Ions. The Key Step in the $S_{RN}1$ Mechanism

MATS TILSET and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The relative rate constants for the reactions of *p*-nitrophenyl radical with DMF (i) and halide ions (ii) were determined over a 40 K temperature range.

$$Ar^{\cdot} + S-H(DMF) \xrightarrow{k_H} Ar-H + S^{\cdot} \quad (i)$$

$$Ar^{\cdot} + X^- \xrightarrow{k_X} Ar-X^- \quad (ii)$$

At 273 K the relative reactivity of halide ions in reaction (ii) was observed to be 143 (I^-), 9.4 (Br^-) and 1.0 (Cl^-). Iodide ion was found to be 1.8 times more reactive toward the radical than CBr_4 which is believed to react at a nearly diffusion-controlled rate. The most unexpected feature of the data is the high degree of selectivity for reactions taking place at rates near the diffusion-controlled limit. The activation parameter measurements emphasize the important role of the entropies of activation in determining the rates of very fast reactions. Relative activation energies were observed to be 0 (I^-), 4.2 (Br^-) and 5.1 (Cl^-) for the halide ion-radical reactions leading to expected relative rate constants of 1.2×10^4 (I^-), 5.25 (Br^-) and 1.0 (Cl^-) at 273 K when the differences in activation entropies are neglected. The observed relative reactivities were considerably different.

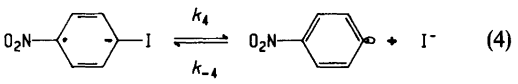
The $S_{RN}1$ mechanism is a chain process involving propagation steps (1)–(3).[1] The term, $S_{RN}1$, was applied to the mechanism by Kim and Bunnett who

$$R-X^{\cdot -} \rightarrow R^{\cdot} + X^- \quad (1)$$

$$R^{\cdot} + Y^- \rightarrow R-Y^{\cdot -} \quad (2)$$

$$R-Y^{\cdot -} + R-X \rightarrow R-Y + R-X^{\cdot -} \quad (3)$$

pointed out the analogy with the S_N1 mechanism, the intermediates of which have one less electron.[2] The reaction sequence was first proposed in 1966 for aliphatic systems[3,4] and a similar mechanism had been proposed to account for phenylation of some carbanions.[5] The first clearly defined report of reaction (2) where R is aryl appeared in 1969 when Lawless and Hawley[6] observed that reaction (4), in the forward direction is inhibited by iodide ion



(4)

implicating the occurrence of the back reaction. Reaction (4) has recently been studied in both the forward[7] and reverse[8] directions and the rate and equilibrium constants have been estimated.

In order to study reaction (2) under conditions where the overall kinetics are not complicated by reactions (1) and (3) it is necessary to fulfill a number of demands. A clean and convenient source of the radical (2) must be available. Reaction (2) must be essentially irreversible under the measurement conditions in order that kinetic measurements refer to the microscopic rate constant for the forward reaction. When the reactions are studied by competition techniques, a well-characterized competing reaction must be available for comparison.

As the source of aryl radicals for reactivity studies, we have chosen the reduction of aryldiazonium ions with iodide ion (5). This reaction and the subsequent

$$Ar-N_2^+ + I^- \rightarrow Ar^{\cdot} + N_2 + \tfrac{1}{2}I_2 \quad (5)$$

reaction of $Ar^{\cdot}$ with iodide ion is one of the early examples of the $S_{RN}1$ reaction in the aromatic series.[9]

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The reaction was applied to the generation of α-naphthyl radical and the estimation of the rate constant of the $S_{RN}1$ reaction with thiophenolate ion.[10] A preliminary report of the kinetics of the reactions of 4-nitrophenyl radical with halide ions has recently appeared.[8] We have recently studied the reactions of 4-nitrophenyl and α-naphthyl generated in reaction (5), with a series of bromomethanes in some detail.[11]

In order to study the relative reactivities of halide ions in reaction (2), the aryl group must be selected carefully. The cleavage of aryl halide anion radicals is a facile reaction.[6,12-17] When the aryl group is phenyl, 4-cyanophenyl, α-naphthyl, or 4-benzoylphenyl, cleavage of halide ion (6) is so rapid that it would not be possible to study the corresponding

$$Ar-X^{\cdot -} \rightarrow Ar^{\cdot} + X^{-} \quad (6)$$

aryl radical–halide ion reactions as essentially irreversible processes. In fact, halide ions are the most common leaving groups in the $S_{RN}1$ reaction.[1,2] The cleavage of 4-halonitrobenzene anion radicals takes place at rates low enough, *i.e.* less than 10 s^{-1},[6,7] that reaction conditions can be found where the reactions are essentially irreversible. For the case where the aryl radical is generated in reaction (5) and reacts with halide ion in (7), two very rapid reactions (8) and (9) are available to

$$Ar^{\cdot} + X^{-} \rightarrow Ar-X^{\cdot -} \quad (7)$$

$$Ar-X^{\cdot -} + ArN_2^{+} \rightarrow Ar-X + Ar^{\cdot} + N_2 \quad (8)$$

$$Ar-X^{\cdot -} + \tfrac{1}{2}I_2 \rightarrow Ar-X + I^{-} \quad (9)$$

immediately oxidize the anion radical. The oxidation potentials of the 4-halonitrobenzene anion radicals are more negative than 1 V *vs.* S.C.E. while reduction of ArN_2^{+} occur at about 0 V[18] and of I_2 at about +0.5 V. Thus, both (8) and (9) are expected to be diffusion controlled.

The reaction of the aryl radical with DMF (10) is a convenient competitive reaction to compare relative rates of (7) with.[8,10] Reaction (10) is rapid

$$Ar^{\cdot} + S-H\ (DMF) \rightarrow Ar-H + S^{\cdot} \quad (10)$$

enough so that significant amounts of Ar–H are formed in competition with Ar–X so that the product distribution can readily be determined by GLC.

In this paper we report the details of the reactions of 4-nitrophenyl radical with iodide, bromide and chloride in DMF in the presence of tetrabutylammonium fluoroborate.

RESULTS

The analysis procedure involved GLC determinations of the composition of the reaction mixtures obtained after generation of 4-nitrophenyl radical by reduction of the corresponding diazonium ion in DMF in the presence of the appropriate halide ion.[8,10,11] Relative rate constants for reactions (10) and (7) were calculated using eqn. (11) where the mol ratio of reactants (n_X/n_{DMF}) remained unchanged

$$k_H/k_X = (A_H/A_X)(r_H/r_X)(n_X/n_{DMF}) \quad (11)$$

Table 1. Competition kinetic data for the reactions of 4-nitrophenyl radical with halide ions and DMF.[a]

X^-	n_{DMF}/n_{X^-}[b]	T/°C	$10^3(k_H/k_{X^-})_{obs}$[c]	$10^3(k_H/k_{X^-})_{corr.}$[d]
I^-	545	0	0.853	0.865
I^-	545	22.0	1.40	1.35
I^-	545	39.9	1.82	1.86
Br^-	272	0	13.0	13.1
Br^-	272	21.9	11.7	11.6
Br^-	272	39.8	10.6	10.7
Cl^-	54.5	0	122	122
Cl^-	54.5	21.6	94.3	94.9
Cl^-	54.5	39.8	79.3	79.0

[a] The radicals were generated by the reduction of $ArN_2^{+}BF_4^{-}$ with I^- in DMF. [b] The mol ratio of DMF and the halide ion. [c] The observed rate constant ratio. [d] The rate constant ratio calculated from the Arrhenius correlation line.

Table 2. Differential activation parameters for the reactions of 4-nitrophenyl radicals with DMF and halide ions.[a]

X^-	ΔE_a[b]/(kcal/mol)	$\Delta\Delta S_{273}$[b]/(cal/K mol)
I^-	3.3	−2.0
Br^-	−0.87	−11.8
Cl^-	−1.84	−10.9

[a]Rate constant data from Table 1. [b]The activation parameters are those of DMF minus those for the halide ion reactions.

during the reaction and the ratio of GLC peak areas (A) was determined in each experiment. The sensitivity factors r were determined by measurements on mixtures of known composition. Measurements were carried out at three different temperatures over a 40 K range for the reactions of I^-, Br^- and Cl^- with 4-nitrophenyl radical. The data are summarized in Table 1.

Differential activation energies (ΔE_a) were obtained by Arrhenius correlations. The last column in Table 1 gives k_H/k_X obtained from the correlation. Only one value, that for I^- at 22 °C, differed from the experimental value by more than 2 %. In general, the relative rate constants obtained from the correlation were within 1 % of the experimental value. The ΔE_a values along with the differential entropies of activation ($\Delta\Delta S^{\neq}_{273}$) obtained using eqn. (12)

$$\Delta\Delta S^{\neq}_T = R \ln (k_H/k_X) + \Delta E_a/T \qquad (12)$$

are listed in Table 2. Rate constant ratios, ΔE_a and $\Delta\Delta S^{\neq}_{273}$ for the reactions of Br^-, Cl^- and CBr_4[11] taking the reaction of the radical with I^- as the reference are summarized in Table 3.

DISCUSSION

The relative rate constants reported previously[8] for the reactions of 4-nitrophenyl radical with halide ions at 22 °C in DMF were measured in the presence of alkali metal counter ions. The values, 1.00 (I^-), 0.11 (Br^-) and 0.005 (Cl^-) are in accord with those found in this study in which Bu_4N^+ was the only counter ion present; *i.e.* 1.00 (I^-), 0.12 (Br^-) and 0.014 (Cl^-) with the exception of the latter which is somewhat greater than reported previously.*

The data gathered in Table 3 for the reactions of 4-nitrophenyl radical at 0 °C are instructive. The activation energies for the reactions with Br^- and Cl^- are considerably greater than that for I^-, 4.2 and 5.1 kcal/mol, respectively. Neglecting the entropies of activation the ΔE_a data lead to the prediction of a much larger range of relative rate constants than was observed, 1.2×10^4 (I^-), 5.25 (Br^-) and 1.0 (Cl^-). The reason for this is that the entropies of activation are very much more positive when the nucleophile is either Br^- or Cl^-. Data are also included for the bromide abstraction from CBr_4[11] since this reaction is expected to be near the diffusion controlled limit in analogy to the corresponding reaction of phenyl radical.[19]

The data in Table 4 are gathered in order to give estimates of the absolute rate constants and activation parameters for the reactions of 4-nitrophenyl radical in DMF at 22 °C, a temperature common to all of the data reported. As a reference point, the most rapid reaction that has been studied, that with I_2 (13)[7] was assumed to have an encounter-con-

*The last entry in Table 1 of Ref. 8 is apparently in error. Under comparable conditions,[7] k_{rel} has been observed to be close to unity which is in accord with the results of this study and also gives a better comparison with the reaction in the presence of potassium ion.

Table 3. Relative rate constants and differential activation parameters for the reactions of 4-nitrophenyl radical with halide ions and carbon tetrabromide.[a]

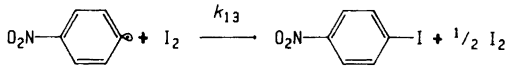
Reactant	k_I/k_X	$\Delta E_{a(I-X)}$/(kcal/mol)	$\Delta\Delta S_{273}$/(cal K^{-1} mol^{-1})
I^-	1.0	0	0
Br^-	15.2	4.2	9.8
Cl^-	143	5.1	8.9
CBr_4	1.8	1.3	4.0

[a]All reactions referred to those with iodide ion, data for CBr_4 are from Ref. 11.

Table 4. Estimated bimolecular rate constants and activation parameters for the reactions of 4-nitrophenyl radical.[a]

Reactant	Data from	$10^9k/M^{-1}s^{-1}$	E_a/kcal mol^{-1}	ΔS_{295}/cal K^{-1} mol^{-1}
I_2	*b,c*	5.8	2.4	−7.7
I^-	*d,c*	2.5	2.4	−9.4
CBr_4	*e*	1.4	3.7	−6.1
Br^-	*d*	0.16	6.6	−0.6
Cl^-	*d*	0.018	7.5	−1.9
DMF	*b,d*	0.0035	5.7	−11.2

[a] For reactions in DMF. The rate constant for the reaction between the radical and iodine was calculated from the Smoluchowski equation and all others are from values relative to that rate constant. [b] Ref. 7. [c] Ref. 21. [d] This work.



(13)

trolled rate constant. The magnitude of k_{13} was estimated from the Smoluchowski equation (14)[20] where r (the sum of the effective radii) was taken to be 5Å and the mutual diffusion coefficient D_{AB} twice

$$k_{en} = 4\pi\kappa r D_{AB} N/1000 \quad (14)$$

the average experimental value[21] for a number of aromatic compounds in DMF. This resulted in a value of 5.8×10^9 $M^{-1}s^{-1}$ for k_{13}. The activation energy was taken to be that for diffusion in DMF and here again an average experimental value,[21] 2.4 kcal/mol was assumed. The entropy of activation $\Delta S^{\ddagger}_{295}$ was then calculated using eqn. (15). Since the differential activation energy for reaction (13)

$$\Delta S^{\ddagger}_{T} = R(\ln k - \ln ek/h - \ln T) + E_a/T \quad (15)$$

has not been determined, E_a for the reaction with iodide ion, reverse reaction (4), was also taken to be 2.4 kcal/mol. The latter seems reasonable in view of the fact that the rate constants for the two reactions only differ by a factor of about 2. All other E_a values could then be assigned from the differential values determined in this study or in the case of CBr_4, previously.[11]

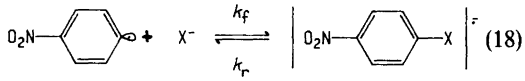
The origin of the large negative apparent entropies of the activation for diffusion-controlled reactions remains uncertain.[21] The significantly more positive $\Delta S^{\ddagger}$ for the reactions of Br^- and Cl^- most likely arises from the desolvation of the halide ions on going from reactants to transition state (16). The entropy change for reaction (17) can be predicted to be strongly positive. The standard entropies of

$$\underset{\text{(weakly solvated)}}{Ar^{\cdot}} + \underset{\text{(strongly solvated)}}{X^-} \rightarrow \underset{\text{(diffusely solvated)}}{|Ar^{\delta-}\text{---}X^{\delta-}|^{\neq}} \quad (16)$$

$$Ar^{\cdot} + X^- \underset{}{\overset{K_{17}}{\rightleftharpoons}} Ar\text{–}X^{\cdot -} \quad (17)$$

Br^- and Cl^- in DMF have been reported to be −43.3 and −46.7 cal/K mol.[22] The value of ΔS for the formation of nitrobenzene anion radical has been observed to be much less negative, −11.7 cal/K mol at 273 K.[23] Thus, assuming that ΔS for 4-nitrophenyl radical in DMF is the same as that of nitrobenzene, ΔS for reaction (17) can be estimated to be +32 and +35 cal/K mol when X^- is bromide and chloride, respectively.

We now have adequate data to use in the consideration of equilibrium (18) in some detail. The pertinent data are gathered in Table 5. The values



(18)

Table 5. Rate and equilibrium constants for the reactions of 4-nitrophenyl radical with halide ions in DMF.[a]

X	$k_f/M^{-1}s^{-1}$ [b]	k_r/s^{-1} [c]	K/M^{-1}
I	2.0×10^9	6.7	3×10^8
Br	1.3×10^8	1.4×10^{-3}	9×10^{10}
Cl	1.4×10^7	1×10^{-6}	1×10^{13}

[a] Refer to equilibrium (18). [b] Values from Table 4 referring to 295 K. [c] Values for I and Br from Ref. 7 and that for Cl estimated from the relative rates of cleavage of haloanthracene anion radicals ($k_{Br}/k_{Cl} = 2000$)[24] and of halobenzophenones ($k_{Br}/k_{Cl} = 1000$).[17] The rate constants refer to 298 K.

of the rate constants in the forward direction (k_f) are from Table 4. The rate constants referring to the reverse direction (k_r) have been reported in DMF for the 4-bromo and 4-iodonitrobenzene anion radicals.[7] 4-Chloronitrobenzene anion radical is stable in DMF. However, two series of reactions have recently been investigated which have bearing on the reaction in question. The relative rate constants for the cleavage of bromide and chloride (k_{Br}/k_{Cl}) were observed to be of the order of 2000 and 1000, during the cleavage of 9-haloanthracene[16] and 4-halobenzophenone[17] anion radicals, respectively. Thus, we can estimate k_r when X = Cl to be of the order of 10^{-6} s^{-1} at 298 K. Equilibrium constants estimated for reaction (18) are very large ranging from 3×10^8 to 10^{13}.

This same type of analysis is not possible for the reactions of the halide ions with radicals such as phenyl, naphthyl, *p*-cyanophenyl or *p*-methoxyphenyl since the corresponding anion radical cleavage reactions (6) are too rapid. The kinetics in such cases are then complicated by competition of (6) with (19) and the rate laws are expected to be of the form of (20), where ox and red refer to oxidation

$$Ar-X^{\cdot -} + ox \xrightarrow{k_{19}} Ar-X + red \qquad (19)$$

$$rate = k_{-6}\, k_{20} |Ar^{\cdot}|\,|X^-| / (k_6 + k_{20}|ox|) \qquad (20)$$

states of the oxidant in (19). On the other hand, if one goes to better nucleophiles, and poorer leaving groups, little is to be gained since the reactions will surely be at the diffusion-controlled limit. Thus, we regard the 4-nitrophenyl radical – halide ion reaction and the reverse cleavage as the optimum system to study in order to gain detailed information about the key step in the $S_{RN}1$ mechanism. The results reported here can be used to predict parameters for related systems.

EXPERIMENTAL

The experimental procedures and the analysis of the data were the same as those recently described in detail.[11]

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Unsymmetrical Anodic Coupling of Veratrole with Various Anisole Derivatives. Products and Mechanisms

ALVIN RONLÁN,[a] BJØRG AALSTAD[b] and VERNON D. PARKER[b]

[a] Organic Chemistry 2, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund, Sweden and [b] Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-703 Trondheim NTH, Norway

The coelectrolysis of veratrole (*1*) with anisole (*2a*), phenetole (*2b*), 3-methylanisole (*2c*), 2-methylanisole (*3*), 2,5-dimethylanisole (*4*), and 2,6-dimethylanisole (*6*) have been investigated in trifluoroacetic acid–dichloromethane solvent. With *2a–c* good yields of "tetrameric" 5-aryl substituted triphenylene derivatives (*10a*, *10b* or *11*) were obtained, probably by initial coupling of the veratrol cation radical ($1^{\cdot+}$) with the anisole derivative or its cation radical to an unsymmetrical dimer (*8* or *11*) followed by dimerisation and intramolecular cyclisation to the product. With *3*, *4* or *5* only "trimeric" products from coupling of two veratrole units with one of the anisole units (*15* or *26*) or from coupling of one veratrole unit with two of the anisole units (*17*, *22*, *25* or *27*) were observed.

The oxidative coupling of benzene derivatives to biphenyls is a reaction of great synthetic value. One of the first attempts to achieve this reaction by anodic oxidation of phenol methyl ethers was made by Erdtman.[1] In our laboratories the anodic coupling of hydroxy[2,3] and methoxy[4–8] substituted aromatic compounds has been studied extensively. In particular, symmetrical intermolecular[4] and symmetrical as well as unsymmetrical intramolecular[5–8] anodic couplings of methoxy aromatics have been studied in detail and the synthetic utility of these reactions has been clearly demonstrated. In this study anodic oxidation has been applied to achieve unsymmetrical coupling of veratrole (1,2-dimethoxybenzene, *1*) with various anisole (methoxybenzene) derivatives (*2–5*).

RESULTS

The products formed in the coelectrolysis experiments along with the yields are given in Table 1. The identifications of the products are based on spectroscopic data and previous findings[4–8] concerning the coupling mode of methoxy aromatics. In the coelectrolysis of veratrole with anisole (*2a*), phenetole (ethoxybenzene, *2b*), or 3-methylanisole (*2c*) mass spectral data and elemental analysis indicated that the major product was formed by coupling of two veratrole units with two units of the anisole derivative with formation of a total of four new carbon–carbon bonds. The structures that best fit these data as well as the NMR-spectral data (see Table 2), are *10a*, *10b* and *13*. In the NMR spectra of the compounds corresponding to structures *10a* and *10b* the hydrogens on carbons 11 and 12 (designated H-11 and H-12 in the following; the numbering of the triphenylene ring system is shown in formula *7*) should appear as AB quartets with a coupling constant of about 9 Hz. Hydrogens H-2′, H-3′, H-5′, and H-6′ should appear as AA′BB′ quartets with coupling constants of about 9 Hz. All the other aromatic protons should appear as singlets. All these features were actually observed (Table 2). The observed differences in chemical shifts between the various aromatic and methoxyl protons are also very well explained in terms of structures *10a* and *10b* (in particular the low field shift of H-12 and the high field shift of the C-8 methoxy group). The total absence of spin–spin couplings in the NMR spectrum of the main product from coelectrolysis of *1* and *2c* clearly shows that its structure cannot be *10c* as would have been

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Table 1. Products and yields from the anodic oxidation of veratrole (*1*) and anisole (*2a*), phenetole (*2b*), 3-methylanisole (*2c*), 2-methylanisole (*3*), 2,5-dimethylanisole (*4*) or 2,6-dimethylanisole (*5*); of 4-methylveratrole (*6*) and *2a*, and of 3,3',4,4'-tetramethoxybiphenyl (*14*) and *2a*.[a]

Coelectrolysis of:	Products containing the veratrole unit (yield based on veratrole in %[b])	Other products isolated
1+*2a*	*10a* (60)	4,4'-dimethoxybiphenyl
1+*2b*	*10b* (54)	4,4'-diethoxybiphenyl
1+*2c*	*13* (48)	4,4'-dimethoxy-2,2'-dimethylbiphenyl
1+*3*	*15* (31); *17* (14)	4,4'-dimethoxy-3,3'-dimethylbiphenyl
1+*4*	*28* (34)	
1+*4*[c]	*27* (16)	
1+*5*	*7* (55); *22* (2); *25* (3); *26* (36)	4,4'-dimethoxy-3,3',5,5'-tetramethylbiphenyl
6+*2a*	3,3'4,4'-tetramethoxy-6,6'dimethylbiphenyl (93)	
14+*2a*	*29* (90)	

[a] For details see experimental section. [b] In all electrolysis experiments *1*, *6* or *14* were recovered in an amount which together with the amount of these compounds found in the products well accounted for all starting material. [c] In this experiment, reduction with zinc dust was omitted from the work-up procedure (see Experimental).

expected. Instead, structure *13* is suggested for this product. The isomeric compound with a methoxy group at C-9 and a methyl group at C-11 fits the NMR data as well as structure *10c*. However, from our previous studies[4-8] we know that the position *para* to a methoxy group is the preferred coupling site. In fact, the *ortho-para* coupling observed in the coelectrolysis of *1* with *4* or *5* (in *5* the *ortho* position is activated by a methyl substituent) to our knowledge are the first examples of such a coupling. The very low field shift of H-12 in the NMR spectra of compounds *10a*–*b* and *13* indicates that the triphenylene ring system is twisted due to steric interference between the substituents. The lowest field shift for H-12 is observed in the NMR spectrum of *13* where the steric interference between the

Table 2. NMR data for the compounds *10a*, *10b* and *13*.[a]

Compound	Chemical shift (ppm)											
Aromatic protons												
	C-12	(C-1	C-4	C-6)[c]	C-9	C-10	C-11	C-2' C-6'		C-3' C-5'	(C-2'	C-4'	C-6'
10a	8.48[b]	7.92	7.74	7.68	7.30	–	7.34	7.35[d]		7.02[d]	–	–	–
10b	8.18[b]	7.70	7.54	7.48	7.25	–	7.18[b]	7.18[d]		6.89[d]	–	–	–
13	9.13	7.74	7.63	7.35	–	6.82[e]	–				7.02[e]	6.71	(2H)
Methoxyl substituent													
	(C-2	C-3	C-7	(C-3'))[c]		C-10	C-4'	C-11	C-8				
10a	4.13	4.12	4.06			3.86[e]	3.84[e]	–	3.31				
10b	4.12	4.11	4.04			–[f]	–[f]	–	3.29				
13	4.07	4.04(6H)		4.00		–	–	3.74	3.31				

[a] Unless otherwise stated the shifts refer to singlet absorption peaks. [b] Doublet, $J=9$ Hz. [c] The actual order of assignment of these shifts has not been established. [d] Two proton doublet, $J=8$ Hz. [e] These assignments could be reversed. [f] Ethoxy groups at these carbons in *10b*.

C-8 and the C-9 substituents would be very great in a planar configuration.

Mass spectral and analytical data indicate that one of the two main products from the coelectrolysis of veratrole and 2-methylanisole (*3*) is formed by coupling of two units of *1* and one unit of *3* through three new carbon – carbon bonds, whereas the other is formed by coupling of one unit of *1* with two units of *3* also through three new carbon – carbon bonds. On the basis of NMR data the former product is assigned structure *15*. For the latter product both structure *17* and the isomeric 2,7,10,11-tetramethoxy-3,6-dimethyltriphenylene fit the analytical and spectroscopic data. However, as no coupling between *1* and 4,4'-dimethoxy-3,3'-dimethylbiphenyl occurs in the coelectrolysis of these two compounds we can exclude the second possibility.

Two different products could be obtained in high yield from the coelectrolysis of veratrole and 2,5-dimethylanisole (*4*) depending on whether reduction with zinc dust was used in the work-up or not. Mass spectral data (molecular weight change of plus two mass units) indicated that zinc dust (in combination with TFA) affected hydrogenation of a double bond in the primary electrolysis product. NMR and mass spectral data indicated that the primary product was formed by coupling of one unit of veratrole with two units of *4* through three new carbon – carbon bonds and with, simultaneously, loss of one methyl group from one of the methoxy substituents. Furthermore, the NMR shifts of the methyl groups attached to a carbon atom (C-methyl groups) showed that the aromaticity of one of the coupled rings had been lost, and the IR spectrum showed that the coupling product contained a carbonyl function. On the basis of this evidence structure *27* was ascribed to the primary electrolysis product. The compound obtained by reduction with zinc dust was assigned structure *28* as one of the *C*-methyl groups of the dienone ring gave rise to a doublet ($J = 6.48$ Hz) at 1.45 ppm in the NMR spectrum. This would not have been the case if the other double bond in the dienone ring of *27* had been reduced. The hydrogenation with zinc dust was not stereospecific. The isomer with the two methyl groups of the cyclohexenone ring in the *cis*-configuration (arising from addition of hydrogen from the less hindered side of the dienone ring of *27*) as well as the isomer with these two methyl groups in the *trans* configuration are formed. The ratio between the two isomers was found to be 8:1. Since addition of hydrogen from the less hindered side should be favoured the *cis*-isomer is probably the dominating product. Three different coupling products were obtained from the coelectrolysis of veratrole and 2,6-dimethylanisol (*5*) which were assigned structures *22*, *25* and *26* on the basis of analytical and spectral data. In particular, it should be noted that all the aromatic protons in both compounds *22* and *26* have different NMR shifts and that no proton – proton spin couplings between them are observed. This excludes all structures with a symmetry plane.

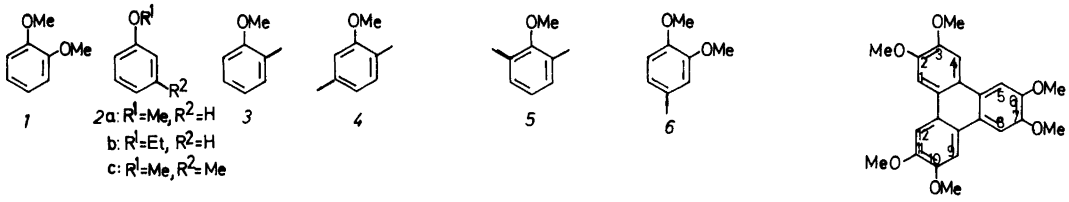
The coupling product from coelectrolysis of anisole and 3,3',4,4'-tetramethoxybiphenyl (*14*) must be *29*. Elemental analysis and the mass spectrum show that only one anisole unit couples with *14* and that two new carbon – carbon bonds are formed. Two structures are possible, *29* and 2,3,7,9,10-pentamethoxytriphenylene. However, the NMR spectrum of the product contains a two-proton AB quartet ($J = 9$Hz) and four one proton singlets which is only compatible with structure *29*.

DISCUSSION

The formation of all the products observed in the coelectrolysis experiments can be rationalised in terms of coupling between veratrole (*1*) or its cation radical ($1^{\cdot+}$), the anisole derivative (*An*) or the cation radical thereof ($An^{\cdot+}$), the cation radical of the veratrole dimer ($1_2^{\cdot+}$), and the cation radical ($1-An^{\cdot+}$) of the coupling product of veratrole and the anisole derivative, followed by intramolecular oxidative cyclisations, in certain cases rearrangement, and deprotonations to give the final products. Each coupling step can involve either two cation radicals or a cation radical and neutral substrate. The product studies do not allow us to distinguish between the two coupling reactions. Furthermore, the various cation radicals can be formed both by heterogeneous electron transfer at the anode and by homogeneous electron transfer in the solution from neutral molecules to a cation radical. The possible reaction pathways leading to the observed products are summarised in Scheme 1 and illustrated in more detail in Schemes 2 – 4.

Depending on the products formed the results of the coelectrolysis experiments can be divided into three groups:

Group one. In this group only tetramers of structure *10* or *13* are observed (coelectrolysis of *1* with *2a*, *2b* or *2c*). Veratrole is 0.3 – 0.4 V more easily

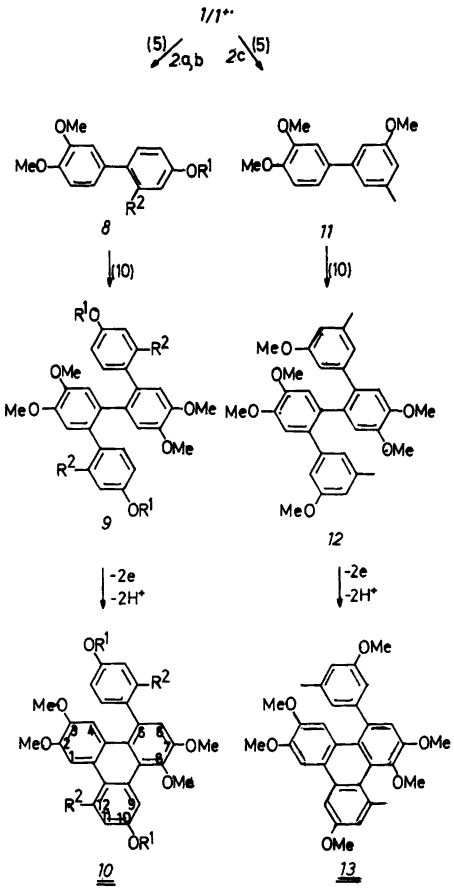


1 2a: R^1=Me, R^2=H; b: R^1=Et, R^2=H; c: R^1=Me, R^2=Me 3 4 5 6 7

1. $1-e \rightarrow 1^{\cdot+}$
2. $An-e \rightarrow An^{\cdot+}$
3. $2\mathit{1}/1^{\cdot+} \rightarrow\rightarrow 1_2^{\cdot+}$
4. $1_2^{\cdot+} + 1/1^{\cdot+} \rightarrow\rightarrow 1_3^{\cdot+} \rightarrow\rightarrow$ *7* (trimer)
5. $1/1^{\cdot+} + An/An^{\cdot+} \rightarrow\rightarrow 1\text{-}An^{\cdot+}$
6. $2An/An^{\cdot+} \rightarrow\rightarrow An_2^{\cdot+} \rightarrow\rightarrow An_2$ (biphenyl derivatives)
7. $1_2^{\cdot+} + An/An^{\cdot+}$
8. $1\text{-}An^{\cdot+} + 1/1^{\cdot+} \rightarrow\rightarrow 1_2\text{-}An^{\cdot+} \rightarrow\rightarrow$ *15, 26* (trimers)
9. $1\text{-}An^{\cdot+} + An/An^{\cdot+} \rightarrow\rightarrow 1\text{-}An_2^{\cdot+} \rightarrow\rightarrow$ *17, 22, 25* (trimers)
10. $2\mathit{1}\text{-}An^{\cdot+}$
11. $1_2\text{-}An^{\cdot+} + An/An^{\cdot+} \rightarrow\rightarrow 1_2\text{-}An_2^{\cdot+} \rightarrow\rightarrow$ *10, 13* (tetramers)
12. $1\text{-}An_2^{\cdot+} + 1/1^{\cdot+}$

Scheme 1. Summary of possible primary reactions for formation of the products observed in the coelectrolysis experiments. *1* is veratrole and $1^{\cdot+}$ is the cation radical of veratrol. *An* is the anisol derivative and $An^{\cdot+}$ its cation radical. $1_2^{\cdot+}$ is the cation radical of the veratrol dimer (*14* = 1_2), 1-$An^{\cdot+}$ is the cation radical of the coupling product between *1* and *An*, *etc.* One arrow corresponds to a simple electron transfer reaction. Two arrows indicate a multistep reaction involving deprotonations, further electron transfers and formation of carbon–carbon bonds (both inter- and intramolecular bonds). The intermediates 1_2, *1-An*, 1_2-*An* and 1_2-An_2 are shown in their cation radical form as they are all considerably more easily oxidised than either *1* or An.

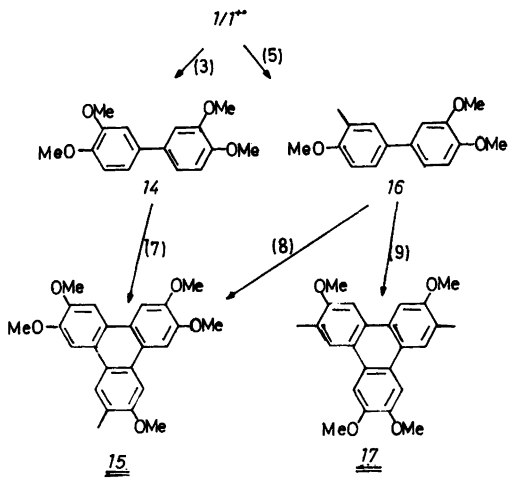
oxidized than are any of the three anisole derivaties *2a–c*.[9] That is, veratrole can be selectively oxidized to its cation radical ($1^{\cdot+}$) in these coelectrolyses. $1^{\cdot+}$ then reacts with *2a*, *2b* or *2c* to give an unsymmetrical dimer (*8* or *9* in Scheme 2; eqns. 1, 2 and 5 in Scheme 1). The absence of *7* or trimers containing two veratrol units (like *15*) clearly indicates that $1^{\cdot+}$ does not dimerise or attack *1* electrophilically under these circumstances. Surprisingly, coelectrolysis of *1* and *2c* does not give the expected tetramer *10c* where the initial bond formation between *1* and *2c* (eqn. 5 in Scheme 1) has occurred between positions *para* to methoxy groups. (Instead the dimer *11* where the new bond is to the position *meta* to the methoxy group of *2c* is formed.) However, we believe that the initial attack of $1^{\cdot+}$ is on the 4-position of *2c* and that the resulting cation radical rearranges faster than it undergoes deprotonation due to the steric interference of the methyl group. Normally deprotonation is a much faster reaction. The final product *13* is formed by dimerisation of *11* to *12* followed by intramolecular cyclisation. Similarily, we believe that *10a* and *10b* are formed *via* dimerisation of the unsymmetrical biphenyls *8a* and *8b*. As shown in Scheme 1 (eqns. 11 and 12) the tetramers *10* and *13* could also be formed by a stepwise coupling of veratrole and anisole units. However, we can exclude these mechanisms since coelectrolysis of *14* and anisole yields only the trimer *29*, and since the results of the coelectrolysis of *1* with *3*, *4* or *5* clearly indicate that the favoured reaction of a *1-*An_2 intermediate is intramolecular cyclisation. No evidence of a reaction such as 12 (Scheme 1) was in fast obtained, although it is evident from the products that intermediates like *1-*An_2 do occur in the coelectrolyses. It is noteworthy that only one of the two possible intramolecular cyclisation reactions of the intermediates *9* and *12* actually occur. The second intramolecular cyclisation would have resulted in a dibenzo[*fg*,*op*]naphthacene derivative. At least compound *13* with an unsubstituted position *para* to a methoxy group in the 5-arylsubstituent should be able to undergo the second cyclisation. The reason for this is probably steric interference between the substituents which would impose a twisted configuration on the dibenzo-



Scheme 2. a, $R^1 = Me$, $R^2 = H$; b, $R^1 = Et$, $R^2 = H$; c, $R^1 = R^2 = Me$. Coelectrolyses of veratrole (*1*) with anisole (*2a*, $R^1 = Me$; $R^2 = H$), or phenetole (*2b*, $R^1 = Et$; $R^2 = H$), or 3-methylanisole (*2c*, $R^1 = R^2 = Me$). Possible mechanisms for the formation of the products. The underlined structures correspond to the compounds actually isolated. The numbers in parentheses refer to the corresponding reaction types in Scheme 1.

[*fg,op*]naphthacene derivative. This diminishes the conjugation between the rings and thereby the aromatic stabilisation energy. Hence, the cation radicals of *10* and *13* are more stable in the "open" than in the cyclised form (in the latter, bonding between C-4 and C-2′ has occurred).

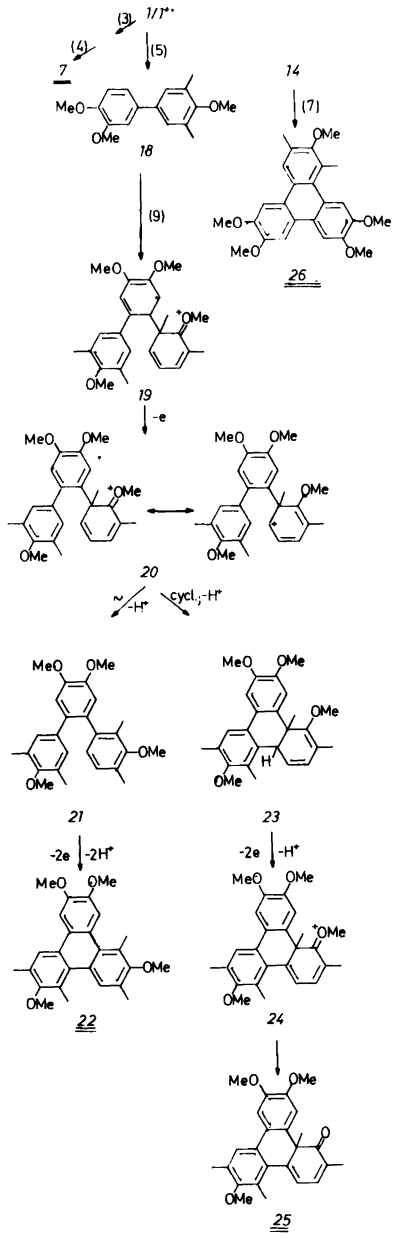
Group two. In this group only trimers of structures *15*, *17* and/or *27* are observed. Coelectrolysis of *1* with *3* or *4*. The oxidation potentials of veratrole and *3* and *4* are quite similar.[9] Therefore, selective



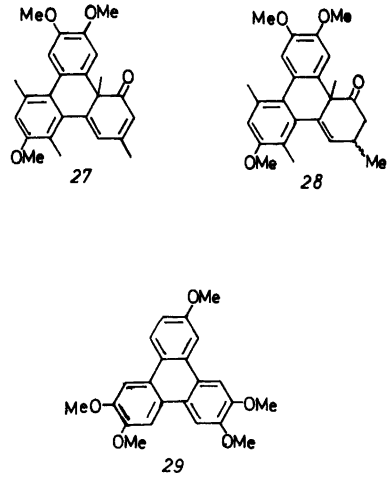
Scheme 3. Coelectrolysis of veratrole (*1*) and 2-methylanisole (*3*). Possible mechanisms for the formation of the products. The underlined structures correspond to products actually isolated. The numbers in parentheses refer to the corresponding reactions in Scheme 1.

oxidation of *1* to $1^{\cdot+}$ is not expected in these coelectrolyses. Instead, the cation radicals of both starting materials are probably formed simultaneously at the electrode and by charge transfer in electron exchange equilibria in the solution. Still, the product studies clearly indicate (products *17*, *22*, *25* and *27*) that unsymmetrical biphenyls (*16* or *18* in Schemes 3 and 4) are intermediates in these coelectrolysis as well. However, in no case was dimerisation of these biphenyl derivatives observed. Only products formed by coupling of three units of starting materials were observed. Possible mechanisms for the formation of the two coupling products, *15* and *17*, isolated from the coelectrolysis of *1* and *3* are shown in Scheme 3 and involve combinations of reactions 1–3 and 5–9 in Scheme 1. The product *17* can only be formed by coupling of *16* or $16^{\cdot+}$ with *3* or $3^{\cdot+}$ (reactions 5 and 9 in Scheme 1), whereas the other product, *15*, can arise both from *16*/$16^{\cdot+}$ by coupling with *1*/$1^{\cdot+}$ (reactions 5 and 8 in Scheme 1) or from *14*/$14^{\cdot+}$ by coupling with *3*/$3^{\cdot+}$ (reactions 3 and 7 in Scheme 1).

In the coelectrolysis of veratrole and *4* only one coupling product containing the veratrole unit was observed (*27* or its hydrogenation product *28*). *27* most likely is formed by the same mechanism as *25* (cheme 4). It should be noted that the sole



Scheme 4. Coelectrolysis of veratrole (*1*) and 2,6-dimethylanisole (*5*). Possible mechanisms for the formation of the products. The underlined structures correspond to products actually isolated. The numbers in parentheses refer to the corresponding reactions in Scheme 1.



reaction of the veratrole cation radical in this case is coupling with $4/4^{\cdot+}$ in the unsubstituted 4-position to form 3,4,4′-trimethoxy-2′,5′-dimethylbiphenyl (or the cation radical thereof). This species couples further with $4/4^{\cdot+}$, now in the methyl substituted 2-position to give – after further oxidation, cyclisation, and demethylation – the final product *27*.

Group three. In this group both trimers of the kind observed in group two and hexamethoxytriphenylene (*7*) are observed; coelectrolysis of veratrole and *5*. As in group two, no selective oxidation of *1* is expected to occur. In fact, this coelectrolysis is the least selective of them all and four coupling products containing the veratrole unit, *7*, *22*, *25* and *26* were isolated. Possible mechanisms for their formation are shown in Scheme 4. It should be noted that 55 % of the veratrole is oxidised to *7* which was not observed in any of the other coelectrolyses.

The coelectrolysis of 4-methylveratrole (*6*) and anisole (see Table 1) gave no unsymmetrical coupling product. Only the dimer of *6* (3,3′,4,4′-tetramethoxy-2,2′-dimethylbiphenyl) was isolated. Apparently, introduction of a 4-substituent in veratrole (as in the intermediates *8* and *9* in Scheme 2) makes the dimerisation reaction very favourable.

CONCLUSIONS

The mechanistic hypothesis that we have made (Schemes 1 – 4) are only meant to signify possible routes by which the observed products may be formed. The differences in oxidation potentials of

all the substrates are not so great that any of the possible electron transfer reactions (*a*) can be excluded. Therefore, all reaction pathways could be controlled by the relative rate constants together

$$Ar_1^{\cdot+} + Ar_2 \xrightleftharpoons{K_{exchange}} Ar_1 + Ar_2^{\cdot+} \qquad (a)$$

with the pertinent concentrations of intermediates and no simple relationship seems to exist between relative oxidation potentials and nucleophilicities of the starting materials and the products obtained. However, it is clear from the product studies that a degree of selectivity is observed and that the mixed coupling provide a useful route to the compound which cannot be obtained by more conventional synthetic procedures.

EXPERIMENTAL

General procedures and apparatus used for voltammetry and coulometry and the purification of solvents were conventional and have been described in previous papers.[2-8] The NMR spectra were recorded in deuteriochloroform with Me_4Si as internal reference. IR spectra were recorded in KBr tablets. The starting materials (*1–5*) were commercial products purified further by distillation.

Preparative electrolyses. General. The veratrole derivative (*1* or *6*, 20 mmol) and phenethol (*1b*) or the anisole derivative (*1a*, *1c*, *3*, *4* or *5*), 60 mmol, were dissolved in TFA–DCM (1:3, 200 ml) containing n-Bu_4NBF_4 (4 g) and transferred to the anode chamber of a closed two-compartment electrolysis cell divided by a glass frit (G-4). The catholyte was pure TFA (50 ml) containing *n*Bu_4NBF_4 (1 g). The anode was a platinum cylinder (150 cm^2) and the cathode a coil of platinum wire. The electrolyses were carried out at constant current (500 mA) with efficient magnetic stirring. The temperature was kept at 12 °C by external cooling and nitrogen was bubbled through the solution during the entire electrolysis. When 6300 Coulomb (corresponding to 3 F per mol of the veratrole derivative) of current had been passed through the cell, zinc dust (9 g) was added and the stirring was continued until the emerald green cation radical colour of the electrolysis mixture had disappeared. Water (150 ml) was added and the organic phase was separated and washed with water (150 ml), saturated bicarbonate solution (2 × 100 ml), and water (150 ml), and then evaporated to dryness. The residue was dissolved in methanol (75 ml) and left at 0 °C for 24 h. If any precipitate had formed it was collected by filtration and analysed. The filtrate was evaporated to dryness and the residue was chromatographed on silica gel (Merck 60, 200 g) with a toluene–diethyl ether gradient. The various fractions were analysed by TLC, NMR and mass spectroscopy. The results are summarised in Table 1.

Coelectrolysis of veratrole (1) and anisole (2a). Treatment of the crude electrolysis product with methanol gave yellow crystals (2.90 g) which were recrystallised from DMC-methanol (3:1) to give greyish crystals (2.70 g), m.p. 201 – 203 °C; identified as 2,3,7,8,10-pentamethoxy-5-(4-methoxyphenyl)-triphenylene (*10a*) by their NMR (Table 2) (360 MHz) δ 8.48 (1H,d(J = 9Hz)), 7.92 (1H,s), 7.74 (1H,s) 7.68 (1H,s), 7.35 (2H,d(J = 8Hz)), 7.34 (1H,d(J = 9Hz)), 7.30 (1H,s), 7.02 (2H,d(J = 8Hz)), 4.13 (3H,s), 4.12 (3H,s), 4.06 (3H,s), 3.84 (3H,s), and 3.31 (3H,s) ppm. Anal. calc. for $C_{30}H_{28}O_6$: C 74.4, H 5.8. Found: C 74.3, H 5.8. M^+ 484 *m/e*.

Chromatography of the methanol soluble part of the electrolysis product gave starting materials, 4,4'-dimethoxybiphenyl, and a trace of *10a*.

Coelectrolysis of veratrole and phenetole (2b). Treatment of the crude electrolysis product with methanol gave brownish crystals (1.07g) which were recrystallised from chloroform–ethanol (3:2) to give greyish crystals m.p. 168 – 170 °C, identified as 10-ethoxy-5-(4-ethoxyphenyl)-2,3,7,8-tetramethoxy-triphenylene (*10b*) by their M^+ 512 *m/e*; NMR (360 MHz) δ 8.18 (1H,d(*J* = 9Hz)), 7.70 (1H,s), 7.54 (1H,s), 7.48 (1H,s), 7.25 (1H,s), 7.18 (2H, d(*J* = 8Hz)), 7.04 (2H,d(*J* = 9Hz)), 6.89 (2H,d(*J* = 8Hz)), 4.12 (3H,s), 4.11 (3H,s), 4.04 (3H,s), 3.95 (2H,q), 3.29 (3H,s), 1.43 (3H,t), and 1.23 (3H,t) ppm; anal. calc. for $C_{32}H_{32}O_6$: C 75.0, H 6.3. Found: C 75.2, H 6.3. Chromatography of the methanol soluble part of the electrolysis product gave starting materials, 4,4'-diethoxybiphenyl (1.62g), and *10b* (1.70g).

Coelectrolysis of veratrole and 3-methylanisole (2c). No precipitate was obtained on treatment of the crude electrolysis product with methanol. Chromatography gave starting materials, 4,4'-dimethoxy-2,2'-dimethylbiphenyl, and 5-(3-methoxy-5-methylphenyl)-2,3,7,8,9-pentamethoxy-11-methyltriphenylene (*13*), 2.50g, m.p. 108 – 110 °C (ethanol–toluene = 2:1), identified by its M^+ 512 *m/e*; NMR δ (360 MHz) 9.13 (1H,s), 7.74 (1H,s), 7.63 (1H,s), 7.35 (1H,s), 7.02 (1H,s), 6.82 (1H,s), 6.71 (2H,s), 4.07 (3H,s), 4.04 (6H,s), 4.00 (3H,s), 3.74 (3H,s), 3.31 (3H,s), 2.36 (3H,s), and 2.17 (3H,s) ppm; anal. calc. for: $C_{32}H_{32}O_6$: C 75.0, H 6.3. Found: C 75.2, H 6.2.

Coelectrolysis of veratrole and 2-methylanisole (3). Treatment of the crude electrolysis product with methanol gave crystals, 2.86 g, which TLC indicated to consist of two different compounds. By preparative TLC these two compounds were isolated in pure form and identified as 3,6,10,11-tetramethoxy-2,7-dimethyltriphenylene (*17*), m.p. 260 – 262 °C) by M^+ 376 *m/e*; NMR (360 MHz) δ 7.70 (2H,s), 7.34

(2H,s), 7.25 (2H,s), 3.93 (6H,s), 3.81 (6H,s), and 2.33 (6H,s) ppm; and 2,3,6,7,10-pentamethoxy-11-methyltriphenylene (*15*, m.p. 240–243 °C) by the M^+ 392 *m/e*; NMR (360 MHz) δ 7.65 (2H,s), 7.29 (2H,s), 7.21 (2H,s), 3.93 (6H,s), 3.81 (3H,s), 3.80 (6H,s), and 2.33 (3H,s) ppm. The molar ratio between *15* and *17* was 1:3 (NMR). Chromatography of the methanol soluble part of the electrolysis product gave starting materials and 4,4'-dimethoxy-3,3'-dimethylbiphenyl, 0.21 g.

Coelectrolysis of veratrole and 2,5-dimethylanisole (4). Treatment of the crude electrolysis product with methanol gave yellow crystals, 2.30 g, m.p. (ethanol – toluene = 1:2) 306–208 °C, identified as a 1:4 mixture of the two isomeric (*cis* and *trans* methyl groups in the hydrogenated dienone ring) dihydro compounds *28* by their M^+ 392 *m/e*; NMR (360 MHz) δ (*cis* isomer) 7.420 (1H,s), 6.947 (1H,s), 6.682 (1H, s) 6.226 (1H,s), 3.985 (3H,s), 3.898 (3H,s), 3.856 (3H,s), 3.0 (2H,m), 2.709 (3H,s), 2.200 (3H,s), 1.7 (1H,m), 1.432 (3H,s), and doublet (J = 6.48 Hz) 1.154 and 1.136 ppm; and (*trans* isomer) δ 7.352 (1H, s), 6.947 (1H,s), 6.716 (1H,s), 6.323 (1H,s), 3.985 (3H,s), 3.898 (3H,s), 3.856 (3H,s), 3.0 (2H,m), 2.709 (3H,s), 2.296 (3H,s), 1.71 (1H,m), 1.473 (3H,s), and doublet (J = 6.48 Hz) 1.173 and 1.155 ppm; IR ν 1675 cm^{-1} (carbonyl); anal. calc. for $C_{25}H_{28}O_4$: C 76.5, H 7.1. Found: C 76.6, H 7.1. Chromatography of the methanol soluble part of the electrolysis product gave starting materials and *28*, 0.65 g.

When the zinc dust reduction at the end of the electrolysis was omitted, treatment of the crude product with methanol gave yellow crystals, 0.40 g, m.p. (EtOH) 138–140 °C, identified as the dienone *27* by M^+ 390 *m/e*; NMR (60 MHz): δ 7.37 (1H,s), 6.70 (1H,s), 6.52 (1H,s), 6.50 (1H,bs), 6.27 (1H,bs), 3.98 (3H,s), 2.72 (3H,s), 1.93 (3H,s), and 1.35 (3H,s) ppm; IR ν 1670 cm^{-1} (carbonyl); anal calc. for $C_{25}H_{26}O_4$: C 76.9, H 6.7. Found: C 76.6, H 6.5. Chromatography of the methanol soluble part of the electrolysis product gave a further 0.85 g of *27*.

Coelectrolysis of veratrole and 2,6-dimethylanisole (5). Treatment of the crude electrolysis product with methanol gave greyish crystals, 1.50 g, identified as *7* by comparison with an authentic sample.[8] Chromatography of the methanol soluble part gave starting materials; 4,4'-dimethoxy-3,3',5,5'-tetramethylbiphenyl, 0.59 g; 2,3,6,10-tetramethoxy-5,7,-9,11-tetramethyltriphenylene (*22*), 0.16 g, m.p. 158–160 °C, identified by its M^+ 404 *m/e*, NMR (60 MHz) δ 8.15 (1H,s), 8.00 (1H,s), 7.87 (1H,s), 7.78 (1H,s), 4.03 (3H,s), 3.99 (3H,s), 3.85 (6H,s), 2.88 (3H,s), 2.85 (3H,s), and 2.48 (6H,s) ppm, and anal. calc. for $C_{26}H_{28}O_4$: C 77.2, H 6.9. Found: C 77.5, H 6.8; the dienone *25*, 0.20 g, m.p. 151–153 °C, identified by its M^+ 390 *m/e*, NMR (60 MHz) δ 7.38 (1H,s), 6.73 (1H,s), 6.53 (1H,s), 6.48 (1H,d(J = 2Hz)), 6.27 (1H,d(J = 2Hz)), 3.98 (3H,s), 3.83 (6H,s), 2.72 (3H,s), 1.95 (6H,s), and 1.35 (3H,s) ppm, IR ν 1670 cm^{-1} (carbonyl), and anal. calc. for $C_{25}H_{26}O_4$: C 76.9, H 6.7. Found C t 6.7, H 6.8; and finally 2,3,6,7,10-pentamethoxy-9,11-dimethyltriphenylene (*26*), 1.46 g, m.p. 180–182 °C, identified by its M^+ 406 *m/e*, NMR (60 MHz) δ 7.95 (1H,s), 7.83 (1H,s), 7.63 (1H,s), 7.50 (1H,s), 7.45 (1H,s), 4.03 (12H,s), 3.82 (3H,s), 2.83 (3H,s), and 2.48 (3H,s) ppm, and anal. calc. for $C_{25}H_{26}O_5$: C 73.9, H 6.4. Found: C 74.0, H 6.3. If the electrolysis was carried out in pure TFA no dienone (*25*) could be isolated. However, a higher yield (0.45 g) of *22* was obtained.

Coelectrolysis of 4-methylveratrole (6) and anisole. No precipitate was formed on treatment of the crude electrolysis product with methanol. Chromatography gave apart from starting materials 3,3',4,4'-tetramethoxy-6,6'-dimethylbiphenyl as the only product.

Coelectrolysis of 3,3',4,4'-tetramethoxybiphenyl (14) and anisole. 14, 10 mmol, and anisole, 60 mmol, were coelectrolysed according to the general procedure above. However, only 4200 Coulomb of current corresponding to 2 F/mol of *14* was passed through the cell. Treatment of the electrolysis product with methanol gave yellow crystals, 3.40 g, m.p. (EtOH – $CHCl_3$ = 2:1) 185–187 °C, identified as 2,3,6,7,10-pentamethoxytriphenylene (*29*) by its M^+ 378 *m/e*; NMR (360 MHz) δ 8.010 (H-12, d(J = 9.17 Hz)), 7.982 (H-9, d(J = 2.45 Hz)), 7.460, 7.332, 7.150, 7.143 (one proton singlets assigned to H-1, H-4, H-5, and H-8; the actual order of the assignment was not determined), 7.191 (H-11, quartet (J_{H-12} = 9.17 Hz, J_{H-9} = 2.45 Hz)), 3.958 (3H,s), 3.924 (3H,s), 3.921 (6H,s), and 3.907 (3H,s) ppm. Anal. calc. for $C_{23}H_{22}O_5$: 84.1, H 5.8. Found: C 84.2, H 5.8. Chromatography of the methanol soluble part gave starting materials and a small amount of 4,4'-dimethoxybiphenyl (0.20 g).

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^{13}C NMR Study and X-Ray Analysis of *N*-(*N*-Acetyl-L-aspartyl)-4-aminobutyric Acid (Ac–Asp–γAbu), $C_{10}H_{16}N_2O_6$. A Comparison of the NMR Results with Semi-empirical Calculations

LARS K. HANSEN, ERIK A. HAGEN, THRINA LOENNECHEN and ARNE J. AASEN

Department of Chemistry, University of Tromsø, P.O. Box 953, N-9001 Tromsø, Norway

The complete assignment of the ^{13}C NMR resonances at 22.5 MHz has been performed for *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid (Ac–Asp–γAbu) in aqueous solution. In the crystalline state the conformation of Ac–Asp–γAbu has been determined by X-ray crystallography. Semi-empirical CNDO/2 calculations based on a model from the solid state have been used to calculate the net charges on the C atoms and these charges show correlation with the ^{13}C NMR chemical shifts found in solution. The synthesis of a deuterium labelled analog *N*-(*N*-acetyl-L-aspartyl)-4-amino-2,2,3,3,4,4-hexadeuteriobutyric acid (Ac–Asp–γAbu-d_6) is also described.

Many ω-amino acids have been isolated from various sources of animals and plants and numerous investigations have been performed to accumulate data concerning their metabolic pathways and/or physiological functions. One of the most important results was that γ-aminobutyric acid (γAbu) was found to have a potent inhibitory action against epileptic seizure.[1] γAbu occurs especially in the mammalian brain where it is enzymatically produced. *N*-Acetyl-L-aspartic acid (Ac–Asp) has also been found in mammalian brain tissue.[2] Recently we reported[3] the synthesis of *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid (Ac–Asp–γAbu, *1*) which has been postulated by Reichelt[4] to be one of the Ac–Asp-containing oligopeptides produced in an amine- and ATP-dependent synthesis in homogenates of mouse cortex.[5,6]

The search for possible structure–activity relationships in such compounds is of considerable interest and we thought it therefore worthwhile to make a combined ^{13}C NMR study and X-ray analysis of Ac–Asp–γAbu and try to correlate the results with semi-empirical calculations.

RESULTS AND DISCUSSION

Ac–Asp–γAbu-d_6 (*2*) was prepared by coupling Ac–Asp(OBzl)[3,7] (*5*) with benzyl 4-amino-2,2,3,3,4,4-hexadeuteriobutyrate (γAbu-d_6–OBzl, *3*) using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as coupling reagents. The protecting benzyl groups were subsequently removed by hydrogenolysis in the presence of acetic acid to prevent cyclization to the corresponding succinimide- or γ-lactam derivatives.

In the paper describing the synthesis of Ac–Asp–γAbu (*1*),[3] we assigned all the ^{13}C resonances except those of the four carbonyl groups which were observed at 174.9, 176.6 (two coinciding resonances) and 180.4 ppm in its noise-decoupled spectrum; *cf.* Fig. 1a. The noise-decoupled spectrum of Ac–Asp–γAbu-d_6 (*2*) exhibited three singlets at 175.0, 176.6 and 176.8 ppm in addition to a barely visible multiplet at 180.4 ppm which could be ascribed to the carboxylic carbonyl resonance of the γAbu-d_6 moiety because of its complexity due to ^{2}H–^{13}C spin couplings and low intensity caused by increased relaxation time. Deuteration of a nearby protonated carbon is known to increase the T_1 of a nonprotonated carbon;[8] *cf.* Fig. 1c.

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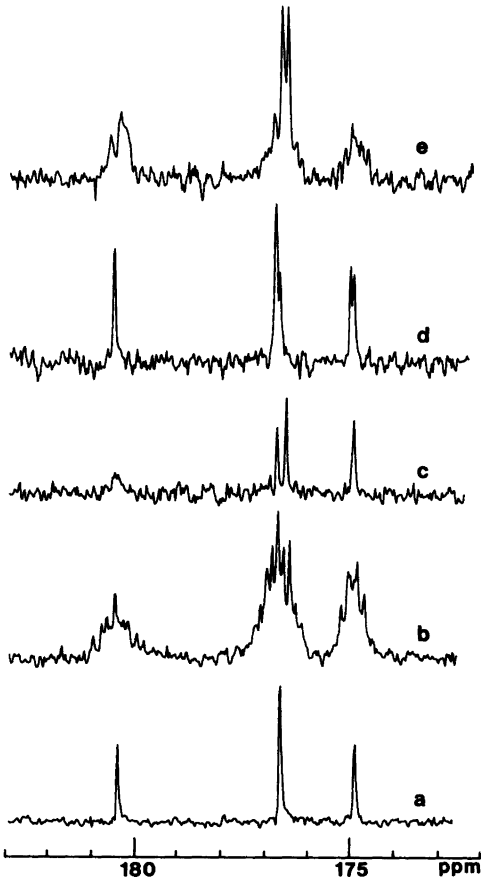

Fig. 1. ^{13}C spectra of carbonyl carbons. a, Ac–Asp–γAbu: ^{1}H-decoupled; b, Ac–Asp–γAbu: single-resonance; c, Ac–Asp–γAbu-d_6: ^{1}H-decoupled; d, Ac–Asp–γAbu in 50:50 (v/v) H_2O–D_2O: ^{1}H-decoupled; e, Ac–Asp–γAbu: selective low-power irradiation at the frequency of the CH_3CO-protons.

When recorded in a 50:50 mixture of H_2O and D_2O as solvent, amide NH protons will, as pointed out by Feeney *et al.*,[9] exchange slowly and amide carbonyl resonances will appear as doublets corresponding to CONH and COND ^{13}C signals which have slightly different δ values due to the deuterium isotope effect. Since no splitting is observed on the carboxylate signals because protons of the carboxylate are in rapid exchange with the solvent, amide and carboxylic carbonyl signals can be distinguished by recording the noise-decoupled spectrum in a mixture of H_2O and D_2O. Thus, the noise-decoupled spectrum of Ac–Asp–γAbu (*1*) in H_2O/D_2O exhibited a singlet at *ca.* 180.4 ppm confirming it being a carboxylic carbonyl resonance. Furthermore, the spectrum revealed a doublet at *ca.* 176.6 ppm partly coinciding with a singlet which consequently could be ascribed to the carboxylic carbon of the Ac–Asp moiety, and a doublet at *ca.* 174.9 ppm; *cf.* Fig. 1d. The isotope shift, 0.08 ppm, observed in the latter doublet is comparable to that observed by Feeney *et al.*[9] The spectrum also displayed doublets at *ca.* 41.2 and 52.9 ppm (isotope shifts 0.14 and 0.08 ppm, respectively) ascribed to the C–4 signal of the γAbu moiety and the C–2 signal of the Ac–Asp moiety, respectively.

Assignments of the two amide carbonyl resonances were readily achieved employing selective low-power irradiation at the resonance frequency of the CH_3CO-protons; *cf.* Fig. 1e. The doublet which appeared at *ca.* 176.6 ppm revealed a residual $^{1}H-^{13}C$ spin coupling constant of 3.1 Hz which is in excellent agreement with the $^{3}J_{^{13}C-C\alpha H}$ spin coupling constant of 3.1 ± 0.1 Hz reported by Feeney *et al.*[9] for a similar system (Ac–Asp). Hence, the final ^{13}C-signal at 174.9 ppm could be assigned to the amide carbonyl carbon linking the Ac–Asp and γAbu moieties (*cf.* Fig. 2).

The conformation of Ac–Asp–γAbu as found in the crystalline state and the numbering of the atoms are shown in Fig. 3. Bond lengths and angles for non-hydrogen atoms as calculated from the atomic coordinates in Table 1, are listed in Tables 2 and 3. Some torsion angles are given in Table 4. The molecule has an open structure with no intramolecular contacts. The different parts of the molecule can be described by four planes P1–P4 comprising the following atoms: P1: O(101), O(102), C(9), C(8) and C(7); P2: C(7), N(2), C(6), O(6) and C(3); P3: C(6), C(3), C(4), C(5), O(51) and O(52); and P4: C(3), N(1), C(2), O(2) and C(1). P1 and P4

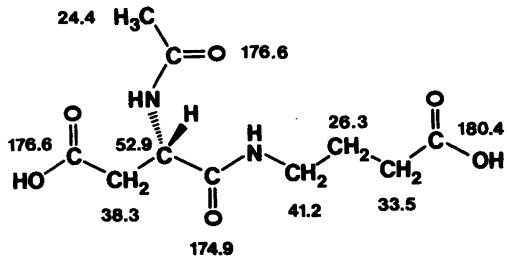

Fig. 2. ^{13}C NMR shift assignments of Ac–Asp–γAbu in H_2O. δ-Values are relative to external TMS.

Table 1. Atomic coordinates and temperature parameters U_{ij}(Å$^2 \times 10^4$) for the oxygen, nitrogen and carbon atoms and U(Å$^2 \times 10^4$) for the hydrogen atoms. The temperature factors are $\exp\{2\pi^2(h^2a^{*2}U_{11}+\cdots 2hka^*b^*U_{12}+\cdots)\}$ and $\exp\{-8\pi^2U(\sin^2\theta/\lambda^2\}$, respectively.

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
C(1)	.2046(14)	.1447(9)	1.2216(6)	405(37)	188(25)	90(21)	0(0)	34(23)	0(0)
C(2)	.1326(11)	.1741(3)	1.0536(5)	315(29)	96(20)	136(22)	66(20)	66(21)	-14(16)
O(2)	-.0660(8)	.1392(2)	.9715(4)	337(23)	132(16)	170(16)	-52(15)	-11(15)	45(13)
N(1)	.2862(9)	.2376(3)	.9946(5)	283(24)	94(16)	114(18)	20(17)	-12(17)	-18(14)
C(3)	.2120(12)	.2734(3)	.8557(5)	315(32)	103(21)	111(21)	-29(21)	14(21)	17(17)
C(4)	.3448(12)	.3636(3)	.8185(6)	367(33)	83(19)	150(22)	-46(22)	88(21)	14(18)
C(5)	.1898(12)	.4350(3)	.9076(6)	357(33)	155(23)	125(22)	-92(22)	-31(22)	37(18)
O(51)	-.0128(11)	.4218(3)	.9787(5)	516(27)	163(18)	256(21)	-28(20)	100(20)	-40(16)
O(52)	.3205(9)	.5115(2)	.8930(4)	457(26)	80(15)	277(21)	-53(16)	72(18)	-22(14)
C(6)	.2964(11)	.2150(3)	.6988(5)	304(29)	87(20)	92(19)	-39(19)	24(19)	-16(16)
O(6)	.1848(9)	.2281(2)	.5626(4)	519(26)	142(17)	95(15)	88(17)	-69(16)	-12(13)
N(2)	.4925(10)	.1547(3)	.7296(5)	289(24)	108(18)	123(18)	-21(17)	14(18)	-29(15)
C(7)	.6083(12)	.0997(3)	.6071(6)	314(30)	97(21)	149(23)	-48(20)	43(21)	11(17)
C(8)	.4300(13)	.0182(3)	.5652(6)	411(35)	131(22)	113(23)	-12(21)	18(23)	-23(18)
C(9)	.4216(14)	-.0463(3)	.7035(6)	439(38)	165(23)	131(23)	-100(24)	-32(24)	30(20)
C(10)	.2787(12)	-.1321(4)	.6651(6)	398(34)	158(22)	134(22)	24(23)	-10(22)	-3(19)
O(101)	.3083(9)	-.1978(2)	.7470(4)	472(25)	127(17)	198(17)	-3(17)	-20(17)	39(13)
O(102)	.1044(10)	-.1299(3)	.5346(5)	582(31)	195(19)	259(21)	-170(21)	-186(20)	71(17)

ATOM	X	Y	Z	U	ATOM	X	Y	Z	U
H(11)	.336(15)	.169(5)	1.273(8)	413(199)	H(2)	.631(13)	.153(4)	.815(7)	280(162)
H(12)	.079(12)	.157(4)	1.280(6)	187(158)	H(71)	.620(13)	.130(4)	.511(7)	342(169)
H(13)	.271(14)	.085(5)	1.210(8)	456(202)	H(72)	.803(10)	.077(3)	.644(5)	528(116)
H(1)	.448(10)	.265(3)	1.055(5)	281(127)	H(91)	.222(10)	.036(3)	.529(6)	571(124)
H(3)	.036(11)	.282(3)	.820(5)	365(113)	H(92)	.516(12)	-.012(3)	.484(6)	479(118)
H(41)	.571(11)	.368(4)	.856(6)	450(131)	H(91)	.623(12)	-.065(4)	.758(6)	512(131)
H(42)	.354(20)	.374(6)	.698(10)	537(524)	H(92)	.325(10)	-.017(3)	.787(6)	426(113)
H(52)	.199(10)	.559(3)	.925(5)	475(121)	H(102)	-.015(19)	-.171(5)	.532(9)	567(248)

Table 2. Bond lengths *l* in *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid. Standard deviations in parentheses.

Bond	*l*(Å)
C(1)–C(2)	1.499(7)
C(2)–O(2)	1.250(6)
C(2)–N(1)	1.322(7)
N(1)–C(3)	1.464(6)
C(3)–C(4)	1.521(7)
C(3)–C(6)	1.524(7)
C(4)–C(5)	1.529(7)
C(5)–O(51)	1.172(7)
C(5)–O(52)	1.329(6)
C(6)–O(6)	1.247(6)
C(6)–N(2)	1.319(7)
N(2)–C(7)	1.456(7)
C(7)–C(8)	1.530(7)
C(8)–C(9)	1.523(7)
C(9)–C(10)	1.499(8)
C(10)–O(101)	1.219(6)
C(10)–O(102)	1.332(7)

Table 4. Selected torsion angles (°) in Ac–Asp–γAbu.

Torsion angle	(°)
C(1)–C(2)–N(1)–C(3)	−175.3(4)
O(2)–C(2)–N(1)–C(3)	5.0(7)
C(2)–N(1)–C(3)–C(4)	159.9(5)
C(2)–N(1)–C(3)–C(6)	−76.8(6)
N(1)–C(3)–C(4)–C(5)	−75.6(5)
N(1)–C(3)–C(6)–O(6)	162.7(5)
C(4)–C(3)–C(6)–O(6)	−73.5(6)
C(4)–C(3)–C(6)–N(2)	104.2(5)
C(6)–C(3)–C(4)–C(5)	158.3(4)
C(3)–C(4)–C(5)–O(51)	−1.5(7)
C(3)–C(4)–C(5)–O(52)	177.9(4)
C(3)–C(6)–N(2)–C(7)	−174.8(5)
O(6)–C(6)–N(2)–C(7)	2.7(8)
C(6)–N(2)–C(7)–C(8)	−84.4(6)
N(2)–C(7)–C(8)–C(9)	−65.5(6)
C(7)–C(8)–C(9)–C(10)	−173.3(5)
C(8)–C(9)–C(10)–O(101)	162.3(6)
C(8)–C(9)–C(10)–O(102)	−20.2(7)

are almost parallel with an angle of 18.5° between them. P2 is almost perpendicular to both P3 and P4, and the values are 84.2 and 84.4°, respectively. The angle between P3 and P4 is 74.3°.

Table 3. Bond angles ∠(*ijk*) in *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid.

i *j* *k*	∠(*ijk*)°
C(1)–C(2)–O(2)	121.0(5)
C(1)–C(2)–N(1)	118.0(5)
O(2)–C(2)–N(1)	121.0(5)
C(2)–N(1)–C(3)	120.4(5)
N(1)–C(3)–C(4)	110.2(4)
N(1)–C(3)–C(6)	114.0(5)
C(4)–C(3)–C(6)	109.3(5)
C(3)–C(4)–C(5)	112.7(5)
C(3)–C(6)–O(6)	118.8(5)
C(3)–C(6)–N(2)	118.0(4)
C(4)–C(5)–O(51)	123.6(5)
C(4)–C(5)–O(52)	110.0(5)
O(51)–C(5)–O(52)	126.4(5)
O(6)–C(6)–N(2)	123.2(5)
C(6)–N(2)–C(7)	123.3(4)
N(2)–C(7)–C(8)	114.0(5)
C(7)–C(8)–C(9)	112.7(5)
C(8)–C(9)–C(10)	115.5(5)
C(9)–C(10)–O(101)	124.1(5)
C(9)–C(10)–O(102)	113.9(5)
O(101)–C(10)–O(102)	122.0(6)

The crystal structures of γ-aminobutyric acid and aspartic acid have been solved[10–15] and a comparison can therefore be made with the γAbu and Ac–Asp parts of Ac–Asp–γAbu. γAbu has been solved at low temperature[10] as well as at room temperature[11] and γAbu · HCl has also been solved.[12,13] Our results show that the γAbu part of Ac–Asp–γAbu is in a planar *trans* conformation with respect to the C_α–C_β bond C(9)–C(8). This is in more accordance with γAbu · HCl than with γAbu where a *gauche* conformation is found with respect to the C_α–C_β bond. For aspartic acid two previous investigations have been made,[14,15] one of L-aspartic acid[14] and the other of DL-aspartic acid.[15] In both structures the carbon chain is nearly planar with an average deviation from planarity of 0.01 and 0.03 Å, respectively. A least squares plane made up of the same four atoms C(6), C(3), C(4) and C(5) in the present molecule gives a mean deviation from the plane of about 0.1 Å. The atoms C(3), C(4), C(5), O(51) and O(52) lie, however, in the same plane to within 0.01 Å, with the atoms C(6) and N(1) −0.52 and 1.36 Å, respectively, out of the plane. A comparison of bond lengths in Ac–Asp–γAbu with relevant bonds in γAbu and Asp is given in Table 5. The packing of Ac–Asp–γAbu is illustrated in Fig. 4.[16] The crystal structure is stabilized by hydrogen bonds, one for each hydrogen atom covalently bonded to nitrogen

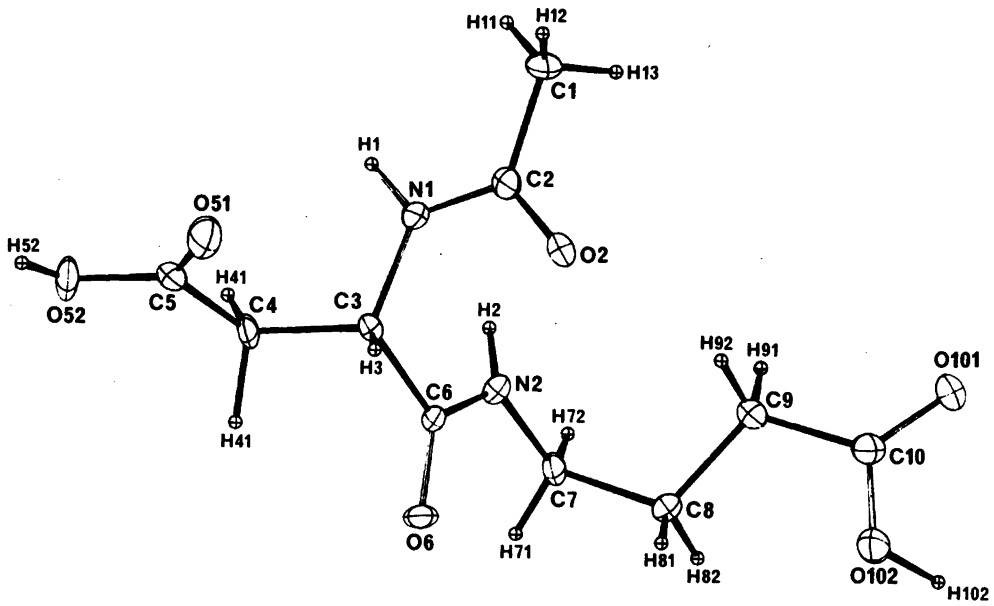

Fig. 3. ORTEP[16] drawing of the title compound with the numbering of the atoms in the molecule. Thermal ellipsoids for the non-hydrogen atoms are drawn at the 50 % probability level.

or oxygen. Hydrogen bond distances are given in Table 6.

CNDO/2 calculations – correlation with the ^{13}C NMR results. The results obtained from the X-ray study have been used to calculate net charges for the C atoms by semi-empirical CNDO/2 calculations.[17] The net charges are calculated from an analysis of the electron density-bond order matrix,

$$P_{\mu\nu} = 2\sum_{i}^{occ} C_{\mu_i\nu_i},$$

where C_{μ_i} are LCAO coefficients and the summation being over all occupied molecular orbitals. The bond lengths and angles found in the X-ray crystallographic work were used as a model for the theoretical calculations, except for the C–H, O–H

Table 5. Comparison of bond lengths (Å) in Ac–Asp–γAbu with relevant bonds in related compounds. The numbering of the atoms refer to the present work.

Bond	This work	Ref. 15	Ref. 14	Ref. 11	Ref. 10	Ref. 13
N(1)–C(3)	1.464(6)	1.491(3)	1.495(4)			
C(3)–C(4)	1.521(7)	1.519(3)	1.518(4)			
C(4)–C(5)	1.529(7)	1.505(3)	1.512(4)			
C(5)–O(51)	1.172(7)	1.219(3)	1.202(4)			
C(5)–O(52)	1.329(6)	1.305(3)	1.306(4)			
C(3)–C(6)	1.524(7)	1.538(3)	1.543(4)			
C(6)–O(6)	1.247(6)	1.250(3)	1.242(4)			
N(2)–C(7)	1.456(7)			1.469(5)	1.497(4)	1.49(2)
C(7)–C(8)	1.530(7)			1.502(6)	1.520(5)	1.56(2)
C(8)–C(9)	1.523(7)			1.519(6)	1.528(5)	1.52(2)
C(9)–C(10)	1.499(8)			1.520(6)	1.522(6)	1.53(2)
C(10)–O(101)	1.219(6)			1.226(6)	1.249(4)	1.22(2)
C(10)–O(102)	1.332(7)					1.32(2)

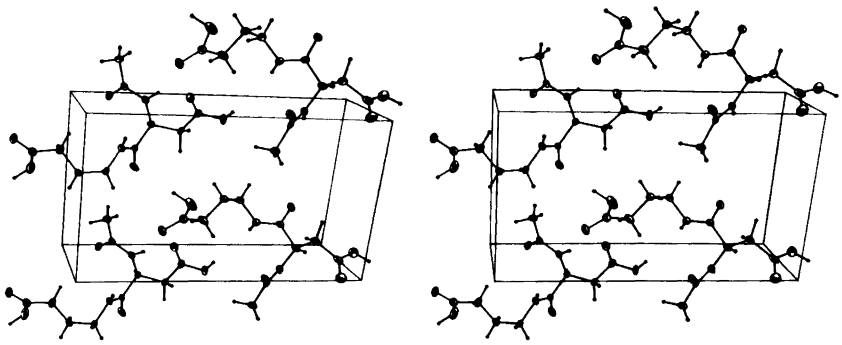

Fig. 4. A stereoscopic view of the arrangement of molecules in the unit cell.

and N–H bond lengths which were given the values 1.09, 0.96 and 1.01 Å, respectively.[18] The net charges have been plotted against the ^{13}C NMR chemical shift found on corresponding atoms, Table 7, and the plot is given on Fig. 5. The plot shows that there is some correlation between the calculated charges and the observed chemical shifts. The correlation coefficient from the least squares linear regression fit[19] is 0.93 if all ten carbon atoms are included.

A comparison of the results from the aspartic part of Ac–Asp–γAbu can be made with the theoretical work of Momany *et al.*[20] They have used CNDO/2 calculations to find the energetically most favoured conformations of polypeptides and proteins and our CNDO/2 results show a good agreement with their calculations. The largest difference in net charge is on nitrogen atom N(1), *cf.* Fig. 3; this work gives a value of −0.203 compared to the value of −0.356 calculated by Momany *et al.* This difference may be explained by the fact that in the present work an acetyl group is attached to N(1) and this will lead to a decreased charge on N(1) relative to a nitrogen atom with only a hydrogen atom attached to it as in the work of Momany *et al.*[20]

EXPERIMENTAL

Melting points (uncorrected), rotations, IR and mass spectra were recorded on Mettler FP61, Perkin-Elmer 241, Beckmann and Micromass 7070H instruments, respectively, ^{1}H (89.55 MHz) and ^{13}C (22.5 MHz) NMR spectra were obtained on a Jeol FX90Q instrument operating in the pulsed-Fourier transform mode. Unless otherwise stated, ^{13}C NMR spectra were recorded using a pulse width of 5.5 μs (45° pulse), a spectral width of 5000 Hz (16K data points), an acquisition time of 0.998 s, and with TMS as external reference. Pulse delays are stated below. Analytical TLC and column chromatography were performed on silica gel: F_{254} plates and Merck Kieselgel 60 (0.040–0.063 mm), respectively.

Tosylate of benzyl 4-amino-2,2,3,3,4,4-hexadeuteriobutyrate (γ-Abu-d_6-OBzl tosylate, 3*).* γAbu-d_6

Table 6. Hydrogen bond distances (Å) and angles (°). Symmetry code:

(i) x, y, z
(ii) $1+x, y, z$
(iii) $-x, \frac{1}{2}+y, 1-z$
(iv) $1-x, \frac{1}{2}+y, 2-z$
(v) $-x, y-\frac{1}{2}, 2-z$

A–H···B	A–H	H···B	A···B	∠AHB
$N(2)^{i}-H(2)\cdots O(2)^{ii}$	0.94	1.90	2.84	180
$N(1)^{i}-H(1)\cdots O(101)^{iv}$	0.98	2.05	2.98	158
$O(52)^{i}-H(52)\cdots O(2)^{v}$	0.97	1.65	2.58	159
$O(102)^{i}-H(102)\cdots O(6)^{iii}$	0.84	1.89	2.66	152

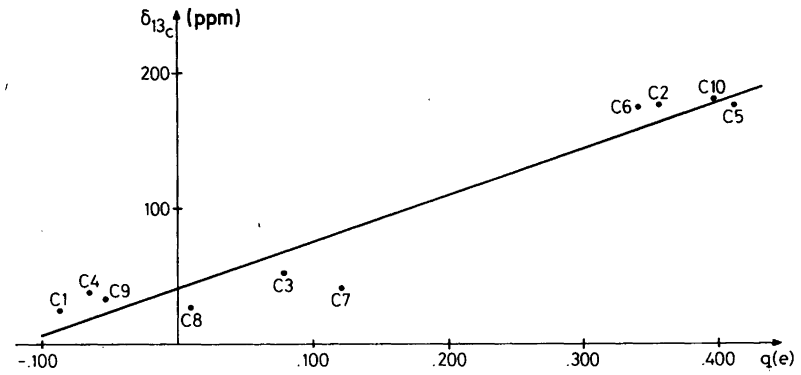

Fig. 5. Correlation of net charges (q) calculated using CNDO/2 and ^{13}C NMR chemical shifts (δ_{13C}) in Ac–Asp–γAbu.

(242 mg, 2.22 mmol, *4*) was esterified with benzyl alcohol-d_1 (656 mg, 6.08 mmol) in refluxing benzene (7 ml) in the presence of *p*-toluenesulfonic acid-d_1 (554 mg, 2.92 mmol). The labile protons of the benzyl alcohol and the *p*-toluenesulfonic acid had in advance been exchanged with deuterium by shaking with D_2O and subsequent removal of the water by distillation *in vacuo*. Water was removed from the reaction mixture by azeotropic distillation (20 h). The solvent was removed and the residue recrystallized from ethanol–ether (763 mg, 93 %). M.p. 100–101 °C: R_F 0.3 ($CHCl_3$); ^1H NMR peaks at (D_2O) δ 2.38 (3H, s), 5.17 (2H, s), 7.38 (2H, d, *J ca.* 8.5 Hz), 7.44 (5H, s), 7.67 (2H, d, J *ca.* 8.5 Hz); ^{13}C NMR (D_2O, pulse delay: 2 s): δ 23.1 (q), 69.7 (t), 128.0, 130.1, 130.9, 131.3, 131.4, 131.5, 132.0, 138.3, 145.1; δ-values are relative to sodium 3-(trimethylsilyl)-propansulfonate.

Ac–Asp(OBzl)–γAbu-d_6*–OBzl (6).* A mixture of Ac–Asp(OBzl) (576 mg, 2.2 mmol, *5*), γAbu-d_6–OBzl tosylate, 733 mg, 2.0 mmol, *3*), dicyclohexylcarbodiimide (1018 mg, 4.9 mmol), 1-hydroxybenzotriazole (800 mg, 5.9 mmol) and *N*-ethylmorpholine (227 mg, 2.0 mmol) in CH_2Cl_2 (10 ml) was stirred for 5 h at −15 °C and left in the refrigerator overnight. Acetic acid (0.5 ml) was added and the mixture filtered after 5 min. The solid material (dicyclohexylurea) was washed with CH_2Cl_2 (30 ml) and the combined filtrates were washed twice with 1 M HCl and water, respectively, dried over Na_2SO_4 and evaporated. The residue was chromatographed twice on silica gel columns and eluted with CH_2Cl_2, 1 % CH_3OH and 2 % CH_3OH in CH_2Cl_2 yielding pure Ac–Asp(OBzl)–γAbu-d_6–OBzl (540 mg, 61 %, *6*). M.p. 91–92 °C; R_F 0.3 (3 % CH_3OH in $CHCl_3$); $[\alpha]^{20}$ (*c* 1.23, $CHCl_3$) −15.5° (589 nm), −16.5° (578 nm), −19.3° (546 nm), −39.5° (436 nm), −78.2° (365 nm). ^1H NMR peaks at ($CDCl_3$) δ 1.99 (3H, s), *ca.* 2.7 (1H, dd, J *ca.* 6.6 and 16.8 Hz, B-part of an ABMX-system), *ca.* 3.0 (1H, dd, J *ca.* 4.4 and 16.8 Hz, A-part of an ABMX-system), *ca.* 4.8 (1H, m, M-part of an ABMX-system), 5.11 (4H, s), *ca.* 6.7 (2H, m), 7.33 (10H, s); ^{13}C NMR ($CDCl_3$, pulse delay: 1.5 s): δ 23.1 (q), 36.0 (t), 49.5 (d), 66.4 (t), 66.9 (t), 128.2, 128.3, 128.4, 128.6, 135.9, 136.0, 170.2 (s), 170.4 (s), 171.8 (s), 173.1 (barely visible multiplet); δ-values are relative to internal TMS; *m/e* (%): 446 (M^+, 0.4), 91 (100), 79 (49), 43 (47), 108 (40), 77 (31), 107 (30), 204 (29), 231 (24), 172 (21). The deuterium-content of $6-d_6$ was calculated on the basis of the intensities of the ions of $6-d_6$ around *m/e* 204 and 231 in relation to corresponding ions of $6-h_6$ around *m/e* 198 and 225 observed in the spectrum recorded for a mixture of the two compounds: d_6 *ca.* 92.4 %, d_5 *ca.* 6.8 %, d_4 *ca.* 0.8 %.

Ac–Asp–γAbu-d_6 *(2).* Ac–Asp(OBzl)–γAbu-d_6–OBzl (470 mg, 1.1 mmol, *6*) dissolved in CH_3OH

Table 7. Net charges (q) from CNDO/2 calculations and ^{13}C NMR chemical shifts (δ) in Ac–Asp–γAbu.

Atom	q(e)	δ (ppm)
C(1)	−0.087	24.4
C(2)	0.355	176.6
C(3)	0.078	52.9
C(4)	−0.065	38.3
C(5)	0.411	176.6
C(6)	0.340	174.9
C(7)	0.121	41.2
C(8)	0.010	26.3
C(9)	−0.054	33.5
C(10)	0.396	180.4

(5 ml) and acetic acid (0.2 ml) was hydrogenated at room temperature and atmospheric pressure in the precence of 10 % Pd/C (40 mg) by bubbling hydrogen through the reaction mixture for 100 min. The solution was filtered through celite and evaporated to yield a crystalline product which was recrystallized from CH_3OH-ether (197.1 mg, 71 %). M.p. 157–158 °C; R_F 0.8 (ethanol–H_2O=14:1); $[\alpha]^{20}$ (*c* 0.64, CH_3OH) −33.6° (589 nm), −35.1° (578 nm), −40.5° (546 nm), −76.1° (436 nm), −136.4° (365 nm); ^{1}H NMR peaks at (TMS, CD_3OD) δ 1.99 (3H, s), *ca.* 2.8 (1H, dd, *J ca.* 16.6 and 7.0 Hz, B-part of an ABX-system), *ca.* 2.7 (1H, dd, *J ca.* 16.6 and 6.3 Hz, A-part of an ABX-system), *ca.* 4.7 (1H, dd, *J ca.* 6.3 and 7.0 Hz, X-part of an ABX-system); ^{13}C NMR (D_2O, pulse delay: 12 s): δ 24.5 (q), 38.5 (t), 53.0 (d), 175.0 (s), 176.6 (s), 176.8 (s), *ca.* 180.4 (barely visible multiplet); *m/e* (%): M^+ not visible, 248 (M−18, 0.3), 43 (100), 85 (34), 70 (9), 46 (8), 187 (7), 186 (6), 92 (6), 91 (6), 201 (6), 112 (5), 229 (3).

Ac–Asp–γAbu (1).[1] ^{13}C NMR spectra (D_2O except in the case where a 1:1 mixture of H_2O–D_2O was employed) were recorded using the following conditions: ^{1}H-decoupled using a pulse delay of 6 s, single-resonance using a pulse delay of 3 s and acquisition time of 1.638 s, selective decoupling using a pulse delay of 1.5 s, and a pulse delay of 2 s in the case where H_2O–D_2O was used as solvent.

Crystal data. *N*-(*N*-Acetyl-L-aspartyl)-4-aminobutyric acid (Ac–Asp–γAbu), $C_{10}H_{16}N_2O_6$, M = 260.25. Monoclinic space group $P2_1$. a=4.720(2), b=15.242(4), c=8.383(3) Å, β=93.19(3)°, Z=2, D_m=1.45 g cm^{-3}, D_x=1.435 g cm^{-3}, μ(Mo$K\alpha$)= 1.29 cm^{-1}. Cell dimensions were found from a least squares refinement of the 2θ values of 25 reflections.

Data collection. A crystal of size 0.5 × 0.2 × 0.2 mm was used for all data collections. Unit cell dimensions and intensity data were measured on an automatic Enraf-Nonius CAD4 diffractometer using graphite monochromator and Mo$K\alpha$ radiation (λ=0.71069 Å). The data was collected at about −150 °C using liquid nitrogen equipment for cooling. Three-dimensional intensity data for 2103 independent reflections within $2\theta<64°$ were collected by the $\omega-2\theta$ scan technique and with $\Delta\omega$= 0.60+0.35 tan θ. After data reduction including Lp-correction but no absorption correction, 1512 reflections had net intensities $>2.0\sigma(I)$, where $\sigma(I)$ is the standard deviation from counting statistics. These were regarded as observed reflections and were used in the refinement procedure.

Structure solution and refinements. The structure was solved using the MULTAN system,[21] which revealed the 18 non-hydrogen atoms of the molecule. After a few full matrix least squares refinement cycles, the 16 hydrogen atoms were found from difference maps. The atomic parameters were thereafter refined by full matrix least squares to an R of 0.066. At the end of the refinement the average shift/error ratio was 0.04.

The form factors used in the structure factor calculations were those of Stewart *et al.*[22] for hydrogen and Cromer and Mann[23] for the other atoms. Final atomic coordinates and temperature parameters are listed in Table 1.

All the calculations mentioned above were carried out on the Cyber 171MP computer at the University of Tromsø. The programs used were mainly those of the X-RAY 76 program system.[24] The data reduction programs used were adopted for the Cyber 171MP computer by L. K. Hansen and L. J. Sæthre, this University.

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Short Communications

Aggregation of Lipid Vesicles (Liposomes). A Versatile Method to Study Sugar Exposure on Biological Membranes and Sugar Affinity of Bacteria *

K.-E. MAGNUSSON,[a] L. ÖHMAN,[a] O. STENDAHL[a] and E. KIHLSTRÖM[b]

[a] Department of Medical Microbiology and [b] Department of Clinical Bacteriology, University of Linköping, S-581 85 Linköping, Sweden

Sugar ligands exposed on biological membranes are important for recognition by bacteria, viruses, toxins and hormones.[1] Thus, the GM_1-ganglioside has been shown to be the receptor for Vibrio cholerae toxin,[2] and a GM_2-like glycoconjugate the receptor for the adhesins CFA/I and K99 of *Escherichia coli*.[3] In the urinary tract the sugar moiety, α-D-Gal-(1→4)-β-D-Gal-, of the P-blood group antigen has been recognized as the binding site for uropathogenic *E. coli*.[4,5] We have employed aggregation of liposomes to characterize (a) sugar exposure on glycolipids of rat intestinal membranes, by using different lectins, and (b) sugar affinity of *E. coli* and *Actinomyces* bacteria, by using appropriate glycolipid-containing liposomes.

Experimental. Determination of sugar exposure on rat intestinal glycolipids. The mucosa of distal ileum in Spraque-Dawley rats (♀; 200 g), was gently scraped off with a curette and put in ice-cold deionized water. It was then freeze-pressed with the X-press[6,7] at −25 °C and about 250 MPa, freeze-dried and extracted according to Karlsson *et al.*[8] The alkaline and neutral lipids were used to prepare lipid vesicles (liposomes) using the reverse phase evaporation method.[9,10] 2 ml lipid extract (≈20 μmol) was mixed with 50 μl 0.1 mM fluorescent lipid *N*-(4-nitrobenzo)-2-oxa-1,3-diazole (NBD)-phosphatidylethanolamine and dried in a rota-evaporator and then dissolved in 15 ml chloroform–diethylether, 1:1, and an emulsion made with 4 ml phosphate-buffered (0.01 M) saline, pH 7.3 using an MSE-sonicator. The vesicles were then formed by evaporation of the organic solvent under vacuum, followed by brief ultrasonic treatment (2 × 30 s) to achieve sonicated unilamellar vesicles (SUVs).[9] To characterize the sugars exposed on the vesicles prepared from intestinal glycolipids, 10 μl lectin (0.25 mg/ml) was mixed with 50 μl liposome suspension at room temperature, and investigated under the epifluorescence microscope with excitation around 480 nm and emission around 517 nm.

Sugar affinity of bacteria. (a) *Aggregation of bacteria with glycolipid-containing fluorescent liposomes.* Unilamellar large liposomes (5 μmol per ml PBS) were prepared by the reverse phase evaporation method (REVs).[9] The vesicles had the following composition: maltobionamide (MB)-vesicles, hexadecylmaltobionamide[11] – phosphatidylglycerol – egg phosphatidylcholine – cholesterol – NBD – phosphatidylethanolamine, 0.7 (or 4 or 10):1:9:9:0.1, and lactosylceramide (LC)-vesicles, *N*-stearoyl-dihydrolactocerebroside – phosphatidylglycerol – egg phosphatidylcholine – cholesterol – NBD-phosphatidylethanolamine, 1:0.5:10:10:0.1. The MB-vesicles were agglutinad only with concanavalin A (ConA, mannose-specific), which was inhibited with α-methyl-mannoside (2 % w/v), LC-vesicles were aggregated with *Ricinus communis* agglutinin -I (D-gal and lactose specific) but not with conA. Aggregation of bacteria with the vesicles was performed at room temperature in microtiter plates on 200 μl of bacteria (10^9 per ml) and 10–100 μl of lipid vesicles (5 μmol per ml). To assess the effect of inhibiting sugars 100 μl bacteria (10^9 per ml) were incubated together with 100 μl PBS containing 2 % appropriate sugar, and 25 or 50 μl lipid vesicles (5 μmol per ml). Spontaneous aggregation of bacteria was studied with 100 μl bacteria (10^9 per ml) and 100 μl PBS. The aggregation was scored after 1 h; ++ = significant agglutination, + weak agglutination, and 0 = no agglutination.

(b) *Agglutination of bacteria and guinea-pig erythrocytes* was studied at room temperature on glass slides using a 3 % (v/v) guinea-pig erythrocyte suspension and 10^9 bacteria per ml PBS (pH 7.2) or two-fold dilutions of this concentration.[12]

(c) *Agglutination of bacteria and yeast cells*

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Table 1. Aggregation of fluorescent intestinal glycolipid vesicles with different lectins.

Lectin[a]	Sugar specificity	Aggregation
RCA-1	β-D-Gal	+
SBA	MD-Gal, α-NAcGal	(+)
WGA	$[\beta(1\rightarrow4)$-D-GlcNAc$]_2$ (sialic acid)	++
UEA-1	α-L-fucose	+
PNA	Gal-$\beta(1\rightarrow3)$-GalNAc	(+)
Control	–	0

[a] RCA-1 = *Ricinus communis* agglutinin I;
SBA = *Glycine max* agglutinin = soybeen agglutinin;
WGA = Wheat germ agglutinin = *Triticum vulgaris* agglutinin;
UEA-1 = *Ulex europaeus* agglutinin I;
PNA = Peanut agglutinin = *Arachis hypogaea* agglutinin.

(Saccharomyces cerevisiae) was done at room temperature in microtiterplates on a 1 % (v/v) yeast cell suspension incubated for 2 h with 10^9 bacteria per ml or two-fold dilutions of this concentration.[12]

Hydrophobic interaction chromatography (HIC). HIC was performed on Octyl-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by *Öhman et al.*[13]

Bacteria. E. coli bacteria isolated from urinary tract infections were grown in suspension for 48+48+16 h at 37 °C in Nutrient Broth (NB; Oxoid No. 2) without and with 0.75 % (w/v) glucose to promote and depress type I-fimbriae formation, respectively.

Actinomyces naeslundii and *A. viscosus* bacteria were grown in Brain Heart Infusion Broth (BHI; Oxoid) for 16 h. The bacteria were washed twice in PBS before being analyzed.

Chemicals. N-Stearoyldihydrolactocerebroside, phosphatidylglycerol, egg-yolk phosphatidylcholine, cholesterol, galactose, α-methylmannoside, mannose and maltose were obtained from Sigma (St. Louis, Mo.). The fluorescent lipid, NBD–phosphatidylethanolamine, was obtained from Polar Lipids, Inc. (Birmingham, Ala.). The lectins were a product of E.Y.Lab.s (San Mateo, Ca.), and concanavalin A of Pharmacia Fine Chemicals (Uppsala, Sweden) Hexadecylmaltobionamide was a generous gift from Dr. Francis Szoka (School of Pharmacy, UCSF, San Fransisco). It was synthetized as described by Williams *et al.*[11]

Results. Sugar exposition on rat intestinal glycolipids. The agglutinability of intestinal lipid vesicles with different lectins is shown in Table 1. Assuming that the degree of aggregation is proportional to the amount of sugar presented as glycolipid on the liposomal membrane, *N*-acetylglucosamin, galactose and fucose should be the dominant sugars.

Sugar affinity of bacteria. The mannose-sensitive agglutination of type 1 fimbrated *E. coli* is shown in Table 2, along with the tendency to hydrophobic interaction with Octyl-Sepharose. It is evident that glucose (a) reduces the hydrophobicity, and (b) abolishes the affinity for mannose-residues presented on MB-vesicles, yeast cells or guinea-pig erythrocytes. When the percentage of the *E. coli* retained in the gel (*y*-value) was plotted against the $-{}^2\log$ of the maximum bacterial dilution (*x*-value) yielding positive guinea-pig hemagglutination, there was a linear relationship, $y=12.6x+16.7$ with $r=0.82$.

When increasing the relative amount of hexadecylmaltobionamide (=mannose-equivalent) in the MB-vesicles from 0.7 to 4 on 10 on molar basis, which corresponds to about 2, 17 and 34 % of the total lipid content, it was found that the intermediate concentration agglutinated most efficiently the type 1 fimbrated *E. coli* bacteria.

When the concentration of MB-vesicles in the assay was varied, agglutination was most apparent in the system with 100 μl bacteria (10^9 per ml PBS), 100 μl PBS (or PBS with 2 % (w/v) mannose or maltose for inhibition and 25 μl MB-vesicles (5 μmol per ml PBS) containing about 17 % hexadecylmaltobionamide.

No aggregation of the *E. coli* bacteria was observed with LC-vesicles.

For the *Actinomyces* bacteria the sugar affinity was done only on liposome-bound ligands (Table 3). Some of the *Actinomyces* bacteria showed some spontaneous aggregation, which, however, could be diminished by dilution of the bacteria from 10^9 to 10^8 per ml. It is apparent that the LC-vesicles increased the aggregation of the *Actinomyces* bacteria, whereas MB-vesicles had no effect.

Discussion. Host-parasite interaction mediated *via* sugar ligands on the animal cell and lectin-like

Table 2. Mannose-sensitive agglutination *E. coli* bacteria with MB-vesicles, yeast cells, and guinea-pig erythrocytes, and tendency to hydrophobic interaction with Octyl-Sepharose.

Bacterial strain	% bacteria retained in column	Mannose-sensitive agglutination		
		MB-vesicles	Yeast cells	Guinea-pig erythrocytes
Culture condition: −glucose				
PN3	67	++	++	++
PN7	64	++	++	++
PN8	69	++	++	++
PN12	72	++	++	++
CU10	72	++	++	++
CU15	40	+	+	+
Culture condition: +glucose				
PN3	3	0	0	0
PN7	5	0	0	0
PN8	26	0	0	0
PN12	10	0	0	0
CU10	10	0	0	0
CU15	42	(0)	(0)	(0)

Table 3. Aggregation of *Actinomyces* bacteria with LC-vesicles (MB-vesicles controls).

	Exp 1		Exp 2	
Bacteria	Bacteria	Bacteria+ liposomes	Bacteria (1:10)	Bacteria (1:10) +liposomes
A. viscosus				
11B2	(0)	(+)	0	+
Be66	++	++	(+)	++
B236	(0)	+	(+)	++
W1053	(0)	(+)	0	++[a]
A. naeslundii				
W752	(+)	++	0	++[a]
B74	(0)	+	(0)	+
12104	0	+	0	++
398A	0	++	0	++[a]
A14	0	+	0	++[a]
A18	(0)	++	(+)	++

[a] No aggregation with MB-vesicles visible in wells, or in the microscope.

appendages (fimbriae) on the microorganism have recently come into focus.[1,14] There is, therefore, a need for simple assays that enable characterization of sugars presented, and of the sugar specificity of the bacterial fibriae. Carbohydrate receptors occur on two classes of molecules, glycolipids and glycoproteins. The sugar affinity is generally tested by using inhibiting sugars. We have used liposomes as vehicle carrying, (i) an unknown glycolipid from rat intestine, and directly tested the sugar exposure as aggregation with well-defined lectins, or (ii) a known glycolipid, and assayed the bacterial affinity for the carbohydrate moiety with the agglutination.

It is evident that a variety of sugars are presented on the intestine (Table I), possibly enabling bacteria with different sugar-binding properties to attach to mucosal membranes. Studies are in progress comparing fluorescent-lectin binding to sections of

intestinal mucosa, and aggregation of liposomes prepared from intestinal glycolipids.

The affinity of type 1 fimbriae from *Escherichia coli* for mannose-residues,[14] and of *Actinomyces* fibrilliae for galactose, or lactose residues[15] is confirmed using the liposome-aggregation assay (Tables 2 and 3). Compared to other assayes available,[12] embedding of defined glycolipids into liposomal membranes focuses on one type of ligand at a time, while keeping other parameters unchanged. Using natural, as well as synthetic glycolipids and fluorescent lipid analogs, for preparation of liposomes of varying size offers a very versatile, and inexpensive, method to study sugar-dependent host–parasite interaction, or cell–cell recognition in general.

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Organolithium-induced Ring-opening of 3-Halo-2,5-dimethylthiophen-1,1-dioxides

J. OLLE KARLSSON, SALO GRONOWITZ and ANDERS HALLBERG *

Division of Organic Chemistry 1, Chemical Center, University of Lund, P O. Box 740, S-220 07 Lund, Sweden

Thiophen-1,1-dioxides have in recent time attracted the attention as interesting precursors in organic synthesis [1-6] and the research in this field is increasing. However, to our knowledge the reactions of thiphen-1,1-dioxides with organolithium reagents have not been investigated. It can be mentioned that 2,5-dihydrothiophen-1,1-dioxides react with Grignard [7,8] and organolithium reagents [9] giving 1,3-butadienes. We now wish to report our results concerning the reactions of 3-bromo-2,5-dimethylthiophen-1,1-dioxide (*1*) and 3-chloro-2,5-dimethylthiophen-1,1-dioxide (*2*) [10] with butyllithium and phenyllithium.

Compounds *1* and *2* were both treated with two equivalents of butyllithium (*vide infra*) at −70 °C (Fig. 1). A considerable amount of butyl bromide (61%, GLC) was formed starting from *1* but no butyl chloride could be found starting from *2* (GLC-MS). This can be rationalized by a halogen–metal exchange, since vinyl bromides undergo this kind of reaction at low temperature, while vinyl chlorides react much slower. The vinyllithium derivative *3* could not be trapped however, neither with carbon dioxide nor with methanol at −70 °C. After

* Author to whom correspondence should be addressed.

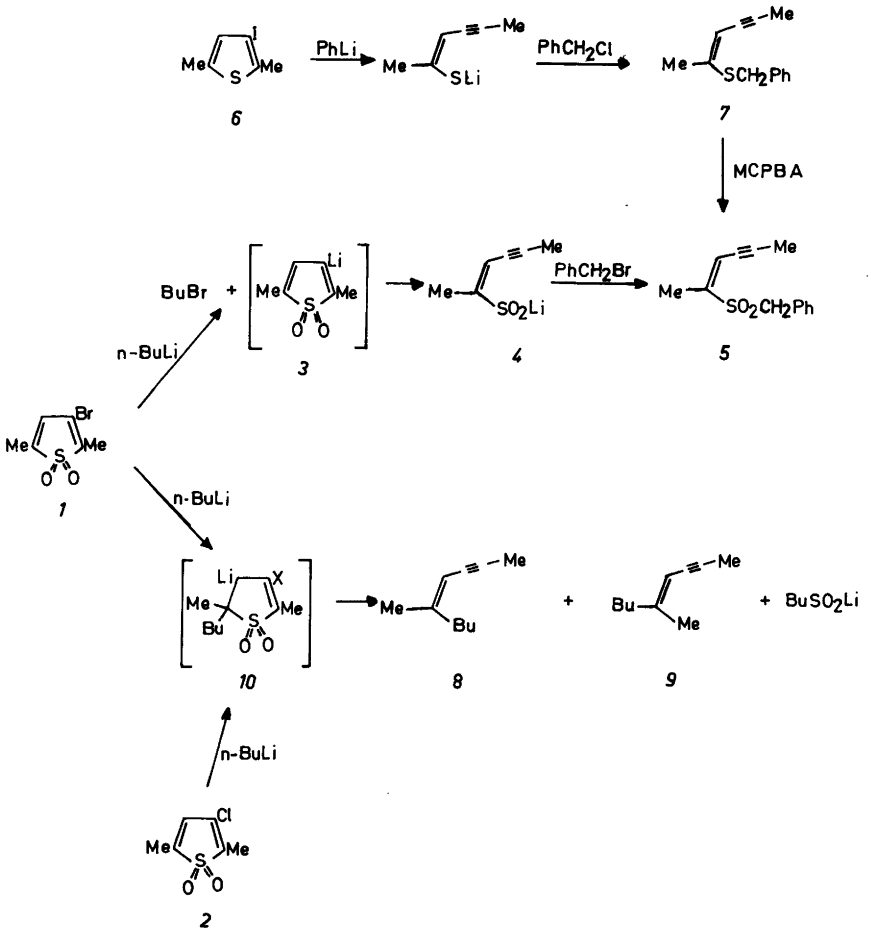

Fig. 1.

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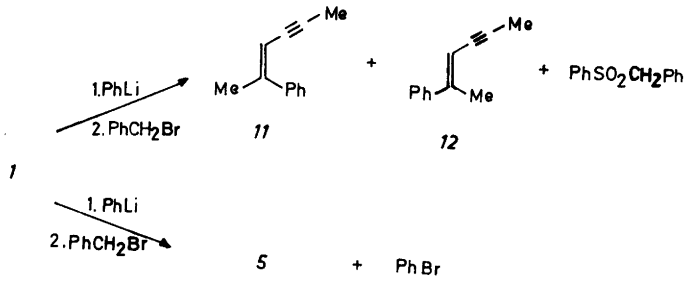

Fig. 2.

evaporation of the solvent from the reaction mixture and subsequent reflux of the residue in a mixture of methanol and excess benzyl bromide, the benzyl hexenyne sulfone *5* was obtained (GLC-MS). To confirm its structure, *5* was also prepared from (*Z*)-2-benzylthiohex-2-en-4-yne (*7*) which in turn was obtained from 2,5-dimethyl-3-iodothiophene (*6*) by a ring-opening reaction.[11] The formation of *5* is presumably due to a fast E1cB type reaction of *3* to give the lithium sulfinate *4* leading to *5* after benzylation.

More interesting though was the formation of two isomeric hexenynes, *8* and *9*, in the same ratio (1.0:1.7) from both *1* and *2* although in different yields, 35 and 80% (GLC), respectively. The hexenynes were formed rapidly, within 5 min at −70 °C.

When *1* or *2* was treated with only one equivalent of butyllithium, much of the starting material remained, especially of compound *2* (~50%), suggesting that some of the butyllithium was consumed in another reaction.

Considering these facts, we propose that *8* and *9* were formed *via* a nucleophilic attack on the 5-carbon of *1* and *2* leading to a carbanionic species, *10*. This is rapidly equilibrated and ring-opens to give *8* an *9* and lithium halide, the sulfur dioxide portion of the molecule being trapped by a second equivalent of butyllithium. The resulting butylsulfinate could not be trapped with benzyl bromide under the conditions mentioned above, but alkanesulfinates are known to be rapidly polymerized, whereas arenesulfinates are more stable.[12,13]

After treatment of *1* with two equivalents of phenyllithium and subsequent benzylation (Fig. 2), benzyl phenyl sulfone could be identified together with *5* by GLC-MS and coinjection of authentic materials. Also, bromobenzene was formed (57%, GLC) together with the hexenynes *11* and *12* (25%) in a 0.77:1.00 ratio. The structures of *8* and *9* together with *11* and *12* were deduced from MS, IR, NMR (360 MHz) and elemental analyses of the isolated compounds.

The last experiment clearly illustrates the conclusion of this report, namely that the bromo-substituted thiophen-1,1-dioxide (*1*) is attacked by the organolithium reagents by two competing processes: a, *via* halogen-metal exchange, and b, *via* nucleophilic attack on the 5-carbon. The chloro derivative, however, follows path b virtually exclusively giving a high yield of enynes.

We are currently working at developing a versatile and stereoselective enyne synthesis based on this reaction by modifying the substitution pattern of the starting materials.

Elemental analyses and spectral data for all new compounds were in accordance with the proposed structures.

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Monoclonal Antibodies Against Mammalian Ribonucleotide Reductase *

YLVA ENGSTRÖM

Department of Biochemistry I, Medical Nobel Institute, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden

Ribonucleotide reductase is present in all dividing cells, furnishing the cell with a balanced supply of the four deoxyribonucleotides needed for DNA synthesis. The reductase from calf thymus consists of two nonidentical subunits: protein M1, which has been purified to homogeneity, and protein M2. The substrate specificity and activity of the mammalian ribonucleotide reductase are controlled by nucleoside triphosphates acting as allosteric effectors and this regulation has been studied in detail.[1] In addition, the activity of the reductase is regulated during the cell cycle by some unknown mechanism, showing a strong positive correlation to the rate of DNA synthesis.

I have now used the hybridoma technique to produce monoclonal antibodies against mammalian ribonucleotide reductase to be able to simplify the purification of the enzyme, to study the localization of the enzyme in the cell and to study the control of enzyme synthesis during the cell cycle by measuring the amounts of protein M1 and M2 molecules in cell extracts using immunochemical technique.

Experimental. Female 6–8 weeks old BALB/c-mice were immunized with ribonucleotide reductase (material after affinity chromatography on dATP-Sepharose [2]), first subcutaneously using 100 μg of protein mixed with Complete Freunds Adjuvans then, after 4 weeks, intraperitoneally with 50–100 μg of protein mixed with Incomplete Freunds Adjuvans and, finally, after another 4 weeks, intraperitoneally with 100 μg of protein in phosphate buffered saline every day for four days. Fusion was made on the fifth day using spleen cells from one immunized mouse (10^8 cells) and 5×10^7 mouse myeloma cells, strain SP2/0, in the presence of 50% polyethylene glycol, m.w. 4000, as described elsewhere.[3] The cell mixture was diluted into 250 ml of hypoxanthine – aminopterin – thymidine (HAT)-containing tissue culture medium (DMEM) supplemented with 15% fetal calf serum and spread in Costar trays to give 240 independent cultures together with mouse peritoneal macrophages (10^5 cells/well) as a feeder layer. Cells were grown in HAT-medium for 10 days. Before changing to normal culture medium, the cells were grown in HT-medium without aminopterin for two passages. Hybridomas producing antibodies against ribonucleotide reductase were detected using the following modified ELISA-test.[4] Plastic microtiter wells were coated with ribonucleotide reductase and remaining sites in the wells were blocked by careful washing with phosphate buffered saline containing 1% bovine serum albumin. Then cell supernatants were incubated in the wells and after washing with the same solution rabbit antimouse antibodies labelled with horse raddish peroxidase were added followed by addition of a solution containing 2 mM 2,2'-azinodi(3-ethyl-benzothiazolinsulfonate) – 2.5 mM H_2O_2 – 0.1 M sodium acetate – 0.05 M NaH_2PO_4. Positive wells were detected by a green colour after incubation at 25 °C and the cells from such wells were frozen and kept in liquid nitrogen. Finally, cells from positive wells were cloned by limiting dilution in the presence of mouse spleen cells as feeders.

To produce antibodies, hybridoma cells (0.5×10^6 cells/ml) were incubated in DMEM tissue culture medium without serum for 7 days or 10^7 hybridoma cells were injected intraperitoneally into pristane treated BALB/c mice to form ascites tumors. The antibodies in the cells supernatants or ascites fluid were precipitated by the addition of 0.313 g of ammonium sulfate per ml solution. The precipitates were dissolved in a small volume of 0.1 M KCl – 50 mM TRIS – Cl pH 7.6, and then the ammonium sulfate was removed by dialysis or gel filtration on Sephadex G-25 columns.

To determine the specificity of the antibodies, a modified Western blot method was used.[5] A crude preparation of ribonucleotide reductase from calf thymus (material after DEAE chromatography [2] was analyzed by SDS-gel-electrophoresis and then the separated proteins were blotted from the gel to a diazobenzyloxymethyl paper. The paper was cut in strips and each strip incubated with antibodies from a specific hybridoma clone followed by rabbit antimouse-Ig antibodies and finally ^{125}I-labelled protein A. Radioactive bands were detected by autoradiography.

Results and Discussion. Viable hybridoma cells were first seen 4 days after the fusion and at the 14th day hybridoma cells were found in 211 out of 237 possible wells. Of these 237 cultures, 31 were strongly positive and 201 slightly positive in the ELISA-test. A high unspecific background (measured in the same way but with 1% bovine serum albumin instead of ribonucleotide reductase as antigen) was found in 10 cultures from the first group and in 22 cultures from the second group. Eight of the strongly positive, low background

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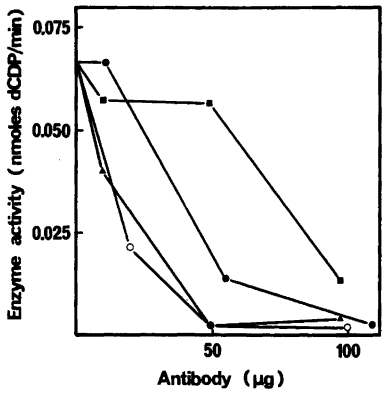

Fig. 1. Inhibition of ribonucleotide reductase activity by the monoclonal antibodies was tested by incubating a series of tubes containing enzyme (15 µg) with increasing amounts of antibodies at 0 °C for 60 min. Then the normal ^{3}H-CDP assay mixture was added and enzyme activity was measured as described earlier.[2] Clone AH 1 (■), AG8 (●), AD203 (▲) and AC1 (○).

cultures, were cloned and named AA–AH, and in Fig. 1 it is shown how 4 of these cloned lines (AC1, AD203, AG8 and AH1) could neutralize the activity of ribonucleotide reductase.

On SDS-polyacrylamide gel-electrophoresis of the same four antibodies, three of them, AC1, AD203 and AG8, gave one heavy chain band with a molecular weight around 55 000 and the fourth one, AH 1, gave two bands with molecular weights around 56 000 and 62 000, indicating that the AC 1, AD203 and AG8 antibodies belong to one of the heavy chain γ class and the AH 1 antibody to the heavy chain γ_{2b} class. The results were further confirmed by Ouchterlony double diffusion technique using subclass specific rabbit antimouse serum (Bionetics, Maryland). Now AD203 and AG8 were shown to belong to heavy chain class γ_1, AC 1 to γ_{2a} and AH 1 to γ_{2b}. All antibodies were of the κ-light chain class. When antibodies from the same four clones were tested on Western blot strips containing an electrophoresed crude mammalian ribonucleotide reductase extract, all gave a single radioactive band at the position of protein M1 of ribonucleotide reductase, while no other bands were detected. This demonstrated in a convincing way the anti-M1 specificity of these hybridomas.

Using the same Western blot method, many supernatants from uncloned cultures with positive ELISA-reaction were tested without finding any antibodies directed against protein M2. This could either be due to the lack of such antibodies or else reflect the fact that the preparation of ribonucleotide reductase used as antigen contains an excess of protein M1 over protein M2[2] and therefore M2 positive clones could be lost in the first ELISA selection.

To get anti-M2 antibodies there is a need of protein M2 antigen, free of protein M1, preferably already when immunizing the mice but even more critical when screening the cultures with the ELISA-test. The supply of such M2 preparations has been very limited earlier but can now be obtained using anti-M1 antibodies coupled to Sepharose to remove M1 from ribonucleotide reductase preparations.

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Structure of D-Erythronic Acid 3,4-Carbonate (DEC)

TERJE O. MOEN*

Department of Chemistry, University of Oslo, Oslo 3, Norway

As part of the work on oxidation of ascorbic acids going on in our laboratory,[1-3] the crystal structure of D-erythronic acid 3,4-carbonate (DEC, $C_5H_6O_6$) has been determined by X-ray diffraction methods. The crystals were found together with crystals of D-erythrono-1,4-lactone obtained by oxidation of D-isoascorbic acid with H_2O_2 in water.[3]

The crystal belongs to the triclinic system with space group P1 and cell dimensions $a=5.007(1)$ Å, $b=5.492(1)$ Å, $c=6.358(1)$ Å, $\alpha=68.20(2)°$, $\beta=82.76(2)°$, $\gamma=68.37(2)°$, $Z=1$. The density was calculated to be $D_x=1.78$ g/cm^3.

1782 independent reflections were measured on an automatic four-circle diffractometer at −150 °C with Mo$K\alpha$-radiation up to 2θ max = 80°. Of these, 1737 reflections were recorded as observed using an observed unobserved cutoff at 3.0σ (I). No corrections for absorption or secondary extinction were applied because the molecule consists of only light atoms and the crystal-size (0.5 × 0.3 × 0.1 mm) is small.

The structure was solved by direct methods,[4] successive use of Fourier-syntheses and finally refined by full-matrix least squares technique.[5] Hydrogen atom positions were refined with isotropic temperature factors while anisotopic temperature factors were introduced for non-hydrogen atoms.

The final ordinary R-value is 5.0 % with R_w = 4.3 %. Final fractional coordinates with estimated standard deviations are given in Table 1. The principal thermal vibration ellipsoids for non-hydrogen atoms correspond to maximum r.m.s. amplitudes between 0.08 and 0.16 Å.

*Present address: Norwegian Defence Research Establishment (NDRE), P.O.B. 25, N-2007 Kjeller, Norway.

Table 1. Final fractional coordinates with estimated standard deviations in parentheses.

Atom	x	y	z
O1	0.1403 (4)	0.3315 (4)	0.5332 (4)
O2	−0.1823 (4)	0.8841 (4)	0.4106 (3)
O3	−0.0253 (4)	1.2342 (3)	0.0136 (3)
O4	−0.2904 (4)	1.0969 (4)	−0.1419 (3)
O5	−0.4080 (4)	1.5529 (4)	−0.2027 (3)
O6	0.5258 (4)	0.4266 (3)	0.3479 (3)
C1	0.2589 (4)	0.4942 (4)	0.4216 (3)
C2	0.1095 (4)	0.8112 (4)	0.3431 (3)
C3	0.1187 (4)	0.9341 (4)	0.0844 (3)
C4	−0.0532 (4)	0.8427 (4)	−0.0357 (3)
C5	−0.2537 (4)	1.3123 (4)	−0.1174 (3)
HO2	−0.228 (9)	0.764 (9)	0.504 (7)
HO6	0.595 (5)	0.255 (5)	0.390 (4)
HC2	0.199 (6)	0.880 (6)	0.403 (5)
HC3	0.304 (6)	0.913 (6)	0.018 (4)
H1C4	−0.117 (6)	0.709 (6)	0.054 (5)
H2C4	0.068 (6)	0.784 (6)	−0.158 (5)

Table 2. Bond distances, angles and dihedral angles with estimated standard deviations in parentheses.

Distance	(Å)	Distance	(Å)
C1−O1	1.212 (2)		
C1−O6	1.322 (2)	C1−C2	1.526 (2)
C2−O2	1.420 (2)	C2−C3	1.531 (2)
C3−O3	1.447 (2)	C3−C4	1.531 (2)
C4−O4	1.448 (2)	C5−O4	1.327 (2)
C5−O3	1.341 (2)	C5−O5	1.207 (2)
Angle	**(°)**	**Angle**	**(°)**
O1−C1−O6	126.0 (1)	O1−C1−C2	122.9 (1)
O6−C1−C2	111.1 (1)	C1−C2−C3	109.2 (1)
C1−C2−O2	111.2 (1)	O2−C2−C3	108.6 (1)
C2−C2−O3	108.3 (1)	C2−C3−C4	113.7 (1)
O3−C3−C4	103.5 (1)	C3−C4−O4	103.9 (1)
C4−O4−C5	109.8 (1)	C3−O3−C5	109.8 (1)
O4−C5−O3	112.7 (1)	O4−C5−O5	124.2 (2)
O3−C5−O5	123.2 (2)		
Dihedral angle		**(°)**	
O1−C1−C2−O2		−2.2 (2)	
O1−C1−C2−C3		−122.0 (2)	
C1−C2−C3−O3		179.7 (1)	
C1−C2−C3−C4		65.2 (1)	
O6−C1−C2−O2		176.5 (1)	
O6−C1−C2−C3		56.8 (2)	
C2−C3−O3−C5		−118.4 (1)	
C2−C3−C4−O4		111.9 (1)	
C3−O3−C5−O5		−178.5 (1)	
C3−C4−O4−C5		6.7 (2)	
C4−C3−O3−C5		2.6 (2)	
O3−C3−C4−O4		−5.4 (2)	
C4−O4−C5−O3		−5.5 (2)	
C4−O4−C5−O5		174.6 (1)	
O2−C2−C3−O3		58.3 (2)	
O2−C2−C3−C4		−56.2 (2)	

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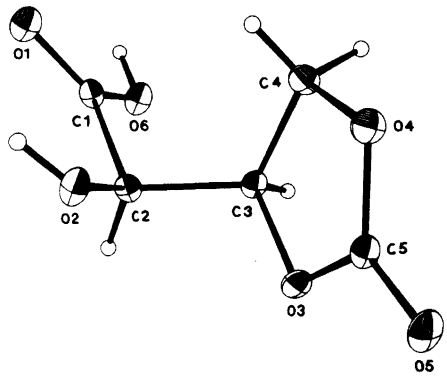

Fig. 1. ORTEP drawing of DEC as seen along the C5 −O5 bond.

Bond distances, bond angles and some dihedral angles for DEC are given in Table 2 with estimated standard deviations in parenthesis. These are calculated from the correlation matrix of the final least squares refinement cycle. Fig. 1 shows the molecule of DEC as seen along the C5−O5 bond.

The bond distances and angles of Table 2 are as expected, but differ slightly from those found in similar structures. Some selected bond distances of DEC compared with those of other structures are given in Table 3. The larger deviations seen from the carbonate group in BDPVC, bis(dimethylphosphatovinyl) carbonate ($C_9H_{16}P_2O_{11}$), might be explained by the noncyclic arrangement of the carbonate group. We also observe the significant differences between bond distances C5−O3 (1.341 Å) and C5−O4 (1.327 Å) which generally are regarded as equal in cyclic carbonates.[6] The bond distances C3−O3 and C4−O4 are greater than the similar distances in ethylene carbonate,[6] but more equal to those of erythritol.[10]

The crystal structure consists of monomers, hydrogen bonded between O2 and HO6 and between O5 and HO2, as shown in Fig. 2. Table 4 gives data for the hydrogen bonding pattern. It is observed that DEC is not built up of dimers as the simple carboxylic acids.[7,8]

The internal contact O2---O1 is 2.715 Å with the distance HO2---O1 being 2.37 Å which could imply a weak internal hydrogen bond, even though the angle O2−HO2−O1 is only 108°. This suggests the existence of a bifurcated hydrogen bond arrangement as earlier observed in 2,5-dihydroxy-1,4-benzoquinone[12] and β-L-lyxopyranose.[13]

The carboxylic acid group defined by O1, O6, C1 and C2, is planar within experimental error with C1 0.007 Å above the least squares plane as greatest deviation. The condensed carbonate-ring system, however, cannot be considered as planar. The best least squares plane is fitted through O3, O4, C5 and O5, with C3 and C4 0.04 and 0.13 Å beneath the plane. This is in agreement with the non-planarity found in the ethylene carbonate molecule.[6] The angle between the best planes through O1, O6, C1, C2 and O3, O4, C5, O5 is calculated to 60.6°.

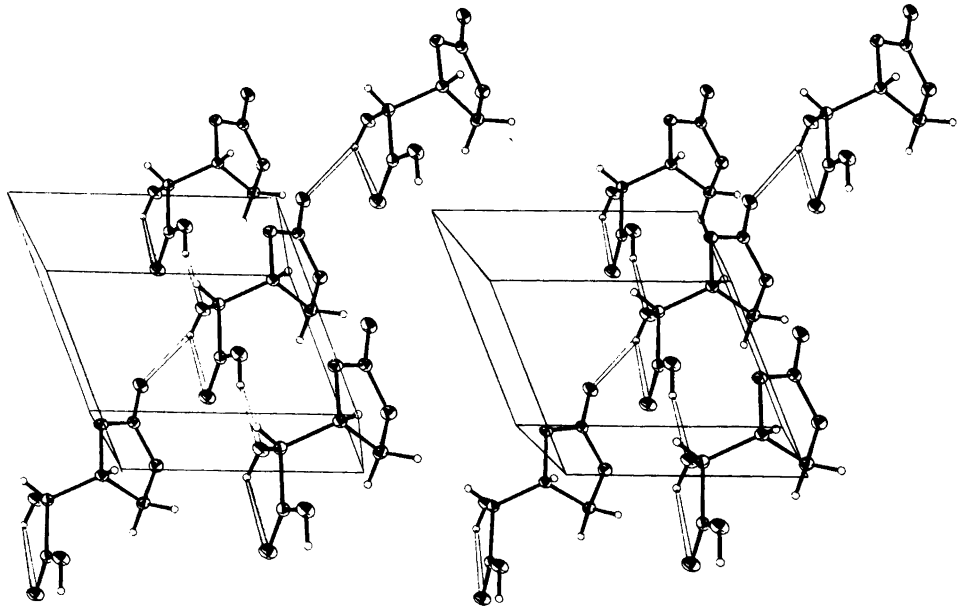

Fig. 2. The hydrogen bonding pattern of DEC as seen along the *a*-axis of the unit cell.

Table 3. Bond distances (Å) in some selected compounds.

Compound	C1–O1	C1–O6	C1–C2	C2–O2	C2–C3	C3–C4	C3–O3	C4–O4	C5–O3	C5–O4	C5–O5
DEC	1.212	1.322	1.526	1.420	1.531	1.531	1.447	1.448	1.341	1.327	1.207
Propionic acid [7]	1.23	1.32	1.50		1.54						
Butyric acid [8]	1.20	1.32	1.52		1.51	1.52					
BDPVC [a]							1.38	1.40	1.36	1.37	1.17
Eryth-ritol [b]			1.523	1.433	1.539	1.523	1.433	1.425			
Ethylene carbonate [6]						1.52	1.40	1.40	1.33	1.33	1.15
Vinylene carbonate [11]						1.236	1.349	1.396	1.284	1.335	1.160

[a] Bis(dimethylphosphatovinyl) carbonate ($C_9H_{16}P_2O_{11}$); Ref. 9. [b] Neutron diffraction investigation; Ref. 10.

Table 4. Data for the hydrogen bonding in DEC.

	Distance	(Å)	Angle	(°)
1	O2---O6	2.688 (2)	O2---HO6–O6	164 (2)
	O2---HO6	1.89 (3)		
2	O5---O2	2.850 (2)	O5---HO2–O2	161 (4)
	O5---HO2	2.09 (4)		
3	O1---O2 [a]	2.715 (2)	O1---HO2–O2	108 (4)
	O1---HO2	2.37 (4)		

[a] Intramolecular.

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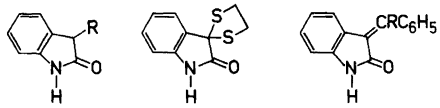
Raney Nickel-induced Alkylation Reactions *

ERNEST WENKERT,** N. V. BRINGI and HARRY E. CHOULETT

Department of Chemistry, Iowa State University, Ames, Iowa 50010, U.S.A.

In connection with studies on the chemistry of oxindoles [2] it became important to develop a simple conversion of isatin (3-oxooxindole) into oxindole (*1a*)*** and hence an investigation of this transformation by the preparation of isatin ethylene thioketal (*2*) and its desulfurization was initiated. The thioketal could be prepared by standard means. Whereas Raney nickel treatment for sulfur removal in benzene or ethanol solution for short periods of time converted the thioketal (*2*) into the desired oxindole (*1a*), longer reaction times in ethanol led to high yields of 3-ethyloxindole (*1b*). Although the extraneous ethyl group could have originated *a priori* from the two-carbon moiety of the thioketal (*2*), its more likely derivation from the solvent was assured by the formation of the 3-alkyloxindoles *1c* and *1d* in the Raney nickel treatment of *2* in methanol and isopropyl alcohol, respectively. These results suggested that the desulfurization preceded the alkylation and that the two reactions were independent of each other. When, as a consequence, the ethylation of oxindole (*1a*) was attempted, *1b* was produced, but after longer reaction time.

It was now clear that the alkylations were identical in nature with the large number of reported Raney nickel-induced N-alkylation [3] and a few C-alkylations [4] and were mechanistically similar to the varied examples of known base-catalyzed alkylations with alcohols.[4] Thus, nickel acted as the oxidizing agent in the conversion of alcohol to aldehyde or ketone, as the base for the condensation of the carbonyl compound and oxindole and as the reducing agent in the reduction of the resultant 3-alkylideneoxindole. Since this view required the nickel surface to be in an intermediate state of oxidation for efficient promotion of the alkylation, it explained readily the more successful ethylation of the thioketal (*2*) than oxindole (*1a*). Presumably the desulfurization lowered the hydrogenation activity of the Raney nickel and hence made it a better agent for the dehydrogenation of ethanol, the first step in the alkylation. On this basis it was predicted, and thereafter verified, that the addition of mercaptans should increase the efficiency of the ethylation of oxindole (*1a*).



1a, R = H
1b, R = Et
1c, R = Me
1d, R = i-Pro
1e, R = $CH_2C_6H_5$

2

3a, R = H
3b, R = Me

Dependence on heterogeneous catalysis for success of the alkylations could be predicted to make the results erratic. Hence it was no surprise that oxindole interaction with methanol gave sometimes 3-methyloxindole (*1c*) and often 3-methyleneoxindole polymer.[5] Reaction with benzyl alcohol led usually to 3-benzyloxindole (*1e*), but in one instance (with the use of old Raney nickel) to 3-benzaloxindole (*3a*). Reaction with α-phenylethanol yielded the alkylideneoxindole *3b*.

The alkylation of active methylene compounds other than oxindole also was investigated.[6] Treatment of ethyl acetoacetate with ethanol and Raney nickel gave merely ethyl β-hydroxybutyrate,[7] whereas similar reaction with nickel, which had been heated for 24 h in refluxing alcohol, yielded ethyl α-ethylacetate. Attempts to ethylate diethyl malonate or 2-tetralone were unsuccessful and a similar undertaking with deoxybenzoin yielded bibenzyl.[8,9]

In view of a reported oxidation of a 1,4-diol to a butyrolactone with Raney nickel [10] it was of interest to effect similar changes of 1,4-butanediol and 1,5-pentanediol. In one run the former was transformed to γ-butyrolactone, but usually low yields of 4-hydroxybutanal and 5-hydroxypentanal, respectively, were obtained.

Since completion of the present work there have been recorded various cases of Raney nickel-induced oxidation-reduction reactions on alcohols [11] and similarly catalyzed *N*-alkylations of amines with alcohols.[12] Furthermore, recent examples of *C*-alkylations with alcohols, albeit without nickel, related to the above alkylations of oxindoles also are on record.[13]

Experimental. Ultraviolet spectra of 95% ethanol solutions were recorded on Beckman DU and Cary 14 spectrophotometers and infrared spectra of

* For a preliminary account of part of this work, see Ref. 1. The present communication is based on H. E. Choulett, M.S. dissertation, Iowa State University, 1960.

** Present address: Department of Chemistry (D-006), University of California – San Diego, La Jolla, CA 92093, U.S.A.

*** After the publication of the preliminary account of this work appeared,[1] Professor David A. H. Taylor (University College, Ibadan, Nigeria) kindly informed the authors that oxindole has been prepared in his laboratory for many years by Raney nickel-induced hydrogenation of isatin in ethanol at 150 °C.

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chloroform solutions on a Perkin-Elmer 137 Infracord spectrophotometer. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Alumina (80 – 200 mesh), activated by being agitated in ethyl acetate for 48 h, washed with water and methanol and dried under an infrared lamp for 48 h, was used for column chromatography. All 2,4-dinitrophenylhydrazones were purified by chromatography on a 4:1 Bentonite-Celite mixture.[14]

Isatin ethylene thioketal (2). A mixture of 500 mg of isatin, 0.5 ml of 1,2-ethanedithiol, 0.5 ml of boron trifluoride etherate in 4 ml of absolute methanol and 10 ml of glacial acetic acid was stirred at room temperature for 30 h. Vacuum removal of the solvents and trituration of the residue with water led to a solid, 630 mg (80%), whose sublimation gave colorless crystals of oxindole *2*, m.p. 200 – 201 °C. Anal. $C_{10}H_9ONS_2$: C, H, N.

General reduction and/or condensation procedure. A suspension of 1 – 5 g of Mozingo, W-2, Raney nickel[15] and 100 – 500 mg of oxindole *1a* or *2* in 20 – 50 ml of the appropriate solvent was refluxed with stirring for the indicated length of time. The catalyst was filtered through Supercel and the filtrate evaporated under vacuum. The residue was crystallized with or without prior alumina chromatography.

*Oxindole (*1a*).* Reduction of thioketal *2* in ethanol or benzene for 4 h gave oxindole (*1a*) (identical in all respects with an authentic sample) in 63 and 79% yield, respectively.

*3-Ethyloxindole (*1b*).* Reduction of thioketal *2* in ethanol for 24 h produced oxindole *1b* [m.p. mmp 104 °C (lit.[16] m.p. 104 °C)] in 83% yield. Nickel-induced ethylation of oxindole (*1a*) in ethanol for 72 h led to the same product in 90% yield, while 24 h nickel treatment of ethanol solutions of *1a*, *1a* and *p*-thiocresol, and *1a* and 1,2-ethanedithiol produced 3-ethyloxindole (*1b*) in 10, 20 and 50% yield, respectively.

*3-Methyloxindole (*1c*).* Reduction of thioketal *2* in methanol for 36 h and chromatography produced oxindoles *1a* and *1c* (identical in all respects with an authentic sample) in 43 and 16% yield, respectively. Nickel treatment of a methanol solution of *1a* for 84 h gave *1c* in 18% yield accompanied by polymer.

*3-Isopropyloxindole (*1d*).* Reduction of thioketal *2* in isopropyl alcohol for 84 h afforded oxindole *1d* [m.p. mmp 106 – 108 °C (lit.[5] m.p. 107 – 108 °C); identical in all respects with an authentic sample] in 32% yield.

*3-Benzyloxindole (*1e*).* A suspension of 500 mg of oxindole (*1a*) and 5.0 g of the nickel catalyst in 20 ml of benzyl alcohol was stirred at 105 °C for 72 h. The mixture was filtered and the filtrate evaporated under vacuum. Chromatography of a benzene solution of the residue on alumina and elution with ether – chloroform mixtures yielded 600 mg (72%) of colorless crystals of oxindole *1e*, m.p. 128 – 129 °C (lit.[17] m.p. 131 °C); IR: C=O 5.84 (s) μm; UV (log ε): 252 (3.95), 277 (sh, 3.13) nm. Anal. $C_{15}H_{13}ON$: C, H, N.

*3-Benzylideneoxindole (*3a*).* On one occasion the same experiment with aged catalyst gave an 81% yield of yellow, crystalline oxindole *3a*, m.p. mmp 177 – 177.5 °C (lit.[17] m.p. 175 – 176 °C); IR: C=O 5.87 (s), C=C 6.20 (m) μm; UV (log ε): 253 (4.14), 323 (4.05) nm (identical in all respects with an authentic specimen).

*3-(α-Methylbenzylidene-)oxindole (*3b*).* A reaction between oxindole (*1a*) and α-phenylethanol was carried out in the manner of the above preparation of *1e*. Elution of the alumina chromatogram with hexane – benzene mixtures yielded a liquid (in an amount equivalent to >10% of the excess alcohol used) whose 2,4-dinitrophenylhydrazone (m.p., mmp 253 – 255 °C) revealed it to be acetophenone. Final elution with chloroform led to 20% recovery of starting oxindole and earlier elution with ether yielded yellow, crystalline oxindole *3b*, m.p., mmp 196 – 197 °C (from hexane); IR: C=O 5.87 (s), C=C 6.20 (m) μm; UV (log ε): 255 (4.22), 260 (4.22), 302 (3.85) nm. Anal. $C_{16}H_{13}ON$: C, H, N.

Ethyl β-hydroxybutyrate. A suspension of 10.0 g of catalyst in 13.00 g of ethyl acetoacetate and 130 ml of absolute ethanol was refluxed with stirring for 72 h. The mixture was filtered and the filtrate evaporated. Distillation of the resultant residue afforded 10.70 g (81%) of liquid hydroxyester [b.p. 83 – 85 °C/10 kPa; IR (CCl_4): OH 2.77 (w), C=O 5.79 (s) μm], whose 3,5-dinitrobenzoate, m.p., mmp 82 – 83 °C (from aqueous ethanol) was identical in all respects with an authentic sample.

Ethyl α-ethylacetoacetate. A suspension of 10.0 g of catalyst in 130 ml of absolute ethanol was refluxed with stirring for 26 h, whereupon a solution of 13.0 g of ethyl acetoacetate in 20 ml of absolute ethanol was added over a 15 min period and the stirring and heating continued for 47 h. The mixture was filtered and the filtrate evaporated. Distillation of the residue gave colorless, liquid ketoester, b.p. 85 – 87 °C/12 kPa; IR (CCl_4): C=O 5.79 (s), 5.86 (s), C=C 6.18 (m) μm. Anal. $C_8H_{14}O_3$: C, H.

Ketonic cleavage of the β-ketoester by standard means and derivatization of the product yielded 2-pentanone 2,4-dinitrophenylhydrazone, m.p., mmp 143 – 144 °C.

Bibenzyl. A suspension of 15.0 g of the catalyst in a solution of 2.00 g of deoxybenzoin in 60 ml of absolute ethanol was refluxed with stirring for 72 h. The mixture was filtered and the filtrate evaporated. Chromatography of the residue on alumina and elution with hexane yielded 1.40 g (71%) of crystalline bibenzyl, m.p. mmp 51 – 52 °C (identical in all respects with an authentic sample).

4-Hydroxybutanal. A suspension of 10.0 g of the catalyst in 50 ml of absolute ethanol was refluxed for 24 h. The liquid then was decanted and the catalyst washed exhaustively with benzene. The solid was suspended in 20.0 g of 1,4-butanediol and the mixture stirred at 115 °C for 45 h. It then was filtered and the filtrate distilled fractionally, yielding 18.0 g of a colorless liquid, b.p. 114–116 °C/10 kPa. Treatment of 640 mg thereof with 2,4-dinitrophenylhydrazine gave 350 mg (20%) of orange, crystalline γ-hydroxybutyraldehyde 2,4-dinitrophenylhydrazone, m.p. 119–120 °C (lit.[15] m.p. 120 °C); IR: NH 3.02 (w), C=N 6.12 (s) μm. Anal. $C_{10}H_{12}O_5N_4$: C, H.

5-Hydroxypentanal. The same reaction and work-up with 10.0 g of catalyst and 20.0 g of 1,5-pentanediol yielded 17.0 g of colorless liquid, b.p. 120–122 °C/11 kPa, derivatization of 570 mg of which gave 200 mg (15%) of reddish orange, crystalline δ-hydroxyvaleraldehyde 2,4-dinitrophenylhydrazone, m.p. 105–106 °C (lit.[16] m.p. 119 °C); IR: NH 3.00 (w), C=N 6.14 (s) μm. Anal. $C_{11}H_{14}O_5N_4$: C, H, N.

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Temperature Effects on Electrode Processes. II. The Entropy of Formation of Ion Radicals of Heteroaromatic Compounds

MORTEN SVAAN and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The temperature dependence of reversible electrode potentials, measured by phase selective second harmonic *a.c.* voltammetry, for the formation of ion radicals of hetroaromatic compounds structurally related to anthracene was determined in order to obtain entropies of formation. All compounds investigated had the anthracene nucleus with the 9 and 10 positions occupied by heteroatoms, S, O, N or Se. The entropy for the formation of the anion radical of phenazine (9 = N = 10) in acetonitrile was observed to be equal to −4.7 cal/K mol, a value in close agreement with that for anthracene anion radical indicating little influence of the nitrogen atoms on the charge distribution of the anion radical. The entropies of formation of cation radicals of the hetroaromatics were very much greater than expected for the anthracene cation radical ranging from +10.0 to +25.3 cal/K mol. Enthalpy changes and reversible potentials, relative to those for the reduction of anthracene, were observed to be linearly related with a slope of 0.907 and a correlation coefficient of 0.999. The deviation of the slope from unity shows the importance of entropy changes in the redox processes.

We have recently shown that the entropy changes during reversible electrode processes (1) are closely

$$A \pm e^- \rightleftarrows B^{\mp} \qquad (1)$$

related to the charge distributions in the ion radicals formed upon charge transfer to or from alternant aromatic hydrocarbons (AAH).[1] The entropy changes were determined from the temperature coefficients of the reversible electrode potentials assuming that the enthalpy changes were constant over the small temperature ranges employed, as indicated in eqn. (2). Appreciable charge

$$dE^{rev}/dT = \Delta S/nF \qquad (2)$$

delocalization is a characteristic of the ion radicals of AAH and the ΔS values observed were relatively small.

Reversible electrode potentials are often correlated with gas phase ionization potentials and electron affinities as well as with molecular orbital parameters.[2-12] Invariably, entropy effects are considered to be negligible, a reasonable approximation for the gas phase reactions but somewhat questionable for the corresponding redox potentials measured in solution. To illustrate this point we have calculated the enthalpy changes relative to that for the reduction of triphenylene from the data previously reported [1] using eqn. (3)

$$\Delta\Delta H = -nF(\Delta E^{rev}) + T\Delta\Delta S \qquad (3)$$

(Table 1). The column headed $T\Delta S$ gives the contribution of entropy to the free energy changes in the reactions at 273.2 K. Correlation of E^{rev} with $\Delta\Delta H$, when expressed in the same units, results in a slope of 0.98 and a correlation coefficient of 0.998. At the level of accuracy achievable in gas phase measurements, the error introduced by the entropy effects for the redox reactions of AAH is of little consequence. However, such correlations have been reported for aromatic compounds in general that do not belong to any closely related group such as the AAH.[8] Correlations have also been reported for non-aromatic nitrogen containing compounds in which the charges on the resulting radical cations are surely localized on the heteroatoms.[7] Reasonably good correlations are found in practice and it is of interest to examine why this is so. Is it that

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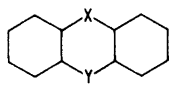
Table 1. Relative contributions of enthalpy and entropy for the formation of alternant aromatic hydrocarbon anion radicals in acetonitrile.[a]

AAH	$E^{rev}_{273.2}$/mV[b]	$-\Delta S_{273.2}$[c]	$-T\Delta S$/kcal mol^{-1}	$-\Delta\Delta H$[d]
Triphenylene	0 (0)	0.87	0.238	0
Perylene	779 (18.0)	2.33	0.637	18.4
Benzoperylene	576 (13.3)	2.47	0.675	13.7
Benzopyrene	604 (13.9)	2.67	0.729	14.4
Pyrene	367 (8.5)	2.74	0.749	8.97
9-Phenylanthracene	530 (12.2)	4.43	1.21	12.0
Anthracene	493 (11.4)	4.54	1.24	11.1
9,10-Diphenylanthracene	571 (13.2)	4.54	1.24	12.9

[a] Electrode potential and entropy data from Ref. 1. [b] Reversible electrode potentials relative to the reduction potential for triphenylene, numbers in parentheses are in kcal/mol. [c] The entropy change in cal/K mol from Ref. 1. [d] The enthalpy change relative to that for triphenylene reduction in kcal/mol.

entropy effects are negligible as commonly assumed? Or, do systematic errors in solution or gas phase data cause a cancellation of the entropy effects? A third intriguing possibility is that changes in ΔH are paralleled by corresponding changes in ΔS resulting in good correlations with significant errors in the slopes when they are assumed only to arise from the ΔH contribution.

We now report the second stage of our investigations of entropy effects on electrode processes. We have chosen another rather closely related series of compounds of structure *1* in order to test the effect of heteroatoms introduced into



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aromatic systems on the entropy changes of the redox reactions. The compounds are related to anthracene and X and Y are O, S, Se, N, N – H and N – Me. The electrochemistry of these compounds has been investigated intensively in recent years.[13-24] It was hoped that this investigation would provide answers to some of the questions posed in the previous paragraph.

RESULTS AND DISCUSSION

The temperature coefficients of the reversible electrode potentials for the reduction of phenazine and the oxidation of all of the other compounds with structure *1* studied were obtained by measurements

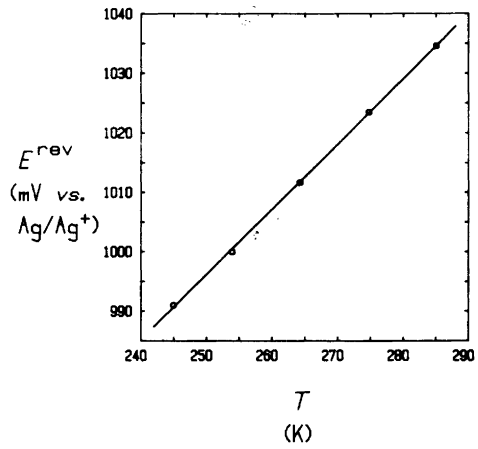


Fig. 1. Correlation of the reversible potential for the oxidation of thianthrene (0.5 mM) in acetonitrile containing Bu_4NBF_4 (0.1 M) with temperature.

over about a 40 K interval in acetonitrile. A typical set of data, that for the oxidation of thianthrene, is illustrated in Fig. 1. Reversible potentials calculated from correlation equations were generally within 0.2 mV of the observed values. In this case the deviation is as great as 0.5 mV at one T. The correlation coefficient in this particular case was 0.9998 and was greater than 0.999 in all correlations. The measurement precision was discussed in detail in Ref. 1. In general, the measurements provide dE^{rev}/dT to ± 0.01 mV/K which corresponds to an error of ± 0.2 cal/K mol in ΔS.

All of the experimental temperature coefficients along with the reversible potentials, ΔS and $\Delta\Delta H$ at

Table 2. Entropies and relative enthalpies of formation of ion radicals in acetonitrile for compounds with structure *1*.

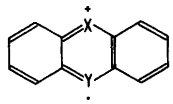
Compound Name	X	Y	(dE^{rev}/dT)/ mV K^{-1} [a]	$E^{rev}_{273.2}$/mV [b]	$\Delta S_{273.2}$ [c]	$-\Delta\Delta H$ [d]
Anthracene [e]	C–H	C–H	−0.197	−2257	−4.54	0
Phenazine	N	N	−0.205	−1957	−4.73	6.97
5,10-Dimethyl-5,10-dihydrophenazine	N–Me	N–Me	0.434	−60	10.0	46.7
N-Methylphenoxazine	N–Me	O	0.497	517	11.5	59.6
N-Methylphenothiazine	N–Me	S	0.530	520	12.2	59.5
Phenoxazine	N–H	O	0.627	429	14.5	56.7
Phenothiazine	N–H	S	0.626	412	14.4	56.4
N-Methylphenoxazine	O	N–Me	0.497	517	11.5	59.6
Phenoxazine	O	N–H	0.627	429	14.5	56.7
Dibenzodioxin	O	O	0.711	1191	16.4	73.8
Phenoxathiine	O	S	0.824	963	19.0	67.8
Phenoxaselenin	O	Se	1.046	926	24.1	65.6
N-Methylphenothiazine	S	N–Me	0.530	520	12.2	59.5
Phenothiazine	S	N–H	0.626	412	14.4	56.4
Phenoxathiine	S	O	0.824	963	19.0	67.8
Thianthrene	S	S	1.098	1021	25.3	67.4

[a] Data obtained in experiments similar to those described in Table 1. Negative values refer to reductions and positive to oxidations. [b] Reversible potential at 273.2 K *vs.* Ag/Ag^+ (CH_3CN). [c] In cal/K mol. The conversion unit for mV/K to cal/K mol is 23.06. [d] The enthalpy change relative to that for the reduction of anthracene in kcal/mol. [e] Data from Ref. 1.

273.2 K are gathered in Table 2. The pertinent data for the reduction of anthracene from Ref. 1 are included for comparison. The $\Delta\Delta H$ values are relative to that for the reduction of anthracene. The compounds are grouped according to structural similarities and some of the compounds fit in more than one series. The two compounds in the first group, phenazine and anthracene, differ from the others in that the central rings are aromatic and their reactions are the only reduction processes. The other groups have X constant while the structural feature Y is varied.

To begin with we will summarize the trends observed in ΔS and leave $\Delta\Delta H$ for later. The first two compounds are planar and the anion radicals formed upon reduction are also planar with only small structural changes expected to take place upon charge transfer. The entropy values are very similar and small in comparison to all of the others in the table. However, we recall that these values are high on the scale observed for the AAH (Table 1).[1] Following the reasoning developed earlier[1] we suggest that the charge distributions in the two anion radicals are similar with some concentration of charge in the 9,10-positions of anthracene and the corresponding 5,10-positions of phenazine. The small difference in entropy changes could reflect the fact that N is more electronegative than C.

When X is N–Me, the entropy changes during oxidation increased in going from Y = N–Me to O to S. The largest difference is the first one. In order for charge to be dispersed into the two carbon containing rings, onium structures *2* and planar



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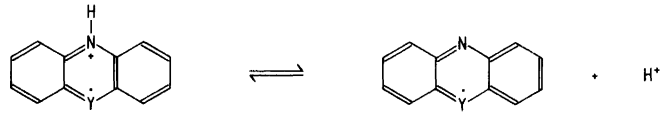
resonance forms must be favorable. The most favorable situation for bond lengths and angles in this ring system is for the parent, anthracene and the corresponding cation radical. Since N^+ is isoelectronic with C, the smallest perturbation in geometry in structures *1* and *2*, relative to



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anthracene is when X=Y=N−R. This is also evident from the comparison in the previous paragraph. Thus, it appears that the entropy changes in this series can be explained by the decreasing contribution of the onium structure *2* to the overall cation radical structure as N−Me is replaced by O and then S. The covalent radii of C, O, S and Se are 0.77, 0.74, 1.04 and 1.17 Å, respectively.[25] As the groups, Y, become larger achieving structure *2* becomes more difficult.

The reasoning carried through in the previous paragraph appears not to be valid for the next two compounds where X=N−H and Y is either O or S. In this case the entropy changes are greater and identical within experimental error. In this case some caution must be exercized in interpreting the results in terms of structure *2*. Equilibrium (4) could contribute significantly to the overall result. The existence of cation radicals *3*, Y=S[26,27] or O,[28] in acidic media was demonstrated by ESR spectroscopy. However, *3* rapidly decays in neutral media and the radical *4*, Y=S, has been identified by ESR measurements.[29] The occurrence of reaction (4) and the structural changes in cation radical *3* due to the weakening of the N−H bond could contribute significantly to the increase in the observed entropy change. The extent to which equilibrium (4) depends on the nature of Y is not known. However, we did not observe any difference in ΔS for the oxidation of phenothiazine when the potentials were measured in acetonitrile containing trifluoroacetic acid (5%). This suggests either that equilibrium (4) does not significantly affect the results or that the acid is not sufficiently dissociated to participate in (4).

Large changes in ΔS are observed in going down the series where X=O. When Y=O as well, ΔS was observed to be 16.4 which is significantly greater than the case where X=Y=N−Me where ΔS was found to be 10.0 cal/K mol. We can use the same reasoning as before in terms of the changing contributions of structure *2* as X and Y are changed. Further large changes in ΔS were observed in going to Y=S and Se.

Large changes in ΔS are also observed when X=S and the same arguments can be applied. In this series, the only compound not appearing in previous series is X=Y=S, thianthrene, the oxidation of which gave the largest entropy change observed in this study, 25.3 cal/K mol.

We can now return to the questions raised earlier regarding the effect of entropy changes on correlations of E^{rev} with gas phase and molecular orbital parameters. The quantity expected to relate to these parameters is ΔH rather than E^{rev}. In the case of correlations involving AAH, the effect of entropy changes was observed to be relatively small. However, the entropy changes observed for the reactions of compounds of structure *1* are very much greater than those for AAH redox reactions. In Fig. 2, $\Delta\Delta H$ is plotted *vs.* E^{rev} for all the processes described in Table 2. Both quantities are expressed in kcal/mol relative to the values for the reduction of anthracene. The slope of the correlation line was observed to be 0.907 with a correlation coefficient of 0.999. In this case it is clear that correlations of E^{rev} with any set of parameters linearly related to ΔH

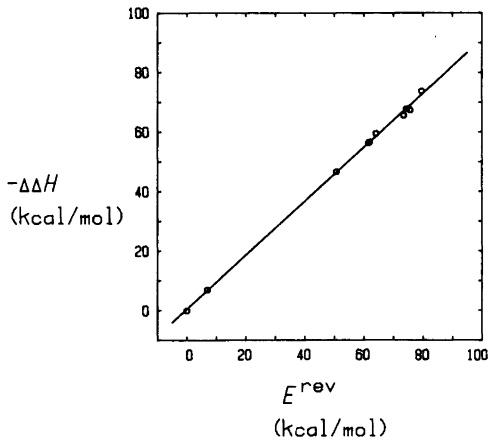

Fig. 2. Correlation of reversible potentials with enthalpy changes for redox reactions of heteroaromatic compounds.

will also appear to be linear. It is also apparent that the resulting slopes will be significantly in error if E^{rev} is assumed to reflect the value of ΔH.

In conclusion, this study has provided further evidence that the factor of overweighing importance in determining the entropy changes in reversible electrode processes is connected with the localization of charge and the accompanying changes in the ordering of the environment around the ions and the neutral molecules.

EXPERIMENTAL

The experimental procedures and data handling operations were described in detail in the first paper in this series.[1] Most of the compounds used in this study were generously provided in highly pure state by Dr. Ole Hammerich, University of Copenhagen.

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Temperature Effects on Electrode Processes. III. Electronic and Steric Effects on the Entropy of Formation of Nitrobenzene Anion Radicals

MORTEN SVAAN and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The effect of multiple substitution and alkyl substituents on the reversible electrode potentials was determined for the reduction of nitrobenzenes in acetonitrile. The entropy of formation of the anion radicals was observed to be influenced both by steric and electronic effects of the alkyl substituents. The effect of multiple substitution was observed to be dependent upon substitution pattern. The entropies of formation of the anion radicals of dinitrobenzenes were observed to be −7.8 (1,3), −5.3 (1,2) and −5.1 (1,4) as compared to −11.7 cal/K mol for the formation of nitrobenzene anion radical. Mono-, di- and trinitromesitylene anion radical formations were observed to be accompanied by entropy changes of −15.1, −11.0 and −6.8 cal/K mol. The larger entropy values due to steric interactions are further demonstrated by the series, 2,5-dimethyl (−13.0), 2,4,6-trimethyl (−15.1), 2,4,6-triethyl (−19.9), 2,4,6-tri-isopropyl (−21.7) and 2,4,6-tri-*tert*-butyl (−18.6). The steric effect is explained by the decrease in charge delocalization as the nitro groups are forced out of the planes of the benzene rings by the interactions with the *ortho* substituents. Entropy changes and enthalpy changes are correlated with molar extinction coefficients.

In previous papers in this series we have demonstrated that reversible electrode potentials for both the oxidation and the reduction of aromatic compounds can be measured to a very high degree of precision. This enabled us to carry out precise determinations of the temperature coefficients which are related to the entropy of formation of the radical ions by eqn. (1).[1,2] Both studies provided

$$dE^{rev}/dT = \Delta S/nF \quad (1)$$

strong evidence that the origin of the entropy changes is dominated by the reorganization of the environment around the species undergoing electron transfer as the charge is transfered. The study concerned with the redox reactions of alternant aromatic hydrocarbons led to deductions concerning the relationship between the entropy change and the charge distribution in the absence of any steric and substituent effects.[1] The study concerned with the oxidation and reduction of compounds with the anthracene skeleton in which the 9 and 10 positions are substituted with heteroatoms brought in another factor, *i.e.* the ease with which the systems can form planar ion radicals, the most favorable situation for charge delocalization.[2] As the heteroatoms became larger the entropy changes were observed to be larger indicating more concentration of charge on the heteroatoms which in turn requires a greater reorientation of the environment during electron transfer.

Neither of the systems studied so far[1,2] were amenable to the assessment of polar effects, which might contribute to solvation of the neutral as well as the charged species. As the starting point for a systematic investigation of polar and steric effects on the entropy changes of reversible electrode processes, we chose to study the reduction of nitrobenzenes. These compounds are particularly suitable since the anion radicals are stable, steric effects can be tested for by *ortho* substitution, polar effects can be analyzed by *meta* and *para*

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Table 1. The temperature dependence for the reversible reduction potentials of nitrobenzenes in acetonitrile.[a]

Compound No.	Substitutents	$\frac{-dE^{rev}/dT}{mV\,K^{-1}}$	$-E^{rev}_{273.2}$/mV
1	0	0.507	1295
2	2-Methyl	0.569	1327
3	3-Methyl	0.496	1324
4	4-Methyl	0.477	1360
5	2,5-Dimethyl	0.556	1464
5a	2,3-Dimethyl	0.597	1417
6	2,4,6-Trimethyl	0.655	1622
7	2,4,6-Triethyl	0.864	1619
8	2,4,6-Tri-isopropyl	0.941	1617
9	2,4,6-Tri-*tert*-butyl	0.805	1662

[a] In solvent containing Bu_4NBF_4 (0.1 M). Measurements by phase selective second harmonic *a.c.* voltammetry at a mercury electrode at temperatures ranging from about 255 to 295 K.

Table 2. The temperature dependence of the reversible reduction potentials of di- and trinitrobenzenes in acetonitrile.[a]

Compound No.	Nitro Substituents	Other Substituents	$\frac{-dE^{rev}/dT}{mV\,K^{-1}}$	$-E^{rev}_{273.2}$/mV
10	1,2	0	0.228	1018
11	1,2	4-Methyl	0.194	985
12	1,3	0	0.338	1097
13	1,3	4-Methyl	0.376	1119
14	1,3	2,5-Dimethyl	0.408	1150
15	1,3	4,6-Dimethyl	0.392	1254
16	1,3	2,4,6-Trimethyl	0.476	1411
17	1,3	2,4,6-Tri-*tert*-butyl	0.669	1466
18	1,4	0	0.219	873
19	1,3,5	2-Methyl	0.117	902
20	1,3,5	2,4,6-Trimethyl	0.295	1209

[a] The measurement conditions were the same as described in Table 1.

substitution, and the effect of multiple substitution can be studied.

The electroreduction of aromatic nitro compounds has received a great deal of attention over the years. In recent years they have been studied in aprotic solvents[3] and liquid ammonia.[4] They have served as substrates for the investigation of ion-pairing[5] and for the determination of disproportionation parameters.[6,7] The electron transfer rate constants, both homogeneous exchange[8,9] and heterogeneous at electrode[8,10-12], have been obtained for a variety of nitrobenzenes. The literature concerning these topics is extensive and only the most pertinent papers have been cited here.

RESULTS AND DISCUSSION

The phase selective second harmonic *a.c.* reversible potential determinations for compounds *1–20* were determined over about a 40 K temperature range and the data are summarized in Tables 1 and 2. In general the temperature coefficients obtained by correlation of E^{rev} *vs.* T were based on measurements at from 5 to 6 temperatures spaced between about 255 and 295 K. The measurements provide dE^{rev}/dT to ± 0.01 mV/K which corresponds to an error of ± 0.2 cal/K mol in ΔS. A vast amount of data was gathered and processed and for reasons of space economy, none

Table 3. Entropy and relative enthalpy changes for the formation of methyl substituted nitrobenzene anion radicals.

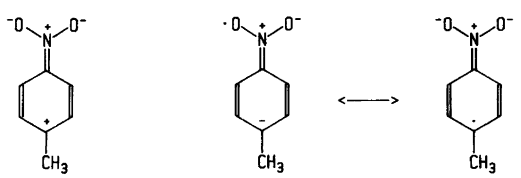
Compound No.	Substituents	$-\Delta S_{273.2}$/cal K^{-1} mol^{-1}	$\Delta\Delta H$/kcal mol^{-1} [a]
1	0	11.7	0
2	2-Methyl	13.1	0.36
3	3-Methyl	11.4	0.75
4	4-Methyl	11.0	1.69
5	2,5-Dimethyl	13.0	3.54
5a	2,3-Dimethyl	13.8	1.69
6	2,4,6-Trimethyl	15.1	6.61

[a] Relative to that for formation of nitrobenzene.

of the raw data will be reported here. Examples of the temperature coefficient determinations have been presented in other papers in this series.[1,2]

Temperature coefficients and reversible electrode potentials for the reduction of alkyl substituted nitrobenzenes and di- and trinitrobenzenes are summarized in Tables 1 and 2. Entropy changes along with enthalpy changes, relative to the reduction of nitrobenzene taken as the standard reaction, are tabulated in groups of similar structural features in Tables 3 to 7. The entropy changes will be discussed first and the relative enthalpy changes are used in comparisons of correlations of reversible potentials and entropy changes with parameters obtained in other types of experiments.

The relative importance of steric and polar effects on the magnitude of the entropy change during the reduction of nitrobenzenes can be assessed from the first four entries in Table 3. As expected, the *ortho* methyl substituent in *2* brings about an increase in the entropy change during reduction relative to unsubstituted nitrobenzene. On the other hand, methyl groups in the *meta* (*3*) or *para* (*4*) positions have the opposite effect. The resonance structures *21* – *23* give an indication of the stabilizing or destabilizing of charge in the neutral (*21*) and anion radical (*22*↔*23*) forms of *4*. Structure *21* suggests that charge separation is enhanced by methyl substitution and to the extent that this contributes to solvation would enhance the latter. On the other hand, the electron donating methyl group would clearly be expected to repel negative charge in the ring and cause the charge to be more localized on the nitro group than in the anion radical of benzene. The same direction in effects are expected when the methyl groups are either *meta* or *ortho* to the nitro group but the magnitude of the effect is expected to be diminished in both cases relative to the *para* substituent, because of weaker polar interactions of *meta* substituents and diminished polar interaction of the *ortho* substituent due to steric interference of planarity. Since the entropy changes are lower for *meta* and *para* methyl substituted nitrobenzenes we conclude that the overriding polar effect is on the solvation of the neutral molecules rather than on that for the corresponding anion radicals. In any event, the contribution of the polar effect to the entropy changes is small with those for *3* and *4* not being very much out of the range of experimental error from that observed for the reduction of nitrobenzene. The small influence of a *meta* methyl substituent is also evident in comparing the entropy change for the reduction of *2* with that of *5*, the values are nearly within experimental error of being identical. The steric effect of two *ortho* methyl groups is apparently greater than twice that of a single one. The decrease in the entropy change in going from *1* to *6* is greater than twice that in going from *1* to *2* in spite of the presence of the *para* methyl substituent which influences the entropy change in the opposite direction.



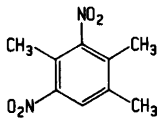
21 *22* *23*

The steric and polar contributions to the entropy change upon the reduction of nitrobenzenes can be incorporated into an empirical equation (2), as can be seen from Table 8, to predict ΔS for nitro, 1,3-

dinitro and 1,3,5-trinitro compounds. In (2) A_z is the entropy change when the nitro compound is

$$\Delta S = A_z + \{0.69(\text{cal/K mol})B + 0.23(\text{cal/K mol})C - 2.08(\text{cal/K mol})D - 5.53(\text{cal/K mol})E\}/z \quad (2)$$

unsubstituted and z is the number of nitro groups. The number of *ortho* and *para* methyl groups are designated by B and that of *meta* by C. The number of nitro groups with one *ortho* methyl and with two *ortho* methyls are specified by D and E, respectively. Thus, structure *24* would be treated in the following manner:



24

$A_z = \Delta S$(*m*-dinitrobenzene)
$z = 2$
$B = 2$, $C = 1$, $D = 1$, and $E = 1$.

The experimental ΔS values for the compounds listed in Table 3 are compared to those calculated using (2) in the upper half of Table 8. With the exception of the value for the reduction of *5a*, the calculated values are within 0.1 cal/K mol of the experimental ones.

Before leaving the mononitrobenzene derivatives and going in more detail into the significance of eqn. (2), we can examine the steric effect of *ortho* substituents a little more closely. The data in Table 4, summarize the effect on ΔS when the 2,6-alkyl substituents are made progressively larger. The entropy change becomes progressively larger as the alkyl groups are changed from methyl to ethyl to isopropyl, in which case the maximum value observed in this study, −21.7 cal/K mol, was recorded. This is qualitativelly the expected trend. As the alkyl group gets larger, the overlap of *p* orbitals on nitrogen with the pi system of the ring becomes progressively less with the charge of the anion radical becoming more and more localized on the nitro group. However, reaction of compound *9* in which the nitro group is flanked by two large *tert*-butyl groups exhibited a smaller entropy change, −18.6 cal/K mol. This result would appear to be inconsistent with our model where localization of charge is accompanied by a more ordered environment and hence a greater loss in entropy. However, when we examined space-filling models of structure *9* we found that the nitro group is effectively buried in the bulky *tert*-butyl groups and solvation cannot possibly be as localized as in the other structures in Table *4*. Thus, the ordering of the solvent around the periphery of the buried nitro group is less than in cases where the solvation can be

Table 4. Entropy and relative enthalpy changes for the formation of 2,4,6-trialkylnitrobenzene anion radicals.

Compound No.	Substituents	$-\Delta S_{273.2}$/cal K^{-1} mol^{-1}	$\Delta\Delta H$/kcal mol^{-1}
6	2,4,6-Trimethyl	15.1	6.61
7	2,4,6-Triethyl	19.9	5.23
8	2,4,6-Tri-isopropyl	21.7	4.69
9	2,4,6-Tri-*tert*-butyl	18.6	6.58

Table 5. Entropy and relative enthalpy changes for the formation of dinitrobenzene anion radicals.

Compound No.	Isomer	$-\Delta S_{273.2}$/cal K^{-1} mol^{-1}	$-\Delta\Delta H$/kcal mol^{-1}
10	1,2	5.26	4.63
12	1,3	7.79	3.50
18	1,4	5.05	7.91
(*11*)[a]	1,2(4-Methyl)	(4.47)	(5.17)

[a] Does not belong in the classes of any of the tables.

more localized. We have observed a similar phenomenon during the study of the entropy of the reduction of some highly substituted benzophenones.[13]

Turning our attention now to the reduction of the dinitrobenzenes, we find that regardless of the substitution pattern a significant lowering of the entropy change upon reduction is observed when a second nitro group is present. A feature of the data in Table 5 that attracts attention is that the ΔS values for 1,2 (*10*) and 1,4 (*18*) dinitrobenzene are nearly the same. This indicates that the polar effect of the nitro group, in contrast to that of the methyl group, is so strong that the steric effect is essentially overcome. Aside from this somewhat surprising feature of the data, the entropy change during the reduction of 1,3-dinitrobenzene (*12*) was intermediate between that of nitrobenzene and the other two dinitrobenzenes. This is to be expected since the *meta* substituent can only exert an inductive effect upon the charge distribution in the corresponding anion radical.

We can now return to the evaluation of polar and steric influences on the entropy change in reduction. The entropy change data for 1,3-dinitrobenzene reductions summarized in Table 6 are compared to the corresponding values calculated using eqn. (2) in the lower half of Table 8. The correspondence is just as good as was observed with the nitrobenzene derivatives with the exception of that for structure *16* where the calculated value is 1.3 cal/K mol more negative than observed. Any attempt to explain the deviation would necessarily be highly speculative and perhaps pointless. A further indication that the entropy effects in this series of reactions parallel those for the nitrobenzenes can be found by considering the differences in entropy changes for the parent compounds (3.9 cal/K mol) and the tri-*tert*-butyl substituted derivatives (3.2 cal/K mol).

The application of eqn. (2) to the reduction processes of the two series of compounds in Table 8 allows us to draw definite conclusions regarding the relative importance of polar and steric effects in determining the entropy changes. The coefficients of *B* and *C*, which relate to the polar effect of the methyl groups, are only 0.69 cal/K mol and 0.23 cal/K mol, respectively, white the corresponding factors for *D* and *E*, which take into account the steric effects, are −2.08 cal/K mol and −5.53 cal/K mol. Thus, the steric influence of an *ortho* methyl group is far greater than the polar effect of a methyl group in any position in the molecule. The signs of the multipliers are also of significance. The positive signs on the factors relating to polar effects can be taken as an indication, as discussed before, that the polar effect exerts a stronger influence on the solvation of the neutral molecule than on the anion radical. Conversely, the negative factors relating to steric effects show that the steric effect is much more important in the charge distribution, and hence solvation, of the anion radicals than of the parent substances.

Table 6. Entropy and relative enthalpy changes for the formation of alkyl substituted 1,3-dinitrobenzene anion radicals.

Compound No.	Substitutents	$-\Delta S_{273.2}$/cal K^{-1} mol^{-1}	$\Delta\Delta H$/kcal mol^{-1}
12	0	7.79	−3.50
13	4-Methyl	8.67	−3.23
14	2,5-Dimethyl	9.41	−2.72
15	4,6-Dimethyl	9.04	−0.22
16	2,4,6-Trimethyl	11.0	2.87
17	2,4,6-Tri-*tert*-butyl	15.4	2.93

Table 7. Entropy and relative enthalpy changes for the formation of methyl substituted 1,3,5-trinitrobenzene anion radicals.

Compound No.	Substituents	$-\Delta S_{273.2}$/cal K^{-1} mol^{-1}	$-\Delta\Delta H$/kcal mol^{-1}
19	2-Methyl	2.70	6.60
20	2,4,6-Trimethyl	6.80	0.64

Table 8. Comparison of experimentally determined entropy changes for mono- and dinitrobenzene reductions with those calculated using an empirical equation.

Compound No.	$-(\Delta S_{273.2})_{exp.}$/ cal K^{-1} mol^{-1}	$-(\Delta S_{273.2})_{calc.}$/ cal K^{-1} mol^{-1} [a]
1	11.7	11.7
2	13.1	13.1
3	11.4	11.5
4	11.0	11.0
5	12.8	12.8
5a	13.8	12.8
6	15.1	15.2
12	7.79	7.79
13	8.67	8.49
14	9.41	9.41
15	9.04	9.18
16	11.0	12.3

[a] Calculated from eqn. (2) in the text.

We have limited data on derivatives of symmetrical trinitrobenzenes. We were unable to obtain reliable response for the parent in the series and the data for *19* and *20* (Table 7) could not be treated directly using eqn. (2). However, it is possible to treat A_z, which is ΔS of the parent, as the unknown in (2) and arrive at a value of -1.7 cal/K mol. Only one of the alternant aromatic hydrocarbon redox reactions, the reduction of triphenylene, proceeds with a lower entropy change, -0.9 cal/K mol.[1] This gives a clear indication that solvation of the anion radicals is very diffuse, and is spread out over the entire framework of the ion. It is of further interest to compare the entropy changes for mono- (*6*), di- (*16*) and trinitromesitylene (*20*) reductions. A smooth trend is observed with values of -15.1, -11.0 and -6.8 cal/K mol recorded for the compounds. This gives further justification for the application of eqn. (2) to determine ΔS for 1,3,5-trinitrobenzene.

It has been pointed out that the steric inhibition of resonance in *ortho* substituted nitrobenzene derivatives is manifested in a decrease in absorption intensity.[16] This is, of course, a reflection of the decrease in conjugation and can be expressed numerically in terms of the molar extinction coefficient, ε. Since this appears to be precisely the factor that we are attributing the relative entropy changes to, a correlation of the available data concerning this parameter is of the utmost interest. In Fig. 1, $\Delta S_{273.2}$ and $\Delta\Delta H$ for the reduction of several nitrobenzenes are plotted *vs.* ε where the latter is the extinction coefficient at the maximum of the absorption band appearing at about 250 nm for all of these compounds. The gratifying result of the correlations is that ε correlates much better with ΔS than with $\Delta\Delta H$ (or E^{rev}). The close correspondence of ΔS and ε provides further evidence for our model based upon entropy and localized charge distribution.

$$A + e^- \underset{k_s}{\rightleftharpoons} A^{\cdot -} \qquad (3)$$

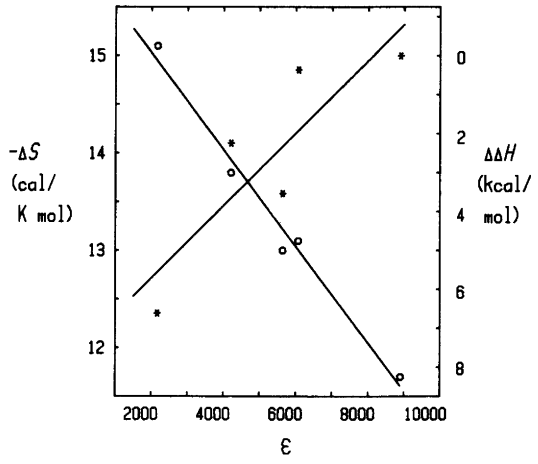


Fig. 1. Correlation of ΔS (circles) and $\Delta\Delta H$ (asterisks) for the reduction of nitrobenzenes in acetonitrile with molar extinction coefficients at the maximum of the band at about 250 nm.

The kinetics of charge transfer reactions (3) and (4) are of interest for comparison with the

$$A^{\cdot -} + A \underset{}{\overset{k_{ex}}{\rightleftharpoons}} A + A^{\cdot -} \qquad (4)$$

thermodynamic quantities for reversible electron transfer. The interesting feature of both (3) and (4) is that under the conditions of the measurements the reactions are at equilibrium and ΔG for (4) is zero. According to electron transfer theory,[17] reorientation of the environment around reactant in going to the transition state plays a dominating role in determining the rate of the reaction. In consideration of this, one might expect a direct correlation between ΔS and k_s or k_{ex}. If such a close relationship could be established it would be of great importance in the study of electron transfer kinetics. This is especially true in the case of k_s where experimentally measured rate constants are not the intrinsic values but are apparent values depending upon the electrode and double layer conditions. Unfortunately, attempts to correlate either ΔS or $\Delta\Delta H$ with available k_s or k_{ex} data were disappointing. No evidence for linear relationships could be found in any case. It is possible that these failures are due to insufficient data as well as to a rather low degree of precision in the kinetic data. Further work is called for along these lines.

The work reported here has reinforced our expectations that the measurement of entropy changes in reversible electrode processes can provide important insight into the details of the reactions.[1,2,13] We are in the progress of studying the effect of reaction conditions such as solvent and counter ion on the magnitude of the entropy changes. Work in progress or planned includes the study of the formation of multiply-charged ions and redox reactions involving other charge types.

EXPERIMENTAL

The experimental procedures and data handling operations were described in detail in the first paper in this series.[1] Nitro compounds were either commercially available or prepared and purified by standard procedures.

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Temperature Effects on Electrode Processes. IV. The Effect of Angular Constraints on the Entropy of Formation of Anion Radicals of Compounds Related to Benzophenone

MORTEN SVAAN and VERNON D. PARKER

Laboratory for Organic Chemistry, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The entropy of the formation of the anion radicals of compounds related to benzophenone was observed to be dependent upon the charge delocalization as reflected by the planarity of the ions. This is demonstrated by the decreasing trend in the entropies as the angles between the aromatic rings and the carbonyl groups increase due to steric restraints in the series 9-fluorenone (−9.8 cal/K mol), 9-anthrone (−10.1 cal/K mol), benzophenone (−12.8 cal/K mol) and 2,6-dimethylbenzophenone (−14.6 cal/K mol). The dependence of the entropy of formation of the anion radicals of benzophenones bearing methyl substituents on structure was observed to be complex and to arise from both steric and electronic effects. The entropies were observed to correlate reasonably well with the angular relationship of the carbonyl group to the benzene rings. It is pointed out that solvation of both the anion radicals and of the neutral ketones must be taken into account in analyzing the entropies of formation of the anion radicals.

Previous papers in this series have established that the entropy changes of reversible electrode processes are highly sensitive parameters depending upon the degree of reorganization of the environment around a species on which the charge is changing.[1-3] Entropy changes ranging from −0.9 cal/K mol for the formation of the highly symmetrical anion radical of triphenylene[1] to 25.3 cal/K mol for the formation of the charge localized cation radical of thianthrene have been evaluated.[2] The recent work[1-3] has invalidated the conclusions, based on limited data,[4,5] that the temperature coefficients of reversible redox potentials of aromatic compounds in aprotic solvents are of the order of 0.4 mV/K which corresponds to about 10 cal/K mol.

The previous paper in this series demonstrated that the contributions of polar and steric effects during the reduction of alkyl substituted nitrobenzenes could be evaluated. The polar effect was found to be mainly manifested in the properties of the neutral molecules while the steric effect exerted the greatest influence on the anion radicals.[3]

Benzophenones and related molecules which are reduced reversibly in proton donor poor solvents offer a further testing ground for the entropy effects in reversible electrode processes. Benzophenone is reduced reversibly to the anion radical in aprotic solvents and in the absence of proton donors the anion radical can be reduced reversibly as well.[6] Halobenzophenone reduction and the subsequent cleavage of the corresponding anion radicals have been studied intensively[6-11] as has reduction of the related compound 9-fluorenone.[12] Both polar and steric effects upon the reversible reduction potentials of methyl substituted benzophenones have recently been studied and compared to the interplanar angles between the phenyl rings and the carbonyl group.[13]

As illustrated in structures *1* to *7*, the average angles between the planes of the two phenyl rings and that of the carbonyl group of benzophenone and methyl substituted benzophenones depend upon the steric interactions of the groups in the 2,6- and 2′,6′-positions. In the structures *1* to *7*, presented by Rekker and Nauta,[14] the circles designate volumes occupied by methyl groups. Even in benzophenone,

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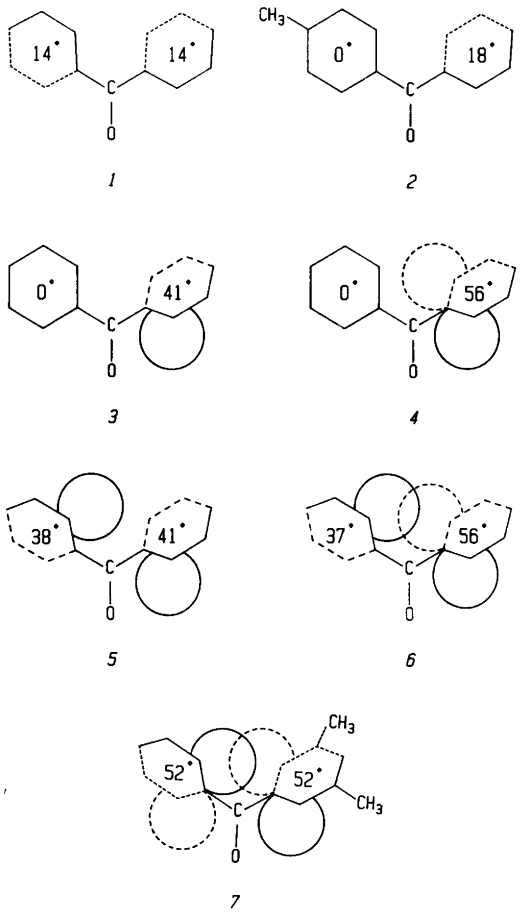


the two phenyl rings cannot be coplanar with the carbonyl group at the same time and structure *1* gives the favored conformation. Successive substitutions with methyl groups bring about increasing deviations from planarity as shown in structures *3* to *7*. The angles shown were calculated from oscillator strengths (*f*) obtained from absorption spectra.[14] Estimates of the interplanar angles have also been made by ^{1}H NMR [15] and ^{13}C NMR [16] spectroscopy.

The thermodynamic parameters for a reversible electrode process identified in eqn. (1) are a

$$-E^{rev} = (\Delta H - T\Delta S)/nF \quad (1)$$

reflection of the energy differences between the neutral and charged species in solution in reaction (2). Thus, the features of importance in the present context are not the conformations of the molecules

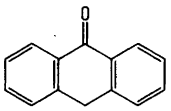
$$\text{Molecule} \pm e^- \rightleftarrows (\text{Ion radical})^{\mp} \quad (2)$$

undergoing charge transfer but rather the differences in conformations between the neutral and charged species. For example, if the favored conformation of benzophenone is *1* and this is also the favored conformation of the anion radical and the same relationship would hold for the substituted compounds and anion radicals, there would be no influence of conformation or ΔH for the reduction. There could still be an effect on ΔS since the solvation of the anion radicals and the neutral molecules differ appreciably. Since the energy of an electronic absorption band gives the difference in energy between an occupied and unoccupied orbital, usually the lowest energy one, in the ground state good correlations are usually obtained between transition energies and electron affinities or ionization potentials.[17] Thus, electrode potentials often correlate with transition energies as well. As we pointed out earlier,[2] there is no fundamental reason why such correlations should exist since the related parameter is ΔH rather than E^{rev}. Data reported for highly substituted nitrobenzenes suggested that steric hindrance to solvation of the anion radicals can give rise to unexpected entropy changes.[3] Structures *4* to *7* for the methyl substituted benzophenones suggest that the reduction of these compounds might provide further insight into the question posed above.

In this paper we report the results of measurements of the temperature coefficients of the reversible reduction potentials for benzophenone, methyl substituted benzophenones, 9-anthrone and 9-fluorenone in acetonitrile.

RESULTS AND DISCUSSION

The reversible potentials and the temperature coefficients for the reduction of compounds *1* to *13* are gathered in Tables 1–3. All dE^{rev}/dT refer to measurements in acetonitrile over about 40 K using phase selective second harmonic *a.c.* voltammetry. As in the previous paper,[3] raw data are not reported but typical data for similar measurements have been presented in other papers.[1,2] The precision in the electrode potential measurements was as before of the order of ± 0.2 mV which gives rise to an error in dE^{rev}/dT of the order of ± 0.01 mV/K or ± 0.2 cal/K mol in ΔS. Details of the measurements and data precision were discussed earlier.[1]



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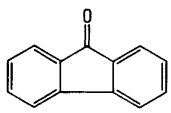

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The degree of coplanarity of the phenyl rings and the carbonyl group increases in going down the series, *1* to *11* to *12*. Since we expect the entropy change to be dependent primarily upon the charge distributions in the resulting anion radicals, any change in geometry resulting in decreased overlap of the carbonyl pi system with those of the phenyl rings is expected to bring about a larger entropy decrease upon charge transfer. Thus, our prediction, based on prior experiences,[1-3] is that the entropy change will become less negative in going from *1* to *11* to *12*. This expectation is fulfilled by the order shown in Table 1. The enthalpy changes, relative to that for the reduction of *12* show no systematic trend.

The thermodynamic parameters for the reduction of unsymmetrically substituted benzophenones are given in Table 2. A slightly lower entropy decrease than for the parent benzophenone was observed during reduction of the 4-methyl substituted isomer *2*. Since substituents in the 4,4'-positions cannot exert a steric effect, this small difference must be a consequence of the polar effect of a methyl group conjugated with the carbonyl group. A 2-methyl substituent (*3*) brought about a greater entropy decrease and that for 2,6-dimethylbenzophenone (*4*) was still greater. Thus, as expected, the steric effect predominates for substituents in the 2,2'-positions. The polar effect of the 4'-methyl substituent in (*10*) resulted in an entropy decrease during reduction intermediate between those for *3* and *4*. The trend observed with *ortho* methyl groups bringing about a larger entropy decrease does not continue with the inclusion of still more substituents. The inclusion of the third *ortho* substituent in *6* was actually accompanied by a lowering in the entropy decrease, as compared to *4*, to a value less than that for benzophenone. Thus, the crowded structures do not fit in the same trend as that observed with the lesser substituted compounds.

It is evident from the values of the thermodynamic parameters for the reduction of benzophenones symmetrically substituted with

Table 1. Entropy and relative enthalpy changes for the formation of anion radicals of aromatic ketones in acetonitrile.

No.	Name	$(-dE^{rev}/dT)$/ mV K^{-1}	$-E^{rev}_{273.2}$/ mV[a]	$-\Delta S_{273.2}$/ cal K^{-1} mol^{-1}	$\Delta\Delta H$/ kcal mol^{-1} [b]
1	Benzophenone	0.557	1967	12.8	9.29
11	9-Anthrone	0.436	1942	10.1	9.45
12	9-Fluorenone	0.426	1529	9.82	0

[a] Measurements at a mercury electrode *vs.* $Ag/Ag^+(CH_3CN)$. [b] Calculated from the reversible potentials and entropy changes relative to that for 9-fluorenone.

Table 2. Entropy and relative enthalpy changes for the formation of anion radicals of unsymmetrically substituted benzophenones in acetonitrile.

No.	Substituents	$(-dE^{rev}/dT)$/ mV K^{-1}	$-E^{rev}_{273.2}$/ mV[a]	$-\Delta S_{273.2}$/ cal K^{-1} mol^{-1}	$\Delta\Delta H$/ kcal mol^{-1}
1	None	0.557	1967	12.8	9.29
2	4-Methyl	0.538	1976	12.4	9.60
3	2-Methyl	0.569	2004	13.1	10.1
10	2,4',6-Trimethyl	0.574	2078	13.2	11.7
4	2,6-Dimethyl	0.633	2018	14.6	9.97
6	2,2',6-Trimethyl	0.537	2104	12.4	12.6

[a] Measurements at a mercury electrode *vs.* $Ag/Ag^+(CH_3CN)$.

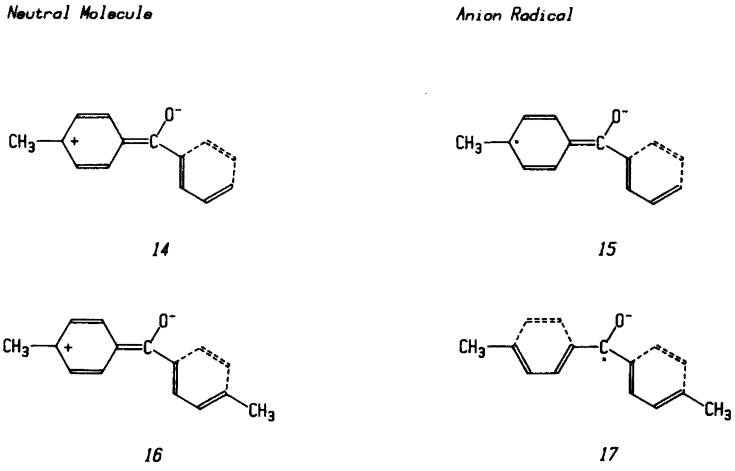
Table 3. Entropy and relative enthalpy changes for the formation of anion radicals of symmetrically substituted benzophenones.

No.	Substituents	$(-dE^{rev}/dT)$/ mV K^{-1}	$-E^{rev}_{273.2}$/ mV[a]	$-\Delta S_{273.2}$/ cal K^{-1} mol^{-1}	$\Delta\Delta H$/ kcal mol^{-1}
13	4,4'-Dimethyl	0.468	2066	10.8	12.1
1	None	0.557	1967	12.8	9.29
8	2,2',4,4'-Tetra-methyl	0.589	2192	13.6	14.3
5	2,2'-Dimethyl	0.630	2149	14.5	13.0
9	2,2',4,4',6,6'-Hexa-methyl	0.367	2366	8.46	19.7

[a] Measurements at a mercury electrode *vs.* $Ag/Ag^+(CH_3CN)$.

methyl groups listed in Table 3 that the effects are not additive. For example, the entropy decrease observed during the reduction of 4,4'-dimethylbenzophenone differs from that of the parent by much more than twice the difference observed with 4-methylbenzophenone. This can be rationalized by considering the features of the resonance structures shown in Scheme 1. Methyl substituents could increase the degree of solvation of the neutral molecules as indicated by favoring the charge separation in resonance forms *14* and *16*. The preferred conformation of 4,4'-dimethylbenzophenone is one in which both of the rings are tilted out of the plane of the carbonyl group by 12°, similar to *1*.[14] This indicates that the two methyl groups can participate equally in stabilizing the polarization of the carbonyl group. If we assume that the polar effect of methyl groups is exerted to the greatest extent on the neutral molecule as appears to be the case for the nitrobenzenes,[3] we must allow for a synergistic effect of the two methyls resulting in larger localization of solvent than twice that resulting from a single methyl group. Resonance structures *15* and *17* indicate that the methyl groups do not cause dispersal of the negative charge into the rings. Thus, the results can be explained by larger entropy of solvation of the neutral molecules as compared to benzophenone with little effect on the anion radicals and an overall lowering of the entropy decrease upon formation of the anion radicals.

An empirical equation in which the polar effects of *ortho* and *para* methyl substituents were taken to be the same has been observed to fit entropy data for



Scheme 1.

the reduction of nitrobenzenes in acetonitrile.[3] The added complication of differing spatial relationships of the two phenyl rings relative to the carbonyl group renders the assignment of values to the polar effects of 4-methyl and 2-methyl groups in the benzophenone series somewhat without meaning. The polar effect is surely dependent upon the degree of overlap between the *p* orbital on the carbonyl carbon with the pi systems of the phenyl rings. In fact, an approximately linear relationship has been observed between electrode potentials and a function of the interplanar angles for 4-methyl substituents.[13] The point of importance in the discussion of our data is that the 2,2'-methyl groups surely have a polar effect in addition to the more obvious steric effect. However, the situation is so complicated by the multitude of possible anion radical conformations that there is little hope of being able to separate polar and steric effects on the basis of the limited data available.

We commented earlier on the fact that the reduction of 2,2',6-trimethylbenzophenone is accompanied by a much smaller decrease in entropy than expected on the basis of a comparison to results from less highly substituted benzophenones. Structure *6* suggests that the crowding caused by the methyl groups could make the solvation shell of the anion radical less ordered than it would be in the absence of the methyl groups. The effect is very much more pronounced during the reduction of 2,2',4,4',6,6'-hexamethylbenzophenone (*9*) in which case $\Delta S_{273.2}$ is 4.3 cal/K mol less negative than that observed during the reduction of benzophenone. The environment of the carbonyl group of *9* would be expected to be very similar to that shown for *7* and the carbonyl group is not easily approached by solvent molecules. Thus, the ordering of the solvent takes place around the periphery of the hindered carbonyl group of the anion radical and results in a much smaller entropy decrease than when the solvation is more intimate.

Finally, we can consider the comparison of the entropy changes for the reductions with the oscillator strengths obtained from electronic absorption spectra. The oscillator strengths,[14] like extinction coefficients,[18] give a measure of the degree of conjugation and were used[14] as the parameters to estimate the interplanar angles shown in structures *1* to *7*. We have attempted linear regression correlations of E^{rev}, $\Delta\Delta H$ and ΔS with f. No correlation was observed between f and E^{rev} or $\Delta\Delta H$, the correlation coefficients were 0.26 and 0.11, respectively. On the other hand, ΔS correlates linearly with f with a correlation coefficient of 0.96 and the results can be incorporated into eqn. (3). The

$$f = 0.0550\ (\text{K mol/cal})\ \Delta S + 1.096 \qquad (3)$$

data are summarized in Table 4. The calculated values in the last column are very close to the f values reported.[14] It may well be that the correlation would be even better with more precise measures of f, the difficulties in the estimation of which has been commented on.[16,19]

This study has provided further evidence for the sensitivity of the temperature coefficients of electrode potentials for reversible processes to changes in the solvation processes. In addition, the discussion amply demonstrates that when comparing the thermodynamic parameters of electrode processes to other quantities it is necessary to consider the features of both the

Table 4. The correlation of the entropy changes for the reversible reduction of methyl substituted benzophenones with UV oscillator strengths.[a]

No.	Substituents	$-\Delta S_{273.2}$/cal K^{-1} mol^{-1}	f_{lit}[b]	f_{calc}[c]
1	None	12.8	0.38	0.39
13	4,4'-Dimethyl	10.8	0.50	0.50
8	2,2',4,4'-Tetramethyl	13.6	0.36	0.35
5	2,2'-Dimethyl	14.5	0.30	0.30
2	4-Methyl	12.4	0.45	0.41
3	2-Methyl	13.1	0.35	0.38
4	2,6-Dimethyl	14.6	0.30	0.29
10	2,4',6-Trimethyl	13.2	0.36	0.37

[a] UV absorption spectral data from Ref. 14. [b] Oscillator strengths f(tot) for measurements in ethanol listed in Table VIII of Ref. 14. [c] Calculated from linear regression correlation eqn. (3).

charged and neutral species. Neglecting the consideration of one or the other of the partners in the redox couple may lead to serious error. All of the papers in this series have shown that there is a great advantage in separating the electrode potentials into the entropic and enthalpic contributions if a maximum of detail is desired.

EXPERIMENTAL

The experimental procedures and data handling procedures were described in detail in the first paper in this series.[1] Some of the methyl substituted benzophenones were graciously provided by Dr. James Grimshaw in a highly pure state. The other benzophenones as well as 9-fluorenone and 9-anthrone were either commercial samples or were prepared by standard methods and purified by recrystallization before use.

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Synthesis of γ-Lactones and 3-Alkylidene-1-indanones

LARS JALANDER and MERETE BROMS

Department of Organic Chemistry, Åbo Akademi, SF-20500 Åbo 50, Finland

γ-Lactones are formed in high yields in the reactions of (*E*)-3-alkyl-3-phenylpropenoic acids in concentrated sulfuric acid if the alkyl groups contain a tertiary γ-hydrogen. If the alkyl groups bear secondary γ-hydrogens 3-alkylidene-1-indanones are formed almost exlusively. Reaction mechanisms for the cyclizations are discussed on the basis of deuteriation experiments.

Acid catalyzed lactonizations of alkylidenemalonitriles[1] and dialkyl alkylidenemalonates[2] have been reported.

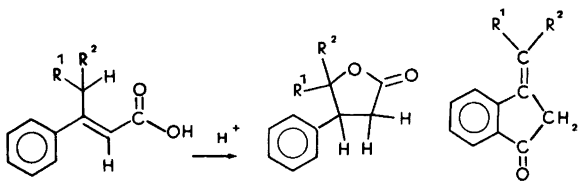
During the course of our investigation concerning the formation of β-alkylated ethyl (*Z*)- and (*E*)-cinnamates from ethyl (*Z*)- and (*E*)-β-chlorocinnamates in CuI catalyzed Grignard reactions, it was of interest to find a method to unambiguously determine the configuration of *Z*- and *E*-isomers. Since NMR measurements of the ethyl β-alkylcinnamates did not always give unequivocal results, we tried some cyclizations in concentrated sulfuric acid with the intention of transferring the *Z*-isomers of the β-alkylcinnamic acids to the corresponding indenone derivatives. In these experiments we found that the *E*-isomers, which contained a tertiary γ-hydrogen, formed γ-butyrolactones and that the *E*-isomers with secondary γ-hydrogens did not cyclisize to lactones but to-3-alkylidene-1-indanones (Scheme 1 and Table 1). β-Methylcinnamic acid did not undergo any cyclization when it was stirred for 48 h in concentrated sulfuric acid at 35 °C. Neither did the ethyl β-alkylcinnamates form lactones, although the alkyl group contained a tertiary γ-hydrogen.

Table 1. Cyclization of β-alkylcinnamic acids in concentrated sulfuric acid according to Scheme 1.

Substituent		Reaction	Product	Yield[a]
R^1	R^2	time h		%
a Me	Me	5	2a	93
b Me	Et	5	2b	86
c C-hexyl		5	2c	87
d C-pentyl		5	2d	81
e H	Et	24	3e	71
f H	i-Pr	24	3f	68
g H	sec-Bu	24	3g	64
h H	H	48	unreacted	0

[a] As determined by ^{1}H NMR spectroscopy.

Several reaction mechanisms are possible for the formation of the lactones. According to the mechanism, reaction proceeds can be elucidated by carrying out the reaction in deuteriated sulfuric acid. If the β-carbon of the lactone does not bear a deuterium atom the cyclization has to proceed by an internal hydride shift (Scheme 2) and not by a double bond migration which should lead to deuteri-



1 2 3

Scheme 1.

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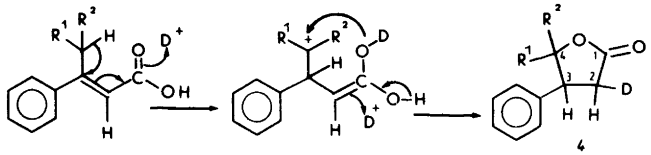
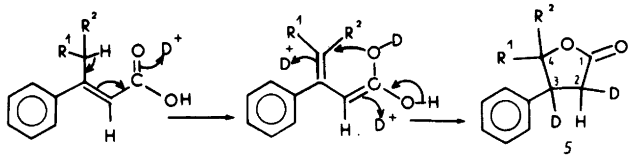
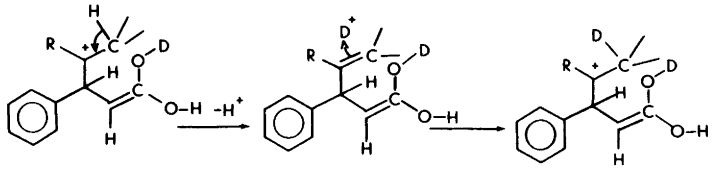


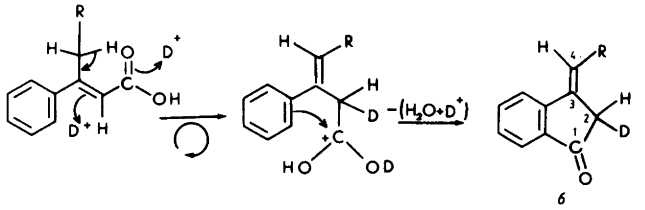
Scheme 2.



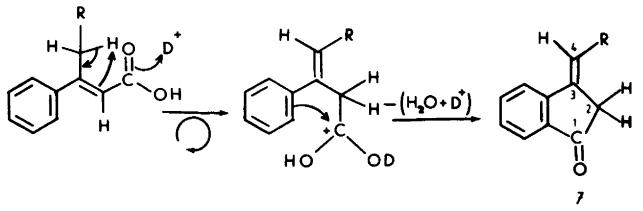
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

ation of the β-carbon (Scheme 3). The high-field part of the proton decoupled ^{13}C NMR spectrum of the reaction products formed when (*E*)-3-isopropylcinnamic acid was allowed to react with deuteriated sulfuric acid is shown in Fig. 1 (spectrum B) together with the spectrum of 4-methyl-3-phenyl-γ-valerolactone (*2a*) (spectrum C). The two upper-field signals in the spectrum of *2a* arise from methyl carbons. The signals at 34.4 ppm and 51.1 ppm belong to C – 2 and C – 3, respectively. The absorptions for C – 2 from the deuteriation experiment appear as a CH_2 singlet and a CHD triplet centered 0.7 ppm upfield from the CH_2 signal. A weak CD triplet can be dicerned, not completely resolved, upfield from the CH signal of C – 3. The low intensity of CD can be due to partial saturation because of long T_1 for CD compared with that of CH. Neither can the relative intensities of CH and CD be compared because of differences in NOE. The deuterium content at C – 3 is, however, small as determined from the decrease in intensity for the CH signal of the deuteriated sample relative to the intensity for the

CH signal of the undeuteriated sample. These results show that an internal hydride shift according, for instance, to Scheme *2* is the dominating pathway, although it cannot be the only reaction mechanism involved because the absorption for C−2 also appears as a CH_2 singlet identical with that from the undeuteriated sample. This shows that an internal hydride or proton shift to C−2 also operates. The presence of two triplets upfield from the methyl signals reveals a ^2H exchange which can take place starting from the intermediary carbocation (Scheme 4). NMR and MS measurements also revealed that a ^2H exhange takes place in the phenyl ring.

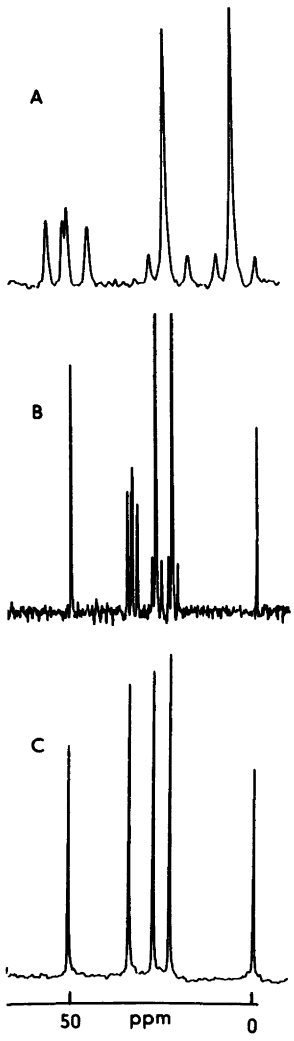


Fig. 1. High-field region of the proton decoupled 15.03 MHz ^{13}C NMR spectra C of 4-methyl-3-phenyl-γ-valerolactone (*2a*) and B its deuterium derivative. Inset A is a 2.4-fold expansion of the methyl groups and C-2 to illustrate the effect of deuteriation.

Why the lactonization did not take place with the cinnamic acids which bear secondary or primary γ-hydrogens can be explained by the lower capacity of only one alkyl group or three hydrogens to stabilize the intermediary positive charge on the γ-carbon. A double bond migration with elimination of a proton from the γ-position is therefore more likely than an internal hydride shift. At least two reaction mechanisms can operate in this cyclization as is shown in the reaction of β-isobutylcinnamic acid with deuteriated sulfuric acid (Schemes 5 and 6). The ^1H and ^{13}C NMR spectra of the deuteriated sample indicate that the reaction mechanism outlined in Scheme 6 is the dominating pathway, although secondary reactions are involved. For example, a complete ^2H exchange is observed at C−4 which most likely is a result of a ^2H exchange after the cyclization, because the same exchange was observed when 3-propylidene-1-indanone (*3e*) was treated with deuteriated sulfuric acid. A complete ^2H exchange at C−2 was also observed when β-propylcinnamic acid was stirred with deuteriated sulfuric acid for 36 h. This exchange has to be the result of a tautomerization.

EXPERIMENTAL

Mass spectra were recorded on an LKB 9000 instrument (70 eV) equipped with a gas chromatograph (50 m × 0.2 mm glass capillary column, stationary phase SE-30). ^1H NMR spectra were obtained on a Jeol FX-60 FT NMR spectrometer at 59.75 MHz and ^{13}C spectra on the same instrument operating at 15.03 MHz. $CDCl_3$ was used as solvent and TMS as an internal standard.

The (*E*)-β-alkylcinnamic acids were prepared by alkaline hydrolysis of the corresponding ethyl cinnamates which were prepared in good yields from ethyl (*Z*)-3-chloro-3-phenylpropenoate in CuI catalyzed Grignard reactions.

General procedure for the preparation of γ-lactones and β-alkylidene-1-indanones. The β-alkylcinnamic acid (1 g) was added to concentrated sulfuric acid (20 cm^3) at 0 °C. The reaction mixture was stirred for 5 h while the reaction temperature was allowed to reach 20 °C. The reaction mixture was poured into ice and water, followed by extraction with di-

ethyl ether. The organic phase was extracted with sodium bicarbonate solution, dried with Na_2SO_4 and the diethyl ether evaporated. The γ-butyrolactone was recrystallized from a mixture of light petroleum and diethyl ether. The β-alkylideneindanones were prepared according to the same procedure except that the reaction time at 20 °C was prolonged to 24 h.

4-Methyl-3-phenyl-γ-valerolactone (2a). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.04 (CH_3, s), 1.55 (CH_3, s), 2.93 ($CHCH_2$, q, *J* 9.3 Hz), 3.55 ($CHCH_2$, q, 9.3 Hz).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 23.2 (CH_3), 27.7 (CH_3), 34.4 (C-2), 51.1 (C-3), 87.1 (C-4), 127.8, 128.6, 136.8 (aromatic), 175.3 (C-1); *J* (C-5, H-5) 127 Hz, (C-2, H-2) 134 Hz, (C-3, H-3) 134 Hz.

MS [IP 70 eV; *m/e* (% rel. int.)]: 190 (16, M), 175 (20, M-CH_3), 162 (46, M-CO), 132 (10), 131 (11), 104 (100, Ph-CH=CH_2), 91 (10), 78 (19), 77 (18), 43 (16).

4-Methyl-3-phenyl-4-hexanolide (2b). Two diastereomers were formed in the ratio 1:3 as determined by ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum of these diastereomers is not easily interpreted. H_2-2 and $H-3$ gave rise to an AB_2 system, in which the signals of the diastereomers appear as different peaks in the A part whereas the signals of the diastereomers coincide in the B_2 part. H_2-5 appears as a distorted quartet with the highest peak at 1.8 ppm.

^{1}H NMR (59.75 MHz $CDCl_3$): δ 1.0 (CH_3, s) 0.9, 1.1, 1.2 (CH_3-CH_2, distorted triplet), (CH3-CH_2, distorted quartet between 1.6–2.0 ppm), (CH-CH_2, AB_2 system between 2.8–3.8 ppm), 7.1–7.4 (aromatic). The signals of the carbon atoms of the diastereomers appear, with some exceptions, as different peaks, *i.e.*, the signals of C–1, C–2 and the aromatic carbons except C-*ipso* coincide.

^{13}C NMR of the diastereomer which was formed in higher yield (15.03 MHz, $CDCl_3$): δ 8.1 (C-6), 21.1 (C-5), 32.9 (C-5), 34.6 (C-2), 48.5 (C-3), 89.6 (C-4), 127.7, 127.9, 128.7, 137.1 (aromatic), 175.7 (C-1). ^{13}C NMR of the diastereomer which was formed in lower yield (15.03 MHz, $CDCl_3$): δ 7.8 (C-6), 24.1 (C-5), 28.7 (C-5), 34.6 (C-2), 51.6 (C-3), 89.3, 127.7, 127.9, 128.7, 136.8 (aromatic), 175.7 (C-1).

MS [IP 70 eV; *m/e* (% rel. int.)]: 204 (3, M), 189 (1, M–CH_3), 176(5), 175 (8, M–C_2H_5), 132 (5, $PhCHCH_2CO$), 131 (7, PhCHCHCO), 115 (2), 105 (18), 104 (100, $PhCHCH_2$), 91 (6), 78 (10), 77 (9).

4-Spirocyclohexane-3-phenyl-γ-butyrolactone (2c). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.5–1.8 (C-hexyl, broad), 2.8–3.4 (CH-CH_2, AB_2-system), 7.2–7.4 (aromatic).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 21.7, 22.6, 24.9, 32.3, 36.8, 88.6 (C-hexyl), 34.7 (C-2), 51.2 (C-3), 127.6, 128.1, 128.6, 137.1 (aromatic), 175.9 (C-1); *J* (C-3, H-3) 132 Hz, *J* (C-2, H-2) 134 Hz, *J* (C-2, H-3) 5.5 Hz.

MS[IP 70 eV; *m/e* (% rel. int.)]: 230 (9, M), 202 (9, M–CO), 187 (2), 169 (3), 132 (18, PhCHCHCO), 105 (34), 104 (100, $PHCHCH_2$), 103 (20), 78 (17), 77 (11).

4-Spirocyclopentane-3-phenyl-γ-butyrolactone (2d). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.2–2.2 (cyclopentyl, broad), 2.8–3.6 (CH-CH_2, AB_2 system), 6.8–7.3 (aromatic).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 23.0, 23.3, 33.9, 38.0, 98.6 (C-pentyl), 36.2 (C-2), 48.7 (C-3), 127.7, 127.9, 128.7, 138.0 (aromatic, 176.0 (C-1); *J* (C-2, H-2) 136 Hz, *J* (C-2, H-3) 6 Hz, *J* (C-3, H-3) 136 Hz.

Ms [IP 70 eV; *m/e* (% rel. int.)]: 216 (10, M), 188 (17, M–CO), 169 (6), 132 (21, PhC_2H_3CO), 131 (10, PhC_2H_4CO), 115 (9), 105 (54), 104 (100, PhC_2H_4), 103 (31), 91 (15), 78 (21), 77 (21), 55 (14).

3-Propylidene-1-indanone (3e). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.1 (CH_3, t, *J* 7.2 Hz), 2.2 (CH_2–CH_3, K, *J* 7.2 Hz), 3.1 (CH_2CO, d, 2.0 Hz), 6.2 (CH=C, tt, *J* 7.3 and 2.0 Hz), 7.2–7.9 (aromatic).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 13.7 (C-6), 23.3 (C-5), 39.4 (C-2), 120.7 (C-4), 123.5, 126.5, 128.1, 134.7, 136.7, 150.9 (aromatic), 203.1 (C-1); *J* (C-6, H-6) 126 Hz, *J* (C-5, H-5) 123 Hz, *J* (C-2, H-2) 131 Hz, *J* (C-2, H-4) 8 Hz, (C-4, H-4) 161 Hz.

MS [IP 70 eV; *m/e* (% rel. int.)]: 172 (48, M), 157 (26, M-CH_3), 145 (11), 144 (100, M-C_2H_4), 141(6), 129 (56), 128 (36), 127 (15), 115 (20), 102 (10), 77 (9), 76 (7).

3-Isobutylidene-1-indanone (3f). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.1 (CH_3, d, *J* 6.6 Hz), 1.4 [$(CH_3)_2CH$, m], 3.2 (CH_2CO, d, *J* 2 Hz), 6.1 (CH=C, dt 9.5 Hz and 2 Hz), 7.2–7.8 (aromatic).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 22.6 [$(CH_3)_2$], 29.6 (C-5), 39.3 (C-2), 120.7 (C-4), 123.5, 128.1, 131.9, 134.8, 136.7, 151.0 (aromatic), 203.2 (C-1); *J* (C-6, H-6) 126 Hz, *J* (C-5, H-5) 125 Hz, *J* (C-2, H-2) 131 Hz, *J* (C-2, H-4) 8 Hz, *J* (C-4, H-4) 161 Hz.

MS [IP 70 eV; *m/e* (% rel. int.)]: 186 (33, M), 171 (48, M–CH_3), 158 (8, M–CO), 144 [100, M–$(CH_3)_2C$], 143 [39, M–$(CH_3)_2CH$], 128 (35), 115 (27), 102 (8), 77 (8), 77 (6).

3(2-Methylbutylidene)-1-indanone (3g). ^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.0 (CH_3CH_2, t, *J* 7.1 Hz), 1.1 (CH_3C, d, *J* 7.1 Hz), 1.4 (CH_3CH_2, m), 2.4 (CH_3CH, m), 3.2 (CH_2CO, d, *J* 2 Hz), 6.1 (CH=C, dt, *J* 9.7 Hz and 2 Hz).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 12.0 (C-7), 20.4 (CH_3), 30.1 (C-6), 36.6 (C-4), 39.7 (C-2), 120.8 (C-4).

MS [IP 70 eV; *m/e* (% rel. int.)]: 200 (23, M), 185 (4, M–CH_3), 171 (72, M–C_2H_5), 145 (16), 144 (100, M–C_4H_8), 143 (50), 141 (12), 129 (13), 127(11), 116 (10), 115 (25), 77 (9).

Deuteriation experiments. (*E*)-4-Methyl-3-phenyl-

2-pentenoic acid (300 mg) was added to deuteriated concentrated sulfuric acid (10 ml) at 0 °C. The reaction mixture was stirred for 24 h while the reaction temperature was allowed to reach 20 °C. The reaction mixture was poured into ice and water and extracted twice with chloroform. The combined chloroform phases were extracted with sodium bicarbonate solution, dried with sodium sulphate and the chloroform evaporated. NMR and MS analyses showed that the reaction mixture contained several deuteriated products, which could not be separated by capillary GLC. The main reaction product was a derivative of *2-deuterio-4-methyl-3-phenyl-γ-valerolactone* with varying degrees of deuteriation in the phenyl ring.

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.03 (CH_3, s), 1.54 (CH_3C, s), 2.8 – 3.6 (*CH*-*CH*D, an AB-system, not completely resolved).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 23.2 (CH_3), 27.7 (CH_3), 34.2 (C-2), 51.1 (C-3), 87.3 (C-4), 175.4 (C-1); J (C-2, H-2) 132 Hz, J (C-2, D-2) 23 Hz, J (C-3, H-3) 133 Hz.

The main reaction product from the reaction of 5-methyl-3-phenyl-2-propenoic acid with deuteriated sulfuric acid was a derivative of *4-deuterio-5-methyl-3-propylidene-1-indanone* with varying degrees of deuteriation in the phenyl ring.

^{1}H NMR (59.75 MHz, $CDCl_3$): δ 1.15 (CH_3, d, J 6.6 Hz), 2.6 [$(CH_3)_2CH$, sep, J 6.6 Hz], 3.2 (CH_2CO, s), 7.2 – 7.8 (aromatic).

^{13}C NMR (15.03 MHz, $CDCl_3$): δ 22.6 (CH_3), 29.5 (C-5), 39.3 (C-2), 119.7 – 153.8 (aromatic, not completely resolved), 203.2 (C-1).

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Cleavage of β-Aryl Ether Bonds in Phenolic Lignin Model Compounds with Anthrahydroquinone and Anthrone

GÖSTA BRUNOW and KRISTIINA POPPIUS

Department of Chemistry, University of Helsinki, Vuorikatu 20, SF-00100 Helsinki 10, Finland

The equal abilities of anthrone and anthrahydroquinone to cleave β-0–4 bonds in phenolic structural units in lignin have been demonstrated with the aid of two model compounds. The structure of a degradation product, *trans*-isoeugenol, indicates that the cleavage involves an adduct having *erythro* configuration.

The "catalytic" effect of anthraquinone on delignification in alkaline pulping processes is believed [1] to derive, at least in part, from the ability of reduced forms of anthraquinone to cleave the aryl ether bond in phenolic β-0-4 structural units in lignin (for nomenclature, see Ref. 2).

In a previous article [3] we have described the reaction of lignin model compound *1* with anthrahydroquinone in alkaline solution. The aryl ether bond was cleaved cleanly in a reaction that resembles the action of hydrosulfide ion on similar structures in kraft cooking.[3]

The terminal group in the side chain of *1* is methyl. In lignin this is usually a primary alcohol group and for structures like *2* the dominant reaction in alkaline solution is a retro-aldol reaction in which an enol ether is formed without cleavage of the aryl ether bond.[4] To find out whether the reaction with anthrahydroquinone is able to compete with the retro-aldol reaction, we heated model compound *2* with an alkaline solution of anthrahydroquinone under similar conditions as compound *1*.

Recent work [5] has shown that some of the anthrahydroquinone in pulping reactions is reduced to the oxidation level of anthrone and that this may lead to irreversible reactions with quinone methides in lignin.[6a] We have now found that anthrone cleaves the aryl ether bond both in *1* and *2* at rates comparable to those of anthrahydroquinone under similar conditions.

The exclusive formation of the *trans* isomer or isoeugenol from *1* indicates that some stage in the reaction occurs with a high degree of stereospecificity.

RESULTS AND DISCUSSION

Experiments with anthrahydroquinone. The yields of liberated guaiacol (2-methoxyphenol) as a function of reaction time at 140 °C are shown in Fig. 1. As can be seen, the aryl ether bond is cleaved at similar rates in *1* and *2* under the chosen conditions, and the difference between the cleavage rates with and without the additive is larger for *2* than for *1*. The conclusion can be drawn that the retro-aldol

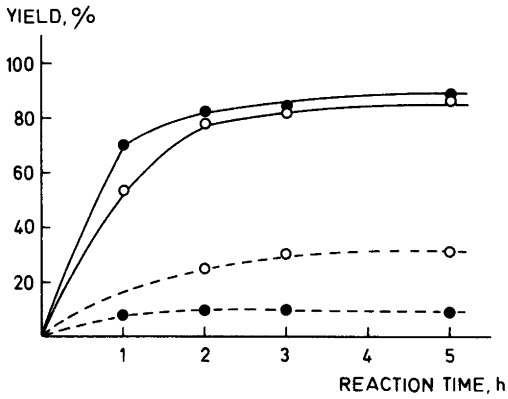

Fig. 1. Formation of guaiacol on treatment of *1* (○) (8.2×10^{-3} mol/l, from Ref. 3) and of *2* (●) (8.2×10^{-3} mol/l) in 1 M sodium hydroxide (40% dioxane) with anthrahydroquinone (———) and without anthrahydroquinone (– – –) at 140 °C.

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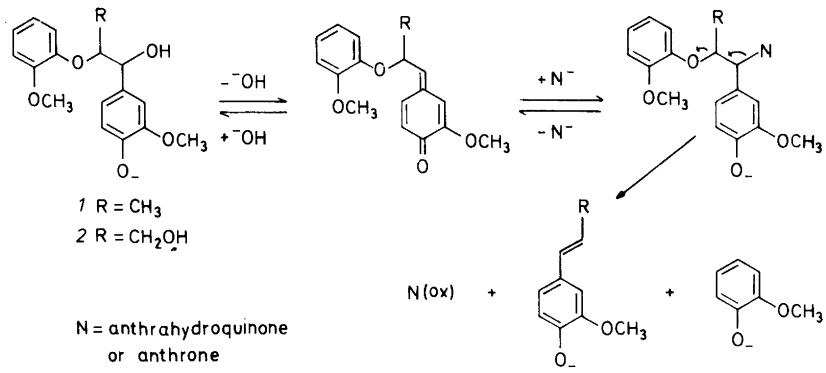


Scheme 1. Phenolic compounds are depicted as anions.

reaction does not compete with the aryl ether cleavage in this case (*cf.* also Ref. 7).

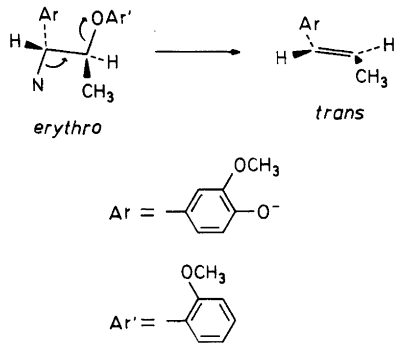
Experiments with anthrone. When compound *2* was heated with an alkaline solution of anthrone at 140 °C for 3 h, the amount of guaiacol released was almost as large as with anthrahydroquinone (Table 1). This demonstrates that anthrone is as effective as anthrahydroquinone in cleaving phenolic β-0−4 ethers. A similar effect can be seen with compound *1* after heating it for 2 and 3 h with anthrone (Table 1). The yield of isoeugenol [2-methoxy-4-(1-propenyl)-phenol] from *1* is significantly lower in the reaction with anthrone, indicating that anthrone reacts irreversibly with some of the isoeugenol or with the quinone methide intermediate (Scheme 1). An adduct between anthrone and a quinone methide intermediate has been prepared [6b] and shown to be more resistant to alkaline degradation than the corresponding anthrahydroquinone adducts, although "extensive" degradation is reported at higher temperatures.[6b] The mechanisms of these reactions are under investigation.

Table 1. Yields of guaiacol and *trans*-isoeugenol on treatment of *1* (8.2×10^{-3} mol/l) and *2* (8.2×10^{-3} mol/l) in 1 M sodium hydroxide (40 % dioxane) with an excess of anthrahydroquinone (AHQ) or anthrone (AN) at 140 °C.

Model compound	Reagent	Reaction time/h	Yield/% Guaiacol	*trans*-Isoeugenol
1	AHQ	3	80.6[a]	67.1[a]
		2	78.5[a]	72.5[a]
	AN	3	84.6	50.3
		2	69.6	56.2
2	AHQ	3	85.1	
	AN	3	80.8	

[a] Ref. 3.

The stereochemistry of the aryl ether cleavage reaction. The isoeugenol formed in the aryl ether cleavage reaction of compound *1* is invariably the *trans* isomer (> 99 %). Since we have not been able to detect any *cis-trans* isomerization of isoeugenol under the present cooking conditions, we conclude that the exclusive formation of the *trans* isomer is a consequence of the stereochemistry of the cleavage reaction. If the cleavage is viewed as a heterolytic fragmentation,[8] as has been suggested,[9] the "electrofugal" and the "nucleofugal" groups will be antiperiplanar in the transition state. Such a transition state will lead to *trans*- isoeugenol only if the intermediate adduct has the *erythro* structure shown in Scheme 2.



Scheme 2. Phenolic compounds are depicted as anions.

EXPERIMENTAL

1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1-propanol (1).[10] The yield of the *erythro* isomer was improved by lowering the temperature of the borohydride reduction step to 6 °C.[11] *1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (2)*[12] was obtained as a 1:1 mixture of *erythro* and *threo* isomers (m.p. 78–85 °C). *Anthrahydroquinone diacetate* was prepared from anthraquinone.[13] The *isoeugenol* (FLUKA, *purum*) was a mixture of *cis* and *trans* isomers (20.5% *cis* according to GLC analysis) and was used as such for the isomerization experiment. Pure *trans* isomer was obtained by recrystallization of the acetylated material and hydrolysis of the *trans* acetate.[14]

The anthrahydroquinone and anthrone cookings. Anthrahydroquinone (AHQ) was generated from the stable diacetate in the reaction mixture. The cookings were run with model compounds *1* (*ca.* 90% *erythro*) or *2* (*ca.* 50% *erythro*) (0.082 mmol) dissolved in peroxide-free dioxane (4.0 ml), an excess of anthrahydroquinone diacetate or anthrone (Koch-Light) (375.0 mg), 2 M sodium hydroxide (5.0 ml) and water (1.0 ml) in Pyrex glass ampoules. Before being sealed, the ampoules were evacuated and flushed twice with oxygen-free nitrogen. In anthrahydroquinone experiments the ampoules were heated in an oil bath at 140 °C for 1, 2, 3, or 5 h. In anthrone (AN) experiments heating was at 140 °C for 3 h in the case of *2* and for 2 or 3 h in the case of *1*. A warm-up time of two minutes was not included in time at temperature.

Cooling was for 5 min at room temperature and then in cold water. Ampoules were opened and the contents immediately neutralized with dilute acetic acid. The precipitate was filtered off and washed with chloroform (2 × 2 ml) and the filtrate was extracted with chloroform (5 × 2 ml). The combined chloroform layers were dried with sodium sulfate, which was filtered off and washed with chloroform (2 × 1 ml). After addition of the internal standard (methyl anisate), the combined solutions were chromatographed without derivatization of the components.

Cookings without any additives were carried out as above but in 0.75 M instead of 1 M sodium hydroxide solutions.

Recovery tests were run in a nitrogen atmosphere with guaiacol (5.9–6.3 mg) and *trans*-isoeugenol (5.7–6.8 mg) dissolved in peroxide-free dioxane (4.0 ml), anthrahydroquinone diacetate or anthrone (375.0 mg), 2 M sodium hydroxide (5.0 ml) and water (1.0 ml). The ampoules were heated at 140 °C for 1.5 h. The alkaline contents were worked up as above. The losses of guaiacol (14.0% with AHQ and 14.1% with AN) and isoeugenol (8.1% with AHQ and 15.8% with AN) in the cooking and extraction processes were taken into account when calculating the results.

The isomerization experiments were run with the commercial mixture of *cis* and *trans* isoeugenol (11.1 mg) dissolved in peroxide-free dioxane (4.0 ml), anthrahydroquinone diacetate (375.0 mg), 2 M sodium hydroxide (5.0 ml) and water (1.0 ml) in glass ampoules. Isomerization experiments without anthrahydroquinone were carried out in 0.75 M sodium hydroxide solutions. The ampoules were heated at 140 °C for 3 h. After working up the reaction mixtures as described above, GLC analysis revealed no significant isomerization. The percentage of *cis* isomer was 20.3% without AHQ and 16.9% with AHQ).

Gas chromatography was performed as described previously.[3]

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Potential Acyl-transfer Agents. Reactions of *N*-Acyl-2-pyridinecarboxamides with Nucleophiles

EVA H. MØRKVED[a] and MARSHALL W. CRONYN[b]

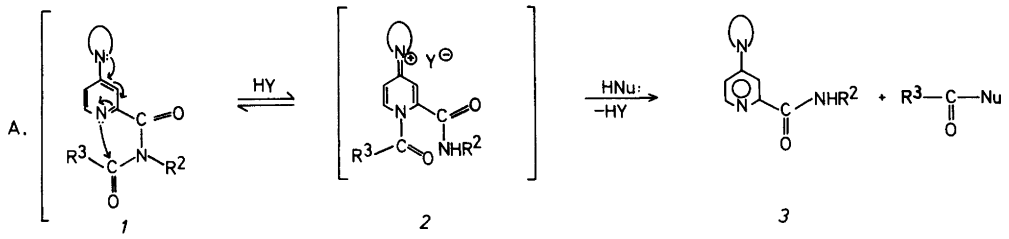
[a]Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and [b]Department of Chemistry, Reed College, Portland, Oregon 97202, USA

A fast reaction is observed between a series of *N*-acyl-2-pyridinecarboxamides and cyclopentylamine or pyrrolidine. Most of the acylamides react exclusively at the pyridine-2-carbonyl group. The selectivity of these reactions is explained by the reaction of the pyridine – nitrogen as a base towards the external nucleophile in a five-ring transition state. The acylamides undergo slow reactions with 4-methylaniline, methanol or water. Several reaction paths are observed with these less reactive nucleophiles. An intramolecular acyl group transfer prior to the reaction with an external nucleophile is indicated for three of the *N*-acylamides which have an *N*,*N*-dialkylamino substituent in the pyridine-4 position. Nucleophilic attack occurs predominantly at the *N*-acyl group of these three compounds which are moderately active acyl-transfer agents.

Several years ago imidazole was shown to enhance the solvolysis of esters and amides through an intramolecular nucleophilic reaction.[1] The present studies are based on those observations and the *N*-acyl-2-pyridinecarboxamides *1* with an *N*,*N*-dialkylamino group as R^1, see Schemes 1 and 2, were visualized as potential acyl-transfer agents. Thus, in protic, weakly basic solutions a resonance stabilized *N*-acylpyridinium salt *2* might be formed by an intramolecular nucleophilic attack on the acyl group $R^3C(O)$ by the pyridine nitrogen of *1*. The intermediate *2* would be an active acyl-transfer agent in the presence of an appropriate nucleophile. The expected transformations are shown as reactions (A) in Scheme 1. The related compounds 4-(*N*,*N*-dialkylamino)pyridines are presently subject to much interest as catalysts in acyl-transfer reactions[2] and the reactive intermediates in these reactions certainly are *N*-acylpyridinium salts.[3] Recent studies of compounds *1* also have shown[4] that *1* with an *N*,*N*-dialkylamino substituent in the 4-position of the pyridine ring react as nucleophiles towards acyl chlorides. Therefore, the nucleophilicity of the pyridine nitrogen of *1* would suffice for an intramolecular acyl-transfer as shown in (A), Scheme 1. Presently reactions of *1* with nucleophiles are reported.

RESULTS

The potential acyl-transfer agents *1* can be prepared by reactions of 2-pyridinecarboximidoyl



Scheme 1.

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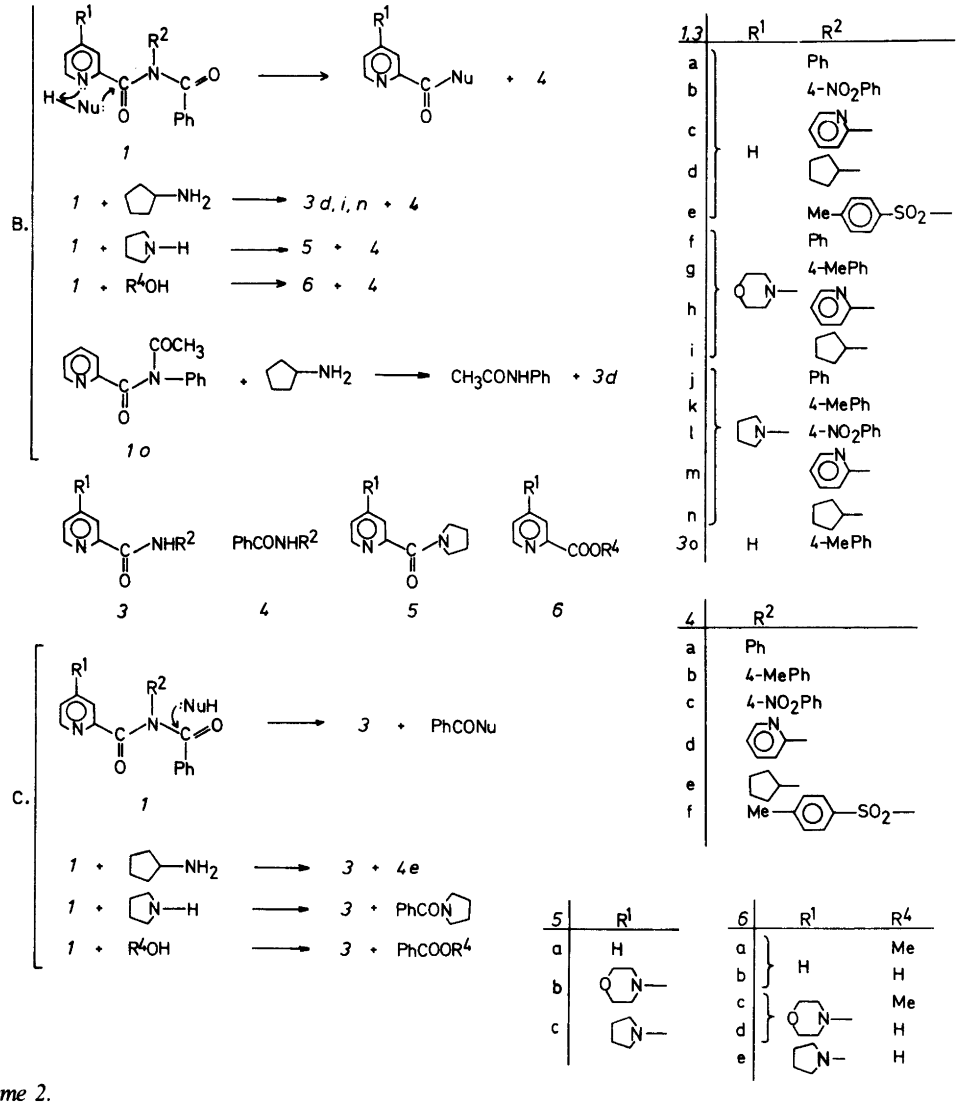
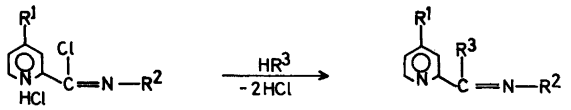
chlorides with salts of carboxylic acids.[5,6] This reaction sequence must be used if the objective is activation and subsequent transfer of the acyl group $R^3C(O)$ which presently is either PhC(O) or MeC(O), see Scheme 2. However, *1* also have been obtained by alternative reaction sequences.[6,7]

The imidoyl chloride hydrochlorides 7 are rather slow reacting towards carboxylate ions.[6] Presently *7a* was reacted with sodium benzoate for 18 h at ambient temperature; cyclopentylamine then was added to the reaction mixture which after another 30 min yielded *1b* (20%), *3b* (16%) and *8a* (58%). Thus, after 18 h about equal amounts of *7a* had reacted with benzoate ions to give *1b* or had hydrolyzed to *3b*. Unreacted *7a* then underwent a rapid reaction with cyclopentylamine to yield *8a*.

Attempts were made to enhance the reactivity of 7 by reacting these compounds with silver tosylate. The intermediate tosyl imidates thus formed are expected to be more reactive than 7 towards carboxylate ions due to the tosylate ion as a leaving group. However, whereas a reaction of *7a* with silver tosylate and cyclopentylamine yields the expected amidine *8a* as the major product, a similar reaction



Scheme 2.



7 8

7	R^1	R^2
a	H	4-NO_2Ph
b	2-pyridyl	4-MePh
c	2-pyridyl	4-NO_2Ph
d	2-pyridyl	Ph
e	3-pyridyl	4-MePh
f	3-pyridyl	4-NO_2Ph

8	R^1	R^2	R^3
a	H	4-NO_2Ph	2-imidazolyl
b	H	4-NO_2Ph	4-morpholinyl
c	2-pyridyl	4-MePh	PhNH
d	2-pyridyl	4-NO_2Ph	PhNH
e	2-pyridyl	Ph	PhNH
f	3-pyridyl	4-MePh	PhNH
g	3-pyridyl	4-NO_2Ph	PhNH

Scheme 3.

of *7f* yields 4-nitroaniline and *N*-cyclopentyl-*p*-toluenesulfonamide instead of the expected product *8g*. Silver tosylate obviously is involved in the formation of 4-nitroaniline from *7f* since a mixture of *7f* and a fourfold molar excess of triethylamine in acetonitrile only produces small amounts of 4-nitroaniline. However, addition of silver tosylate to this reaction mixture produces substantial amounts of 4-nitroaniline and several unidentified compounds.

It will be noted that silver tosylate does not cleave the imidoyl chloride as was observed for *7f* if other reaction conditions are used. The formation of an *N*-acylamide derived from *3l* apparently is enhanced by silver tosylate. Thus, the products isolated from a reaction of *7f-HCl* with triethylammonium acetate, silver tosylate and aniline were *3l* (87%) and acetanilide (56%). These products indicate both the formation of at least 56% of the *N*-acetyl derivative of *3l* and that aniline preferentially reacts at the acetyl – carbonyl of the *N*-acetylamide.

Compounds *7* react with carboxylate ions or aniline at comparable rates; the latter reactions yield amidines *8*. Morpholine and cyclopentylamine, however, undergo rapid reactions with *7* to give *8*. The formation of *8* is shown in Scheme 3.

Reactions of the *N*-acyl-2-pyridinecarboxamides *1* with nucleophiles are summarized in Table 1. The striking feature of these reactions is the predominant attack by the strong nucleophiles cyclopentylamine or pyrrolidine on the pyridine-2-carbonyl group of *1*. Therefore, instead of reaction path (A), Scheme 1, these reactions are best explained by path (B), Scheme 2. The reactions will have a five-ring T.S. where the pyridine – nitrogen reacts as a base by removing a proton from the attacking nucleophile. A similar behavior has been observed[8] for the pyridine – nitrogen of *N*-(2-pyridyl)benzamides; an enhancement of the rate of basic methanolysis was explained by the reaction of the pyridine – nitrogen as an intramolecular base in a six-ring T.S.

The third mode of reaction which has been considered for compounds *1* is shown as path (C) in Scheme 2. *N*-Acylamides are generally not very effective acylating agents,[9] and, therefore, these reactions are expected to proceed at a slow rate. A product analysis will not distinguish between reaction paths (A) and (C). Reactions of *1* with 4-methylaniline, methanol or water are quite slow. For instance, the reaction of *1c* with 4-methylaniline was not yet completed after 72 h. Also, during the long reaction periods of about 200 h compounds *1* might both hydrolyze and react with the added nucleophile as was observed for the reaction of *1i* with 4-methylaniline. However, a change from aprotic to protic solvents has no effect on the product composition from the fast reactions of *1c* or *1g* with cyclopentylamine. A comparison of the reactions of *1a* and *1o* with cyclopentylamine also shows that a change of the *N*-acyl group from benzoyl in *1a* to acetyl in *1o* has no influence on the

Table 1. Reactions of *N*-acyl-2-pyridinecarboxamides *1* with nucleophiles.

Compound	Nucleophile[a]	Solvent	Reaction time, h[b]	Expected products Path (B)	Path (A) or (C)	Observed[c] % Reaction by path (B)
1a	CPA	Benzene[d]	1	*3d*, *4a*	*3a*, *4e*	97
1a	P	EtOH	1	*5a*, *4a*	*3a*, $PhCON(CH_2)_4$	93[e,f]
1b	CPA	Benzene	1	*3d*, *4c*	*3b*, *4e*	99
1c	CPA	MeCN[d]	1	*3d*, *4d*	*3c*, *4e*	95
1c	M	MeCN	72	*3o*, *4d*	*3c*, *4b*	74[g]
1d	P	EtOH	10	*5a*, *4e*	*3d*, $PhCON(CH_2)_4$	65[e,h]
1d	P	MeCN	10	*5a*, *4e*	*3d*, $PhCON(CH_2)_4$	58[e,h]
1d	M	MeCN	200	*3o*, *4e*	*3d*, *4b*	88
1d	MeOH	MeOH	200	*6a*, *4e*	*3d*, PhCOOMe	80
1d	H_2O	Acetone	200	*6b*, *4e*	*3d*, PhCOOH	99
1e	M	MeCN	3	*3o*, *4f*	*3e*, *4b*	42
1f	CPA	MeCN	1	*3i*, *4a*	*3f*, *4e*	95
1f	MeOH	Benzene	240	*6c*, *4a*	*3f*, PhCOOMe	45
1g	CPA	$MeCN/CH_2Cl_2$[i]	1	*3i*, *4b*	*3g*, *4e*	99
1g	P	$MeOH/CH_2Cl_2$	1	*5b*, *4b*	*3g*, $PhCON(CH_2)_4$	86
1g	MeOH	CH_2Cl_2	240	*6c*, *4b*	*3g*, PhCOOMe	60
1h	CPA	MeCN	1	*3i*, *4d*	*3h*, *4e*	97
1i	P	EtOH	10	*5b*, *4e*	*3i*, $PhCON(CH_2)_4$	46[j,k]
1i	P	MeCN	10	*5b*, *4e*	*3i*, $PhCON(CH_2)_4$	47[j,k]
1i	MeOH	MeOH	240	*6c*, *4e*	*3i*, PhCOOMe	30
1i	M	MeCN	240	*3g*, *4e*	*3i*, *4b*	50[l]
1i	H_2O	Acetone	200	*6d*, *4e*	*3i*, PhCOOH	50
1j	CPA	MeOH	1	*3n*, *4a*	*3j*, *4e*	95
1k	CPA	EtOH	1	*3n*, *4b*	*3k*, *4e*	92
1l[m]	CPA	MeCN	1	*3n*, *4c*	*3l*, *4e*	40
1m	CPA	MeCN	1	*3n*, *4d*	*3m*, *4e*	96
1m	M	MeCN	48	*3k*, *4d*	*3m*, *4b*	89[g]
1n	P	EtOH	10	*5c*, *4e*	*3n*, $PhCON(CH_2)_4$	36[j,n]
1n	P	MeCN	10	*5c*, *4e*	*3n*, $PhCON(CH_2)_4$	46[j,n]
1o	CPA	Benzene	1	*3d*, MeCONHPh	*3a*, $MeCONHC_5H_9$	99

[a] Abbreviations used: CPA = cyclopentylamine; P = pyrrolidine and M = 4-methylaniline. [b] All reactions at ambient temperature. [c] Product analysis by GLC at 140 – 300 °C, instrumentation, see Ref. 4. % Reaction by path (B) is calculated from the observed product mixture. Mixed reaction paths, (B) and (A) or (C) give the four products of columns 5 and 6 whereas reactions by path (B) give equimolar amounts of the two products of column 5. % Reactions by paths (A) or (C) are found as the difference between 100 and the number of column 7. [d] Or in methanol and acetone. [e] Glass column for GLC: 3% OV – 225 (213 cm, 2.2 mm i.d.) on Chromosorb W/AW – DMCS 80 – 100 mesh. *5a* and $PhCON(CH_2)_4$ could not be separated. [f] % Reaction by path (B) calculated from the ratio between *4a* and *4a* + *3a*. [g] Incomplete reaction. [h] % Reaction by path (B) calculated from the ratio between *4e* and *4e* + *3d*. [i] Or in methanol and dichloromethane. [j] % Reaction by path (B) calculated from the ratio between *4e* and *4e* + $PhCON(CH_2)_4$. [k] *5b* and *3i* could not be separated even on an SE-30 (35 m, 0.5 mm i.d.) SCOT column. [l] Some *6d* and several unidentified products also present in the reaction mixture. [m] *1l* decomposed on attempted purification; crude *1l* was used. [n] *5c* and *3n* could not be separated.

amount of nucleophilic attack at the pyridine-2-carbonyl group of *1*. Compound *1e* undergoes a fast reaction with 4-methylaniline whereas the other *N*-acylamides give slow reactions with this amine. Nearly 60% of the nucleophilic attack on *1e* occurs at the benzoyl carbonyl group. These observations indicate comparable reaction rates for *1e* by paths (B) and (C). The electron withdrawing arylsulfonyl group probably enhances the reaction by path (C).

The products from the reaction of *1l* with cyclopentylamine also show that 60% of the nucleophilic attack occurs at the benzoyl group. It is

interesting to compare this reaction with that of *1b* with the same nucleophile where no attack at the benzoyl group is observed. Therefore, since the 4-nitrophenyl group of *1b* does not enhance path (C) for the reaction of that compound, path (C) also must be excluded for the reaction of *1l*. Consequently, these results indicate that *1l* reacts with cyclopentylamine by both paths (A) and (B). This also is in accord with the mentioned reactions of *7f-HCl* with nucleophiles where a predominant attack on the acetyl group is observed.

Reactions of *1d*, *1i* and *1n* with cyclopentylamine would give the same reaction products from either path (A), (B) or (C) due to the *N*-cyclopentyl substituent of these compounds. However, since quite similar reaction patterns have been established for *1a* and *1g* with both cyclopentylamine and pyrrolidine, the latter base is used as a substitute for cyclopentylamine in reactions of *1d*, *1i* and *1n*. Only 36 – 65 % of the reactions of these three compounds with pyrrolidine occur by path (B). This is in contrast to the reactions of compounds *1* which have an *N*-aryl substituent. All of those compounds, except *1l*, react with a strong base only by path (B). Compounds *1i* and *1n* with enhanced pyridine-*N* nucleophilicity compared to *1d* show less reaction by path (B) than *1d*. Also, the least amount of reaction by path (B) is observed for *1n* in a protic solvent. These observations indicate that *1n* reacts with pyrrolidine in ethanol mostly by path (A) and that *1i* reacts somewhat less by path (A) under the same reaction conditions.

DISCUSSION

There are two limitations to the use of compounds *1* as acyl-transfer agents. Firstly, the reactivity of the imidoyl chloride hydrochlorides *7* towards carboxylate ions needs to be improved. Attempts to use silver tosylate for this purpose led to erratic results; a reaction of *7f-HCl* with acetate ions was enhanced by silver tosylate as shown by the products isolated after the addition of aniline to this reaction mixture. However, a reaction of *7f* with triethylamine and silver tosylate was shown by GLC to produce substantial amounts of 4-nitroaniline which had been produced through cleavage of the imide bond of *7f*.

The second limitation is the favorable competition in most instances of path (B) with the planned path (A). However, there are some exceptions to this pattern. Compound *1l* reacts predominantly by path (A), and this may be explained by both the electron-withdrawing 4-nitrophenyl group and the enhanced pyridine-*N* nucleophilicity of *1l*. Compound *1n* also reacts mostly by path (A) and *1i* reacts substantially by path (A). Compounds *1i* and *1n* have a pyridine-4 substituent which is expected to promote path (A) but the apparent effect of the cyclopentyl group of these compounds is less obvious. Thus, further studies of compounds related to *1f* – *1n* but with a variety of amide-*N* substituents are indicated.

EXPERIMENTAL

General. The instrumentation has been described.[4] Cyclopentylamine, pyrrolidine, 4-methylaniline, benzanilide and 2-pyridinecarboxylic acid, all *purum*, were obtained from Fluka. Acetanilide and *p*-toluenesulfonamide were obtained from Schuchardt, methyl benzoate from Riedel-de-Haën, hippuric acid and *p*-toluenesulfonic acid from Merck.

Silver tosylate[10] and *N*-cyclopentylacetamide, liq., lit.[11] b.p. 146 – 149 °C/22 mmHg were prepared.

2-Pyridinecarboxamides, 3a – o *and* 5a – c. Compounds *3a*, *3b*, *3o*, *3f*, *3g* and *3j* – *l* have been described,[5] *3c*[4,12] and compounds *3d*, *3h* – *i*, *3m* – *n* also have been described.[4] Compound *3e* was prepared from equimolar amounts of 2-pyridinecarbonyl chloride, *p*-toluenesulfonamide and triethylamine in tetrahydrofuran at ambient temperature. *3e* (60 %) m.p. 134 – 136 °C. IR (nujol): 3300 (s), 1710 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.47 (3H, s), 7.46 – 8.71 (8H, m). MS [*m/e* (% rel. int.)]: 212 (39, M – SO_2).

Compound *5a* was prepared,[13] and *5b* was obtained from a reaction of *6d*,[5] first with thionyl chloride and thereafter with an excess of pyrrolidine in benzene. Chromatography on silica gel yielded *5b* (70 %), m.p. 84 – 85 °C. IR (nujol): 1630 (s), 1595 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.90 (4H, m), 3.29 – 3.84 (12H, m), 6.80 (1H, dd, *J* 2.9 Hz), 7.05 (1H, d, *J* 2.9 Hz), 8.20 (1H, d, *J* 5.7 Hz). MS [*m/e* (% rel. int.)]: 261 (18, M). Mol. wt., obs. 261.1476, calc. for $C_{14}H_{19}N_3O_2$ 261.1477.

Compound *5c* was prepared by heating methyl 4-chloropyridine-2-carboxylate with pyrrolidine (3 mol eq.) at 90 °C for 24 h.[5] The reaction mixture was extracted with benzene. The benzene extract was washed with water, chromatographed on silica gel and *5c* was eluted with chloroform and acetone, 1:1. The liquid product was crystallized from diethyl ether and *5c* (62 %) m.p. 87 – 88 °C was obtained. IR (nujol): 1625 (s), 1600 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.0 (4H, m), 3.5 (4H, m), 6.54 (1H, dd, *J* 2.9 Hz), 6.77

(1H, d, *J* 2.9 Hz), 8.14 (1H, d, *J* 5.7 Hz). MS [*m/s* (% rel. int.)]: 245 (21.7, M). Mol. wt. obs., 245.1521, calc. for $C_{14}H_{19}N_3O$ 245.1528.

Methyl 2-pyridinecarboxylates. Compound *6a* was prepared [14] and was purified by chromatography on silica gel. Compound *6c* was prepared by heating 4-(4-morpholinyl)-2-pyridinecarbonyl chloride hydrochloride with an excess of dry methanol at 60 °C for 30 min. Excess methanol was removed under reduced pressure and the liquid residue was extracted with benzene and triethylamine. Triethylammonium chloride was removed by filtration and the filtrate yielded *6c* (88 %), m.p. 108 – 109 °C (diethyl ether). IR (nujol): 1750 (s), 1600 (s) cm^{-1}. ^1H NMR (CD_3CN): δ 3.3 (4H, m), 3.75 (7H, m), 6.88 (1H, dd, *J* 2.9 Hz), 7.50 (1H, d, *J* 2.9 Hz), 8.30 (d, *J*, 5.7 Hz). MS [*m/e* (% rel. int.)]: 222 (41.7, M). Mol. wt., obs. 222.1003, calc. for $C_{11}H_{14}N_2O_3$ 222.1004.

Benzamides, 4b – f. These compounds were prepared from benzoyl chloride and an amine.

4b, M.p. 157 – 158 °C, lit.[15] m.p. 158 °C.

4c, M.p. 198 – 201 °C, lit.[16] m.p. 199 °C.

4d, M.p. 81 – 82 °C, lit.[17] m.p. 82 – 83 °C.

4e, M.p. 159 – 161 °C, lit.[18] m.p. 157.5 – 158.5 °C.

4f, M.p. 146 – 149 °C, lit.[19] m.p. 147 – 150 °C.

N-Benzoylpyrrolidine, m.p. 51 – 53 °C, lit.[20] m.p. 46 – 47 °C.

p-*Toluenesulfonamides*. *N*-Cyclopentyl-*p*-toluenesulfonamide, m.p. 75 – 76 °C was prepared from cyclopentylamine and *p*-toluenesulfonyl chloride, lit.[21] m.p. 84 °C. *N*-(4-Nitrophenyl)-*p*-toluenesulfonamide, m.p. 188 – 190 °C, lit.[22] m.p. 189 – 190 °C.

N-*Acyl-2-pyridinecarboxamides*, 1a – o. Compounds *1a*, *1f* and *1j* have been described,[7] *1b* and *1o*,[6] *1c – d*, *1h – i* and *1m – n* also are known compounds.[4]

1e (75 %), m.p. 124 – 126 °C was obtained from a reaction of *3e* with equimolar amounts of benzoyl chloride and triethylamine. IR (nujol): 1725 (s), 1710 (s), 1700 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.50 (3H, s), 7.4 – 8.3 (13H, m). MS [*m/e* (% rel. int.)]: 316 (3.1, $M-SO_2$). Anal. $C_{20}H_{16}N_2O_4S$: C, H, S.

1g (63 %), m.p. 203 – 207 °C dec. was obtained[4] from equimolar amounts of *N*-(4-methylphenyl)-benzimidoyl chloride,[23] *6d* and triethylamine. IR (nujol): 1695 (s), 1685 (s), 1600 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.35 (3H, s), 3.35 (4H, m), 3.78 (4H, m), 6.77 (1H, dd, *J* 2.9 Hz), 7.2 – 8.1 (11H, m). MS [*m/e* (% rel. int.)]: 401 (38.2, M). Mol. wt., obs. 401.1746, calc. for $C_{24}H_{23}N_3O_3$ 401.1739.

1k (50 %), m.p. 192 – 196 °C dec. was obtained[4] from *N*-(4-methylphenyl)benzimidoyl chloride,[23] *6e* and triethylamine. IR (nujol): 1695 (s), 1690 (s), 1610 (s) cm^{-1}. ^1H NMR (CD_3NO_2); δ 2.0 (4H, m), 2.30 (3H, s), 3.25 (4H, m), 6.47 (1H, dd, *J* 2.9 Hz), 6.95 – 8.2 (11H, m). MS [*m/e* (% rel. int.)]: 385 (30.9, M). Mol. wt., obs. 385.1790, calc. for $C_{24}H_{23}N_3O_2$ 385.1790.

1l was prepared[4] from equimolar amounts of *N*-(4-nitrophenyl)benzimidoyl chloride,[24] triethylamine and *6e*. The benzene soluble product, which was a liquid, was reacted with cyclopentylamine without further purification. IR (film): 1715 – 1690 (s), 1675 (s), 1600 (s) cm^{-1}.

Amidines, 8. These compounds were prepared by the following procedure. Three molar equivalents of cyclopentylamine or morpholine, or 1.1 molar equivalent of aniline plus two molar equivalents of triethylamine were added to a suspension of the imidoyl chloride hydrochloride[5] *7* in acetonitrile. The reaction mixture was stirred at ambient temperature for 24 h. The solvent was removed under reduced pressure and the residue was extracted with diethyl ether. The diethyl ether soluble amidine was recrystallized from a mixture of hexane and diethyl ether or chromatographed on silica gel.

8a (70 %), m.p. 91 – 93 °C. IR (nujol): 3380 (s), 1610 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.5 – 2.1 (9H, m), 4.1 (1H, broad s), 6.8 – 8.1 (7H, m), 8.7 (1H, d, *J*, 5.7 Hz). MS [*m/e* (% rel. int.)]: 310 (76.4, M). Mol. wt., obs. 310.1427, calc. for $C_{17}H_{18}N_4O_2$ 310.1430.

8b (69 %), m.p. 120 – 122 °C. IR (nujol): 1605 (sh), 1600 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 3.4 – 3.7 (8H, m), 6.7 – 7.9 (7H, m), 8.55 (1H, m). MS [*m/e* (% rel. int.)]: 312 (100, M). Mol. wt., obs. 312.1220, calc. for $C_{16}H_{16}N_4O_3$ 312.1222.

8c (69 %), m.p. 135 – 136 °C. IR (nujol): 3280 (m), 1640 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 2.26 (3H, s), 3.05 (4H, m), 3.65 (4H, m), 6.7 – 7.2 (11H, m), 8.23 (1H, d, *J* 5.7 Hz). MS [*m/e* (% rel. int.)]: 372 (94.2, M). Mol. wt., obs. 372.1950, calc. for $C_{23}H_{24}N_4O$ 372.1950.

8d (50 %), m.p. 117 – 120 °C dec. IR (nujol): 3300 (m), 1640 (m), 1610 (m) cm^{-1}. ^1H NMR (CD_3CN): δ 2.5 (1H, broad s), 3.1 (4H, m), 3.7 (4H, m), 6.6 – 7.2 (10H, m), 8.03 (1H, d, *J* 8.6 Hz), 8.23 (1H, d, *J* 5.7 Hz). MS [*m/e* (% rel. int.)]: 403 (69.5, M). Mol. wt., obs. 403.1634, calc. for $C_{22}H_{21}N_5O_3$ 403.1644.

8e (50 %), m.p. 129 – 132 °C. IR (nujol): 3340 (s), 1640 (s), 1615 (s), 1595 (s) cm^{-1}. ^1H NMR (CD_3NO_2 and CD_3CN): δ 1.95 (4H + CD_3CN, m), 3.0 (4H, m), 6.4 – 7.2 (12H, m), 8.13 (1H, d, *J* 5.7 Hz). MS [*m/e* (% rel. int.)]: 342 (66.2, M). Mol. wt., obs. 342.1843, calc. for $C_{22}H_{22}N_4$ 342.1844.

8f (60 %), m.p. 96 – 98 °C. IR (nujol): 3350 (s), 1635 (s), 1610 (s), 1590 (s) cm^{-1}. ^1H NMR (CD_3NO_2): δ 1.95 (4H, m), 2.27 (3H, s), 3.1 (4H, m), 6.5 – 7.3 (11H, m), 8.12 (1H, d, *J* 5.7 Hz). MS [*m/e* (% rel. int.)]: 356 (79.9, M). Mol. wt., obs. 356.2003, calc. for $C_{23}H_{24}N_4$ 356.2003.

8g (46 %), m.p. 137 – 138 °C. IR (nujol): 3350 (m), 3320 (sh), 1650 (s), 1605 (s) cm^{-1}. ^1H NMR (CD_3CN): δ 1.5 – 2.0 (12H + CD_3CN, m), 3.1 (4H, m), 3.4 (1H, m), 4.14 (1H, broad s), 6.4 – 6.8 (4H, m), 7.9 – 8.2 (3H, m). MS [*m/e* (% rel. int.)]: 379 (63.6, M).

Mol. wt., obs. 379.2011, calc. for $C_{21}H_{25}N_5O_2$ 379.2008.

Reactions of 7a *with nucleophiles.* Silver tosylate (140 mg, 0.5 mmol) was added to a mixture of *7a* (150 mg, 0.5 mmol) and triethylamine (50 mg, 0.5 mmol) in 10 ml of acetonitrile. The reaction mixture was stirred at ambient temperature for 45 min. Cyclopentylamine (85 mg, 1 mmol) was added and stirring was continued for 30 min. Silver chloride was removed by filtration. The filtrate was analyzed by GLC at 300 °C, four compounds were found and were identified as *8a* (0.62 mol eq.), *3d* (0.28 mol eq.), *3b* (0.06 mol eq.) and *N*-cyclopentyl-*p*-toluenesulfonamide (0.04 mol eq.).

A mixture of *7a* (150 mg, 0.5 mmol), triethylamine (100 mg, 1 mmol) and sodium benzoate (72 mg, 0.5 mmol) in 10 ml of dichloromethane and 1 ml of acetonitrile was stirred at ambient temperature for 18 h. The solvents were removed under reduced pressure, 8 ml of benzene were added, the mixture was filtered and cyclopentylamine (45 mg, 0.5 mmol) was added to the filtrate. The reaction mixture was filtered after 30 min at ambient temperature. The benzene insoluble material was treated with water and yielded 20 mg (16%) of *3b*, m.p. 232 – 235 °C. The benzene was removed from the filtrate and diethyl ether (10 ml) was added to the residue. The mixture was filtered and 40 mg (20%) of *1b*, m.p. 160 – 170 °C was removed as insoluble material. The filtrate was concentrated and gave 90 mg (58%) of *8a*, m.p. 91 – 95 °C.

Hippuric acid (90 mg, 0.5 mmol) was added to a mixture of *7a* (150 mg, 0.5 mmol) and triethylamine (120 mg, 1.2 mmol) in 10 ml of dichloromethane. The reaction mixture was stirred at ambient temperature for 18 h. Cyclopentylamine (45 mg, 0.5 mmol) was added and the reaction mixture was analyzed by GLC after 1 h at ambient temperature. The two compounds *3b* and *8a* were present in a molar ratio of 1:5. The solvent was removed from the reaction mixture and 100 mg (64%) of *8a* m.p. 90 – 95 °C was isolated as diethyl ether soluble material, 15 mg (12%) of *3b*, m.p. 235 °C as diethyl ether insoluble material.

Reaction of 7e *with triethylammonium benzoate.* A mixture of *7e* (340 mg, 1 mmol), benzoic acid (122 mg, 1 mmol) and triethylamine (300 mg, 3 mmol) in 10 ml of acetonitrile was stirred at ambient temperature for 40 h. The solvent was removed under reduced pressure, and the benzene soluble fraction of the residue was chromatographed on silica gel. Compound *1k*, 190 mg (49%) m.p. 192 – 196 °C decl. was eluted from the column with acetone.

Reaction of 7f *with nucleophiles.* Silver tosylate (31 mg, 0.11 mmol) was added to a mixture of *7f* (40 mg, 0.11 mmol) and triethylamine (15 mg, 0.15 mmol) in 4 ml of acetonitrile. The reaction mixture was stirred for 10 min at ambient temperature. Cyclopentylamine (26 mg, 0.3 mmol) was added and stirring was continued for 30 min. The suspension was filtered and the filtrate was analyzed by GLC. Three compounds were identified, 4-nitroaniline (0.65 mol eq.), *N*-cyclopentyl-*p*-toluenesulfonamide (0.27 mol eq.) and *3n* (0.08 mol eq.).

In another experiment a mixture of *7f* and a fourfold molar excess of triethylamine in acetonitrile was analyzed by GLC. Only minute amounts of 4-nitroaniline were present in the solution. Silver tosylate (1 mol eq.) was added, the reaction mixture was filtered after 15 min and was analyzed by GLC. Substantial amounts of 4-nitroaniline were found in addition to other unidentified products.

In another experiment a solution of triethylammonium acetate (161 mg, 1 mmol) in 5 ml of tetrahydrofuran was added to a solution of *7f* – *HCl*[5] (331 mg, 1 mmol) in 10 ml of tetrahydrofuran. To this solution was added silver tosylate (279 mg, 1 mmol) in 10 ml of acetonitrile. The reaction mixture was stirred, protected from light and moisture, at ambient temperature for 1 h. Aniline (93 mg, 1 mmol) was added and the reaction mixture was stirred for 23 h. The solvents were removed under reduced pressure and the residue was extracted with 30 ml of benzene and 10 ml of a saturated aqueous sodium hydrogencarbonate solution. Some undissolved material was removed by filtration, and the chloroform soluble part of this solid gave 120 mg (38%) of *3l*, m.p. 219 – 222 °C. The benzene solution was extracted with 10 ml of 5% aqueous hydrogen chloride and the hydrochloric acid extract yielded 153 mg (49%) of *3l* m.p. 205 – 220 °C upon neutralization. The benzene solution was washed with water, dried over magnesium sulfate and yielded 76 mg (56%) of acetanilide, m.p. 105 – 112 °C (benzene and heptane).

Reactions of 1 *with nucleophiles.* Compound *1* was dissolved in a specified solvent (Table 1) and a fourfold molar excess of cyclopentylamine or pyrrolidine, or 1.1 molar equivalent of 4-methylaniline, was added. The solutions were left at ambient temperature before analysis by GLC. Compounds *1* also were reacted with methanol or water (Table 1) and these were used in a large molar excess. Response factors and retention times were found for the expected reaction products by GLC analysis of authentic samples under the same conditions which were used for the product analyses.

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Design and Synthesis of Effective Antagonists of Substance P

KARL FOLKERS,[a] JOACHIM HÖRIG,[a] GEORG RAMPOLD,[a] PATRICIA LANE,[a] SUNE ROSELL[b] and ULLA BJÖRKROTH[b]

[a] Institute for Biomedical Research, The University of Texas at Austin, Austin, Texas 78712, U.S.A. and [b] Farmakologiska Institutionen, Karolinska Institutet, Stockholm, Sweden

The agonist/antagonist activities of four background analogs of substance P (SP) facilitated design and synthesis of 12 new analogs to achieve effective antagonists. (D-Pro^2, D-Phe^7, D-Trp^9)-SP, (D-Pro^2, D-Trp^{7,9})-SP and (D-Arg^1, D-Phe^7, D-Trp^9)-SP showed no agonist activity; 9 analogs showed weak agonist activity of SP.

(D-Pro^2, D-Trp^{7,9})-SP was the most potent antagonist which at a concentration of 10^{-5} required a 3-fold increase in SP to allow a 50 % response by SP. (D-Pro^2, Lys^6, D-Phe^7)-SP and (D-Pro^2, D-pClPhe^7, D-Trp^9)-SP were also potent, and the antagonism was competitive.

For specific pairs of peptides, Lys^6 is a promising substituent. D-Trp^{7,9} was as effective as Lys^6, D-Phe^7. D-pClPhe^7 was three times as effective as D-Phe^7. D-Dln^6 was 1.33-fold better than D-Gln^5. D-Pro^2 and D-Pro^4 were equally effective. D-Pro^2 was 1.5 times as effective as D-Lys^3. D-Pro^2 may not be important. D-pClPhe^9 and D-Trp^9 were equally effective.

Von Euler and Gaddum[1] discovered the physiological actions of an entity in fractions from equine intestinal tissue which they names substance P (SP). Diverse biological and chemical investigations followed including progress on purification. In 1970, Chang and Leeman[2] isolated a sialogogic peptide from hypothalamic tissue which was proven to be identical in physiological activities and chemical properties to those described for SP.

Substance P is the undecapeptide, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, elucidated by Chang *et al.*[3] The physiological activities of SP include the contraction of the guinea pig ileum, the sialogogic effect in the rat,[4] depolarization of motor neurons of the spinal cord, and a lowering of blood pressure.[5]

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Jessell and Iversen[6] reported the release of SP from neurons of the brain stem is inhibited by the action of [D-Ala^2]-Met-encephalin, and interpreted this effect as support for the hypothesis that SP is a neurotransmitter in nociception.

Before the elucidation of the structure of SP, the chemistry of eledoisin and physalemin was elucidated as peptides which were later recognized to be closely related in sequence to that of SP.[7,8] The *C*-terminal pentapeptide moiety of these two tachykinins is similar to the *C*-terminal pentapeptide moiety of SP. The amino acid in position 8 is the only difference. Phe^8 is in SP, Ile^8 is in eledoisin, and Tyr^8 is in physalemin. The analogs of these tachykinins which have been described[9,10,11] are also analogs of SP, and provide information about the sequence – activity relationships of these peptides. These and subsequent studies revealed the importance of the substituents Phe^7, Leu^{10} and Met^{11}.

Despite these years of investigations and the innumerable available analogs, no meaningful progress was made on the design and synthesis of effective inhibitors of SP, eledoisin and physalemin.[12,13] As biological progress was made with the newly avaulable synthetic SP, the need for effective inhibitors of SP became emphasized.

Yamaguchi *et al.*[14] synthesized [D-Phe^7]-SP, which was found to have a weak antagonistic activity in the guinea pig ileum system. Leban *et al.*[15] found that [D-Leu^8, D-Phe^9]-SP antagonized SP, but only at high concentrations. [D-Pro^2]-SP exhibited some antagonistic activity.

Described herein are the syntheses and the chemical and biological properties of newly designed analogs which were based upon the structural features and the antagonistic activities of [D-Phe^7]-SP, [D-Leu^8, D-Phe^9]-SP and [D-Pro^2]-SP.

EXPERIMENTAL

The protected amino acids were purchased from Peninsula Laboratories, Inc., San Carlos, California. The benzhydrylamine hydrochloride resin was purchased from Beckman Inc., Palo Alto, California. All solvents (except TFA and isopropranol) were distilled before use. To check homogeneity, the peptides (5 μg in 5 μl of water) were chromatographed on precoated TLC plates (silica gel, 5 × 20 cm, Merck, Darmstadt, W. Germany) in the following solvent systems: I. $CHCl_3$ – conc. NH_4OH – CH_3OH = 60:20:45; II. EtOAc – Pyr – AcOH – H_2O = 5:5:1:3; III. n-BuOH – EtOAc – AcOH – H_2O = 2:2:1:1; IV. n-BuOH – Pyr – AcOH – H_2O = 30:30:6:24; V. i-PrOH – 1 N AcOH = 2:1. The spots on the developed thin layer plates were detected with the chlorine-o-tolidine reagent.

Synthesis of the peptides. The peptides were synthesized by the solid phase method[17] using a Beckman Model 990 Peptide Synthesizer. The benzhydrylaminehydrochloride resin (BHA-resin) was used as a solid support. The program of the synthesizer was divided into subprograms to increase the versatility of the synthesizer, as follows.

Deprotection: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 50 % TFA in CH_2Cl_2 containing 0.1 % indole 1 × wash, 2 min); 3. 50 % TFA in CH_2Cl_2 (deprotection, 30 min); 4. CH_2Cl_2 (2 × wash). Neutralization: 1. CH_2Cl_2 (2 × wash, 2 min); 2. Et_3N (10 % in CH_2Cl_2) (2 × wash, 2 min); 3. Et_3N (10 % in CH_2Cl_2) (Neutralization, 20 min); 4. CH_2Cl_2 (2 × wash, 2 min). DCC Coupling: 1. CH_2Cl_2 (2 × wash, 2 min); 2. Amino acid derivative in CH_2Cl_2 (delivery, transfer, mix, 2 min); 3. DCC (10 % in CH_2Cl_2) (delivery and mix, 180 min); 4. CH_2Cl_2 (2 × wash, 2 min). Active Ester Coupling: 1. CH_2Cl_2 (2 × wash, 2 min); 2. Amino acid derivative and 1-hydroxybenzotriazole in DMF (delivery, transfer, mix, 180 min); 3. CH_2Cl_2 (2 × wash, 2 min). Final Wash: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 2-ProOH (3 × wash, 2 min); 3. DMF (3 × wash, 2 min); 4. CH_2Cl_2 (3 × wash, 2 min). Wash after TFA treatment: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 2-ProOH (3 × wash, 2 min); 3. CH_2Cl_2 (3 × wash, 2 min). Acetylation: 1. CH_2Cl_2 (2 × wash, 2 min); 2. 10 % Ac_2O and 10 % Pyr in CH_2Cl_2 (1 × wash, 2 min); 3. 10 % Ac_2O and 10 % Pyr in CH_2Cl_2 (Acetylation, 20 min); 4. CH_2Cl_2 (2 × wash, 2 min).

The first amino acid was attached to the resin by the program sequence 2-3-5. Before placing the resin into the reaction vessel, it was washed twice in a separate funnel with 25 ml of CH_2Cl_2/g resin to remove the finer particles. In all couplings, usually a 4 – 5-fold excess of the Boc-amino acid over the nitrogen content of the resin (nitrogen content was about 0.5 mequ/g dry resin) was used. This procedure generally resulted in a complete coupling reaction. If a negative ninhydrin color reaction[18] was not obtained, a second coupling using the same excess of the amino acid derivative was performed (program sequence 3 – 5). Then, the resin was acetylated (program sequence 7 – 5).

The next amino acid was attached by the program sequence 1-6-2-3-5. All amino acid derivatives were used as their Boc derivatives except Arg, which was used as Aoc-Arg(Tos). The ε-NH_3-group of Lys was protected by the Cl-Z group. For DCC coupling, all amino acids were dissolved in CH_2Cl_2 (0.5 – 1 mmol/ml). To dissolve Boc-Trp, it was necessary to add 10 % of DMF to the suspension. Gln was coupled to the resin by its Boc-Gln-ONp derivative using the active ester coupling program sequence 1-6-2-4-5. The Boc-Gln-ONp (4 – 5-fold excess over the N-content of the resin) was dissolved in DMF 1 mmol/ml), and 20 mg/ml 1-hydroxybenzotriazole was added as a catalyst.[19]

The volume of the solvents and the reagents used for washing and performing chemical reactions was about 10 ml/g resin. The acetylation mixture was freshly prepared before each use.

Cleavage of the peptides from the resin. After all of the amino acids had been coupled, the resin was dried overnight, *in vacuo*, by an oil pump. It was then treated with double-distilled and dried (over CoF_3) liquid hydrogen fluoride (10 ml/g resin) containing 10 – 25 % of distilled anisole for 1 h at 0 °C. Then, the HF was evaporated under reduced pressure and the residue was dried overnight, *in vacuo*, by an oil pump. The mixture was then extracted with EtOAc (25 ml/g resin), and then twice with 25 ml of 20 % AcOH, and once with 25 ml water. The combined aqueous layers were pooled and lyophilized to yield the crude peptide.

Purification of the crude peptide. Gel filtration. 200 mg of the crude peptide was applied to a column of Sephadex G-25 (100 × 2.5 cm) which was equilibrated with 6 % AcOH, and then chromatographed with the same solvent. Fractions of 10 ml were collected. The peptide was detected by spotting samples of the individual fractions on silica gel plates and chromatographing them in solvent system II. The fractions containing the product in a partially purified state were pooled and lyophilized. The yield was 60 – 130 mg of dry material.

First partition chromatography. The lyophilized material was applied to a column of Sephadex G-25 (3.5 × 53 cm). Before use, the column was first equilibrated with 1 l of lower phase of BuOH – 0.1 % AcOH – Pyr = 7:10:3, and then with 1 l of the upper phase of the same system. The sample was then dissolved in 3 – 5 ml of the upper phase, applied to the column and chromatographed. Fractions of 10 ml were collected (0.5 ml/min). The peptides, in general, were eluted in fractions 18 – 23 except for peptides V and VI, which were eluted with fractions

60 – 80. The fractions which contained the pure or nearly pure peptide were collected and lyophilized.

Second partition chromatography. When the product was not sufficiently pure, after having been chromatographed in the first two systems, it was further purified on a column of Sephadex G-25 (2.4 × 100 cm) prepared in the same manner as the first partition column, but with the upper phase of the solvent system BuOH-AcOH-H_2O = 4:1:5. Fractions of 10 ml were collected. The peptide was eluted in fractions 21 – 36 (flow rate: 0.5 ml/min).

Column chromatography on Sephadex LH 20. If not sufficiently pure, after the second partition chromatography, the peptide was chromatographed on a column of Sephadex LH 20 (2.5 × 100 cm) with the solvent system BuOH-AcOH-H_2O = 6:10:90. The peptide was eluted with fractions 35 – 45 (fraction size, 10 ml). The fractions containing the pure peptide were lyophilized.

High pressure liquid chromatography. The HPLC was performed on a Waters Liquid Chromatograph equipped with a Waters 660 Solvent Programmer. The samples were chromatographed either on a Waters analytical μ-Bondapak column (3.9 × 300 mm) or a Chrompak Lichrosorb 5 RP C_{18}-column (5 μ) (4.6 × 250 mm). For elution of the analogs, a linear gradient from 0 – 100 % of solvent system B in 25 min was used (solvent A: 0.1 M K-phosphate buffer, pH 3.0; solvent B: 50 % of solvent system A, 50 % CH_3CN). The flow rate was 2.0 ml/min. 10 μl of a 0.1 % solution of the peptide was injected. The eluted peptide was detected by its UV-absorbance at 210 nm.

Amino acid analysis. The automated amino acid analysis was performed on a Beckman Model 119 Automated Amino Acid Analyzer. The peptides were hydrolyzed for 24 h in a sealed glass tube at 110 °C in 6 N HCl. The mixture was then dried, *in vacuo*. The residue was dissolved in 1.5 ml of sodium citrate buffer, pH 2.2 (0.2 N), and 0.2 ml of the solution was applied to the analyzer and chromatographed.

Optical rotation. The optical rotation (α_D) was measured at room temperature (25 °C) with a Perkin Elmer 141 Polarimeter. All peptides were dissolved in distilled MeOH (10 mg/ml).

Properties of background analogs of substance P showing antagonistic activity. The chemical properties of four analogs of substance P which showed some antagonistic activity, and which provided some guidance for the design of the initial analogs of the group of fourteen peptide in Table 1 are as follows. The TLC systems were: Rf^1: BuOH – AcOH – EtOAc – H_2O = 1:1:1:1; Rf^2: EtOAc – Pyr – AcOH – H_2O = 5:5:1:3; Rf^3: BuOH – Pyr – AcOH – H_2O = 30:30:6:24; Rf^4: 2-PrOH – 1 N AcOH = 2:1; Rf^5: $CHCl_3$ – MeOH – AcOH = 60:20:45.

[D-Leu^8, D-Phe^9]-SP showed single spots in five systems in TLC: Rf^1, 0.51; Rf^2, 0.78; Rf^3, 0.39; Rf^4, 0.13; Rf^5, 0.31. Amino acid analysis: Glu, 1.96 (2); Pro, 2.01 (2); Met, 1.01 (1); Leu, 1.92 (2); Phe, 1.99 (2); Lys, 1.11 (1); Arg 1.00 (1).

[D-Pro^2,Ile^7]-SP showed single spots in three systems by TLC: Rf^2, 0.81; Rf^3, 0.53; Rf^5, 0.43. Amino acid analysis: Glu, 2.05 (2); Pro, 1.87 (2); Gly, 1.06 (1); Met, 0.97 (1); Ile, 0.90 (1); Leu, 1.06 (1); Phe, 0.91 (1); Lys, 1.04 (1); Arg 1.00 (1).

[D-Pro^2]-SP showed single spots in three systems by TLC: Rf^3, 0.3; Rf^1, 0.1; Rf^2, 0.8. Amino acid analysis: Glu, 2.10 (2); Pro, 2.00 (2); Gly, 1.04 (1); Met, 0.95 (1); Leu, 1.07 (1); Phe, 1.89 (2); Lys, 0.94 (1); Arg, 1.02 (1).

[D-Pro^2,D-Leu^8,D-Phe^9]-SP showed single spots in three systems by TLC: Rf^1, 0.08; Rf^2, 0.6; Rf^3, 0.4. Amino acid analysis: Gly, 1.97 (2); Pro, 1.97 (2); Met^a, 0.81 (1); Leu, 1.94 (2); Phe, 1.91 (2); Lys, 1.11 (1); Arg, 0.97 (1).

RESULTS AND DISCUSSION

Chemical results. The fourteen peptides in Table 1 were synthesized by stepwise coupling of Boc-amino acids to the growing peptide chain on the benzhydrylamine (BHA)-resin. When the reaction cycles were finished, the peptides were deprotected and cleaved from the resin by liquid HF, and the crude peptides were purified by column chromatography. The data of Table 1 include *Rf* values from five solvent systems by TLC, and the retention times in HPLC on a column of lichrosorb RP-5, estimated purities, and the optical rotations.

The crude peptides were first partially purified by gel filtration on Sephadex G-25, and then further purified by partition chromatography on Sephadex G-25. These two steps frequently resulted in peptides which showed single spots in the five TLC systems, and a sharp symmetrical peak by HPLC with only negligible impurities, as exemplified by Fig. 1 for [D-Pro^2,D-Phe^7,D-D-Trp^9]-substance P. Since analog IX was not sufficiently pure by this two-step purification, it was subjected to a second column of partition chromatography. Analog XIV was not pure even after three-step purification, and was further purified by chromatography on Sephadex LH 20. This system was found to be particularly suitable for the purification of these analogs of SP.

The data in Table 2 summarizes the details of the syntheses of the peptides of Table 1 and the yields which were obtained after the chromatographic purifications. The resin had an N-content of 0.47 –

Table 1. Analogs of substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2.

Analog	*Rf* in solvent I	II	III	IV	V	Retention time in HPLC(min)	Purity (HPLC)(%)	$[\alpha]_D$ (°)
I. [D-Pro^2,Ile^7,D-Leu^8,D-Phe^9]-SP	0.43	0.75	0.02	0.64	0.05	19.5	>97	−21.6
II. [D-Arg^1,D-Pro^2,Ile^7,D-Leu^8, D-Phe^9]-SP	0.47	0.73	0.02	0.59	0.03	19.5	>98	−35.7
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP	0.46	0.76	0.03	0.61	0.04	22.3	>98	−38.2
IV. [D-Arg^1,D-Pro^2,D-Phe^7,D-Trp^9]-SP	0.53	0.75	0.02	0.59	0.04	22.3	>98	−46.1
V. [D-Pro^2,Lys^6,D-Phe^7]-SP	0.3	0.54	0	0.54	0	17.6	>94	−26.6
VI. [D-Arg^1,D-Pro^2,Lys^6,D-Phe^7]-SP	0.35	0.54	0	0.52	0	17.6	>96	−35.4
VII. [D-Pro^2,D-Trp^{7,9}]-SP	0.4	0.78	0.02	0.59	0.03	22.3	>99	−40.1
VIII. [D-Arg^1,D-Phe^7,D-Trp^9]-SP	0.33	0.77	0.03	0.6	0.03	22.3	>95	−71.4
IX. [D-Lys^3,D-Phe^7,D-Trp^9]-SP	0.49	0.74	0.02	0.57	0.03	22.3	>95	−37.9
X. [D-Pro^4,D-Phe^7,D-Trp^9]-SP	0.4	0.75	0.02	0.62	0.03	22.3	>96	−47.1
XI. [D-Gln^5,D-Phe^7,D-Trp^9]-SP	0.44	0.81	0.02	0.62	0.03	22.3	>96	−38.7
XII. [D-Gln^6,D-Phe^7,D-Trp^9]-SP	0.43	0.77	0.02	0.58	0.03	22.3	>95	−51.2
XIII. [D-Pro^2,D-pClPhe^7,D-Trp^9]-SP	0.71	0.78	0.01	0.61	0.03	23.1	>90	−42.0
XIV. [D-Pro^2,D-Phe^7,D-pClPhe^9]-SP	0.74	0.78	0.01	0.62	0.03	23.5	>89	−40.3

0.5 meq/g resin. The first amino acid was coupled to the extent of 60 − 100 % as determined by amino acid analysis. The yields of the crude peptides were 0.2 − 0.5 mmol/g resin, which were 40 − 100 % of the theoretical values. These results show that during deprotection of the Boc-protected peptide in 50 % TFA and CH_2Cl_2 only little or no loss of peptide occurred, which is presumed to have been due to the stability of the amide linkage between the peptide and the resin toward hydrolysis. This stability of the BHA-resin is a great advantage over that of the Merrifield-type resin.

The data from the amino acid analysis of all analogs, which are described herein, revealed the expected ratios of the amino acids. These data alone may not be regarded as a proof for the purity of such

Table 2. Data on syntheses of analogs of substance P.

No.	Resin (g)	Peptide-resin (g)	Peptide after HF (mg)	Peptide for 1st column (mg)	Peptide after 1st column (mg)	Peptide after 1st pat. chrom. (mg)	Peptide after 2nd part. chrom. (mg)
I	3.0	3.48	910	100	78	44	[b]
II	3.0	3.7	990	150	[a]	17	[b]
III	1.0	1.57	324	200	[a]	75	[b]
IV	1.0	1.26	299	200	[a]	42	[b]
V	1.0	2.54	740	200	[a]	92	[b]
VI	1.0	2.39	550	200	[a]	78	[b]
VII	0.8	1.68	380	200	103	50	[b]
VIII	0.64	1.16	348	200	64	24	[b]
IX	0.64	1.11	315	200	120	[a]	25
X	0.64	1.16	280	200	121	68	[b]
XI	0.64	1.28	362	200	122	47	[b]
XII	0.64	1.28	326	200	142	80	[b]
XIII	1.0	2.29	627	200	87	76	[b]
XIV	1.0	[a]	460	200	94	42	[c]

[a] Not determined. [b] Not performed. [c] Peptide was chromatographed in addition on an LH-20 column (yield: 6 ng).

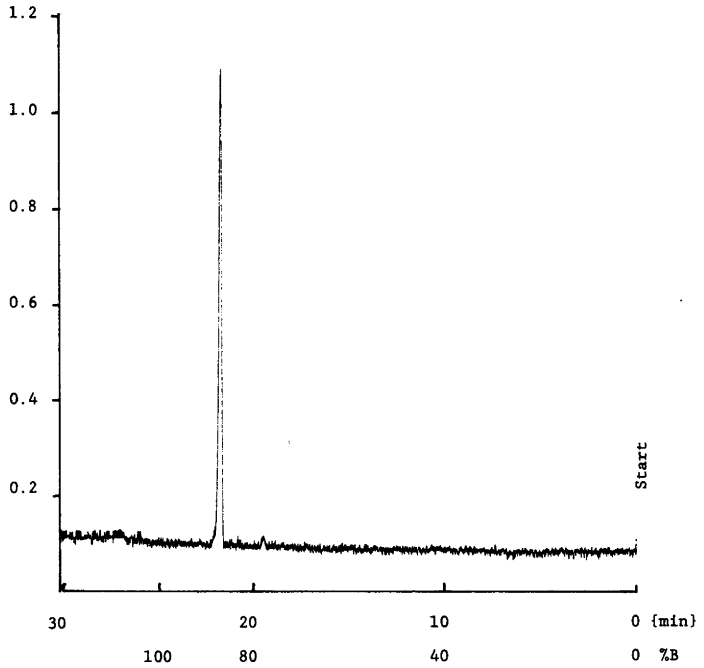

Fig. 1. HPLC of [D-Pro^2,D-Phe^7,D-Trp^9]-substance P. A Lichrosorb 5 RP C_{18} column was used. The flow rate was 2 ml/min. The column was equilibrated with 0.1 m K-phosphate buffer pH 3.0 (solvent A). At "start", the compound was injected and a linear gradient of 0 – 100 % of solvent B (50 % solvent A, 50 % CH_3CN) was applied within 25 min. The diagram shows the absorpance of the effluent at 210 nm. HPLC was performed only on fractions which were selected from column purification for purity rather than yield. Generally, samples of about 10 μg were used for HPLC and which had been found to be adequate to reveal impurities and purity for each peptide.

peptides, since it is common for analyses of crude preparations of peptides after cleavage by HF to reveal acceptable amino acid analytical data. However, these data must be acceptable as a component of the total data of purity.

During the purification by chromatography of the peptides, fractions were selected in such manner that purity was emphasized rather than yield. In the final chromatography step, only fractions which appeared pure by HPLC were taken. This procedure usually gave yields of 12 – 45 % of the pure peptides as calculated on the basis of the weights of the crude deprotected peptides.

Biological results. Twelve peptides (Table 3) were tested for smooth muscle stimulatory or substance P inhibitory actions on the isolated guinea pig ileum, which was suspended in a 5 ml organ bath containing Kreb's solution.

Three of the twelve peptides, (D-Pro^2, D-Phe^7, D-Trp^9)-SP, (D-Pro^2, D-Trp^{7,9})-SP and (D-Arg^1, D-Phe^7, D-Trp^9)-SP did not stimulate smooth muscle even at very high concentrations. All the other analogs very weakly stimulated smooth muscle (Table 3).

At a concentration of 10^{-4}, ten of the peptides showed antagonistic activity requiring concentrations of 3 to 22-fold of SP to allow a 50 % response. Of these ten peptides, (D-Pro^2, D-Trp^{7,9})-SP, (D-Pro^2, Lys^6, D-Phe^7)-SP and (D-Pro^2, D-pClPhe^7, D-Trp^9)-SP were the most potent at a concentration of 10^{-4} by requiring 22, 18 and 17-fold increases, respectively, in the SP concentration to allow a 50 % response.

[D-Pro^2,D-Phe^7,D-pClPhe^9]-SP not only inhibited the smooth muscle contraction induced by SP, but also of histamine, indicating that the inhibitory activity is less specific. None of the other peptides affected the smooth muscle stimulating activity of

Table 3. Assay data from guinea pig ileum system.

Analog	Agonist activity[a] Relative potency SP-100	Analog conc. (M)	Antagonist activity[a] Increase in SP-conc. to give 50 % of max. response
I	0.001	10^{-4}	0
III	0.0007	10^{-4}	6×
		10^{-3}	30×
V	0.034	10^{-4}	18×
VI	0.110	10^{-5}	0
VII	0.0009	10^{-4}	22×
		10^{-3}	430×
VIII	0.0005	10^{-4}	5×
IX	0.003	10^{-4}	4×
X	0.004	10^{-4}	6×
XI	0.001	10^{-4}	3×
XII	0.001	10^{-4}	4×
		10^{-3}	29×
XIII	0.001	10^{-4}	17×
		10^{-3}	[b]
XIV	0.001	10^{-4}	6×

[a] The tests were made on isolated guinea pig ileum suspended in a 5-ml organ bath containing Krebs' solution. [b] Inhibits also histamine.

histamine or acetylcholine, which indicated that these peptides are specific antagonists of substance P.

In the presence of each of the ten peptides (III, V, VII, VIII, IX, X, XI, XII, XIII, XIV) in the bath, the dose-response curve for SP was shifted in parallel to the right with no change in a maximally obtained response, which indicated that the antagonistic activity of these peptides is of a competitive type.

A comparison of the substitution in the sequences of these peptides reveals the following relationships, which may contribute to the design of new analogs or greater potency and perhaps greater specificity of diverse antagonistic effects.

The following style of comparisons of substitutions and antagonism facilitate interpretations of the more effective substitutions.

V. [D-Pro^2,Lys^6,D-Phe^7]-SP; 18-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
Lys^6 is a promising substituent.
VII. [D-Pro^2,D-Trp^{7,9}]-SP; 30-fold/10^{-4}
V. [D-Pro^2,Lys^6,D-Phe^7]-SP; 18-fold/10^{-4}
D-Trp^{7,9} was as effective as Lys^6,D-Phe^7.
XIII. [D-Pro^2,D-pClPhe^7,D-Trp^9]-SP; 17-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-pClPhe^7 was three times as effective as D-Phe^7. D-Arg^1 and L-Arg^1 are equally effective.
XII. [D-Gln^6,D-Phe^7,D-Trp^9]-SP; 4-fold/10^{-4}
XI. [D-Gln^5,D-Phe^7,D-Trp^9]-SP; 3-fold/10^{-4}
D-Gln^6 was 1.33-fold better than D-Gln^5.
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
X. [D-Pro^4,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-Pro^2 and D-Pro^4 were equally effective.
IX. [D-Lys^3,D-Phe^7,D-Trp^9]-SP; 4-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-Pro^2 was 1.5 times as effective as D-Lys^3.
VIII. [D-Arg^1,D-Phe^7,D-Trp^9]-SP; 5-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-Pro^2 may not be important.
XIV. [D-Pro^2,D-Phe^7,D-pClPhe^9]-SP; 6-fold/10^{-4}
III. [D-Pro^2,D-Phe^7,D-Trp^9]-SP; 6-fold/10^{-4}
D-pClPhe^9 and D-Trp^9 were equally effective.

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Chlorination of Esters. IV. Chlorination of Chloromethyl Esters of Aliphatic C_3-C_{12} n-Carboxylic Acids. Determination of Monochloro Products by Gas-Liquid Chromatography and Gas-Liquid Chromatography–Mass Spectrometry

ILPO O. O. KORHONEN

Department of Chemistry, University of Jyväskylä, Kyllikinkatu 1–3, SF-40100 Jyväskylä 10, Finland

The chlorinations with chlorine in the liquid phase at room temperature have been studied, in the absence and in the presence of benzene. The products of ten substrates, 65 compounds altogether, were identified and estimated by gas-liquid chromatography (GLC) and gas-liquid chromatography–mass spectrometry (GLC–MS). The chlorinations yield all possible monochloro isomers, the (ω-1)-chloro isomers being in general the main products in the absence of benzene. The solvent alters the selectivity of the reagent, the main product varying from (ω-1)- to (ω-4)-chloro isomer with an increase in chain length. The amounts of ω-chloro isomers were 1.1–4.3 times greater in the absence of benzene. The isomeric monochloro chloromethyl esters are eluted in direct order from 2- to ω-chloro isomer on a Carbowax 20M glass capillary column. The 2-, 3-, 4-, 5- and ω-chloro isomers can be distinguished by mass spectrometry from the other isomers on the basis of characteristic fragment ions. The mass spectra of the mid-chain isomers were nearly similar to each other.

Numerous papers have appeared on the chlorination of aliphatic methyl esters by various methods, but none on the chlorination of chloromethyl esters.

Recently, the chlorination of straight-chain methyl esters from propanoic to octadecanoic acid has been reported to produce chloromethyl esters as a side product.[1-3]

The present paper describes the chlorination of aliphatic chloromethyl esters from propanoic to dodecanoic acid with chlorine in the liquid phase, in the absence and in the presence of benzene. The monochlorinated isomers formed were determined by gas-liquid chromatography (GLC) and gas-liquid chromatography–mass spectrometry (GLC–MS). The gas-liquid chromatography of combined mixtures of even- and odd-carbon-number chloromethyl monochloro esters has been reported earlier.[4] The mass spectra of the compounds will be published later.

EXPERIMENTAL

Samples. Chloromethyl esters, used as starting materials in the chlorinations, were prepared from the corresponding acid chlorides and paraformaldehyde in the presence of a trace amount of zinc chloride.[5] 2-Chloro C_5-C_{12} acid chlorides,[6,7] monochloro propanoyl[8] and butanoyl chlorides[9-11] and the mixtures of monochloro C_5-C_{12} acid chlorides obtained by chlorination of the parent compounds with chlorine were converted[5] to chloromethyl esters.

The structures of the separately prepared authentic chloromethyl esters were confirmed by ^{1}H NMR and MS.

Chlorinations. Chloromethyl esters were chlorinated with chlorine in the liquid phase at room temperature without solvent and in benzene solution (molar ratio substrate–benzene was 1:10) as described earlier.[1] After removal of excess of chlorine and hydrogen chloride liberated with dry nitrogen, the crude chlorination mixtures were analyzed by GLC and GLC–MS. Variable amounts of unreacted substrates were observed as less than an equimolar amount of chlorine was used.

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The quantities of higher chlorinated products were at greatest a few per cent.

Gas-liquid chromatography. A Varian Model 2400 gas chromatograph with a flame-ionization detector and 3% Carbowax 20M glass capillary column (50 m × 0.3 mm I.D.) was used for the qualitative and quantitative analyses of the chlorination products. The column temperature was programmed from 50 to 190 °C at 4 °C/min and held at 190 °C until the elution of peaks ceased. Nitrogen was used as the carrier gas at a flow-rate of 1 ml/min. The splitting ratio was 1:20 and the temperatures of injector and detector were 220 and 240 °C, respectively. The chromatographic data were analyzed with a Hewlett-Packard Model 3390A Reporting Integrator using standard programs.

Gas-liquid chromatography – mass spectrometry. A Varian MAT-212 mass spectrometer connected with a Varian Model 3700 gas-liquid chromatograph was used. It was equipped with the same column as above with a helium flow-rate of 1 ml/min. Appropriate temperatures were used to separate the isomeric monochloro esters. Electron ionizing energy was 70 eV and ion source temperature 240 °C. Data were acquired and processed on a Spectro System MAT-188.

RESULTS AND DISCUSSION

The chlorination rate of chloromethyl esters seems to be slightly greater than that of the corresponding methyl esters. Thus, the monochlorinated isomers formed react further easily to di- and polychloro compounds. To avoid this and to control the reaction, the use of a diluent is recommended. Benzene was chosen as a solvent, because it has been found to alter the selectivity of the reagent. This leads to a different isomer distribution.[12-14]

Identification. The products were identified by GLC and GLC-MS, through comparison with authentic samples. On a Carbowax 20M glass

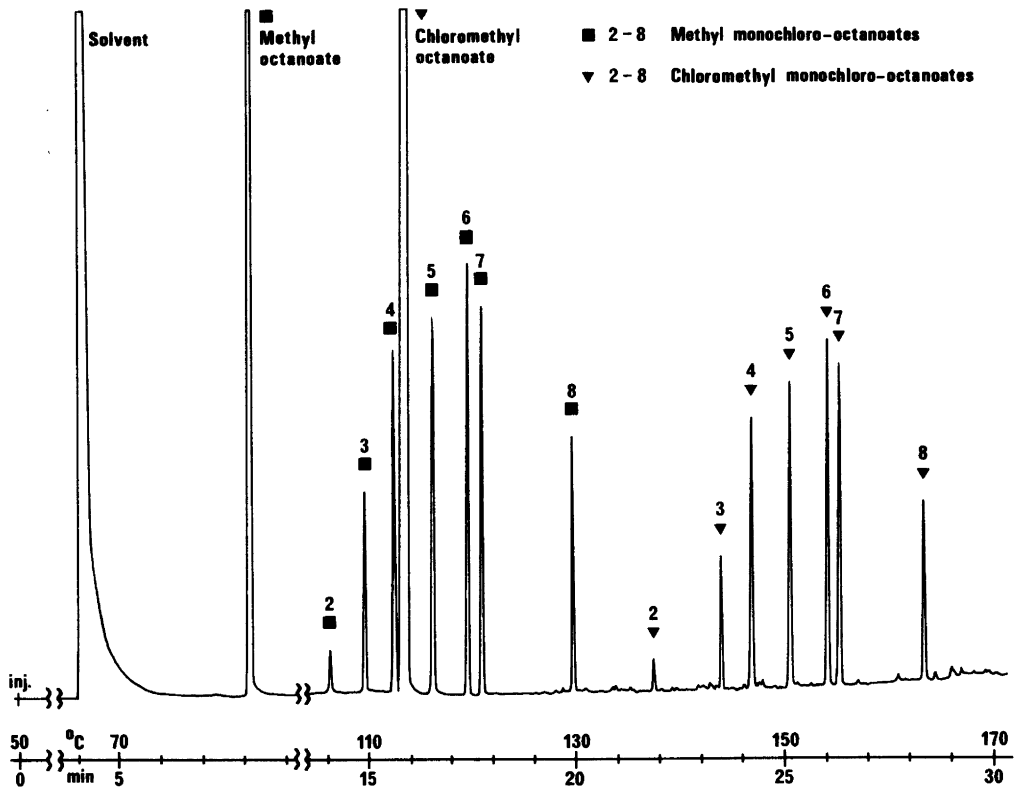

Fig. 1. Chromatogram of a mixture of methyl and chloromethyl monochloro-octanoates. S = solvent; peak number = position of Cl-substituent.

capillary column, which separated the chlorination mixtures better than a non-polar SE-30 column, all of the isomeric monochloro chloromethyl esters were resolvable except for chloromethyl 6-chloro- and 7-chlorododecanoates.[4] The amounts of isomers had to be estimated through comparison with corresponding isomers formed in the chlorination of methyl dodecanoate.[3]

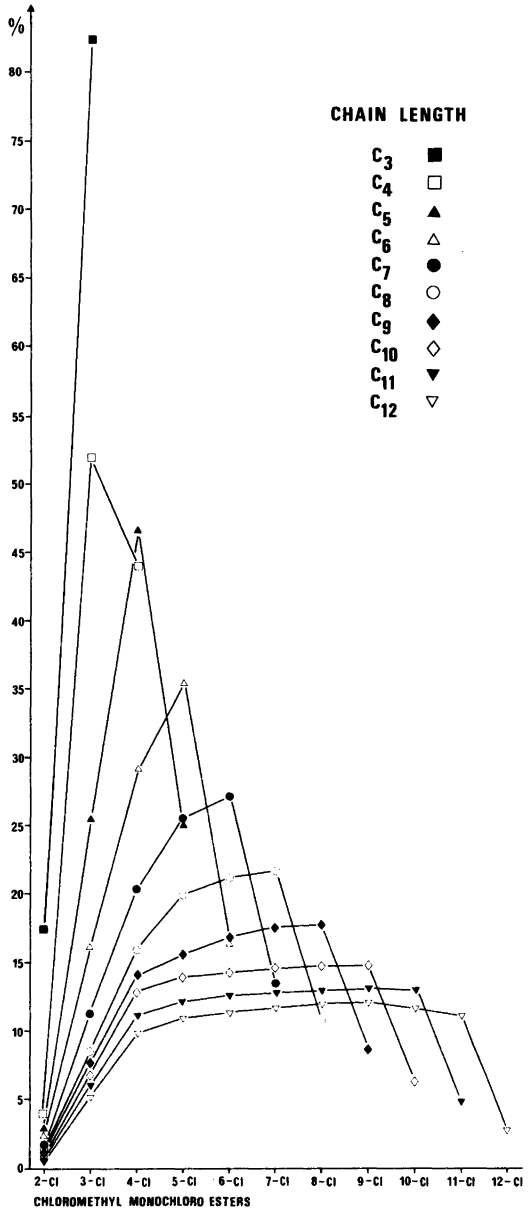

Fig. 2. Isomer distribution of chloromethyl monochloro esters of aliphatic C_3-C_{12} n-carboxylic acids based on GLC analyses. Chlorinations were carried out without solvent with chlorine at room temperature.

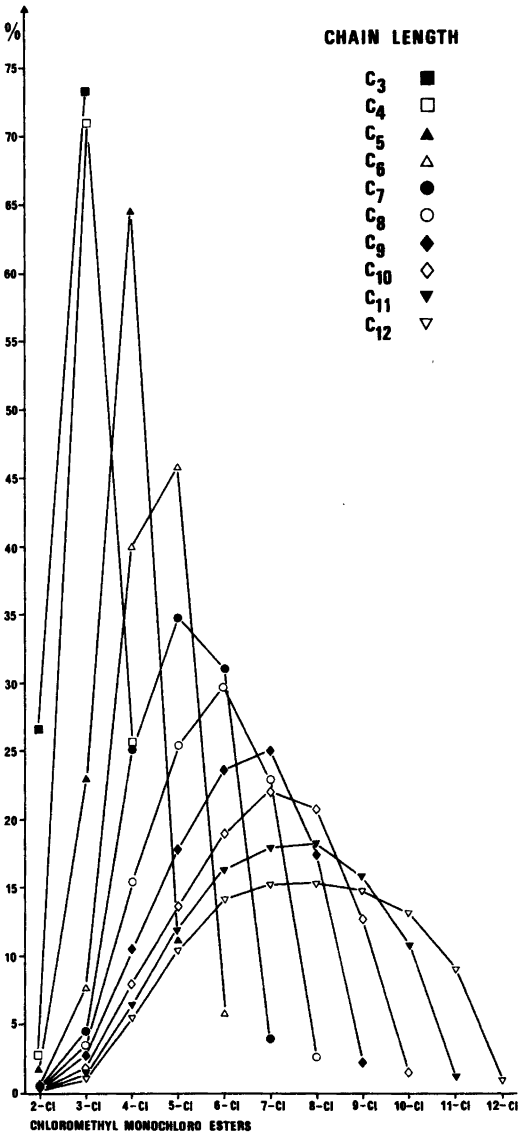

Fig. 3. Isomer distribution of chloromethyl monochloro esters of aliphatic C_3-C_{12} n-carboxylic acids based on GLC analyses. Chlorinations were carried out in benzene with chlorine at room temperature.

As expected, the isomeric monochloro chloromethyl esters are eluted in the direct order from 2-chloro to ω-chloro compound[4] like the corresponding methyl derivatives.[15] In order to confirm this, C_5-C_{12} acid chlorides, which certainly yield all monochlorinated isomers,[1-3] were chlorinated with chlorine in the liquid phase. However, the amounts of 2-chloro isomers formed in the reactions of long-chain ($>C_8$) compounds were negligible, wherefore isomers were prepared separately.[6,7] One part of the chlorination mixtures was esterified with methanol and the other part was converted to chloromethyl esters.[5] The combined mixture of the derivatives was analyzed by GLC. A chromatogram of the mixture of octanoates is illustrated in Fig. 1. The patterns of methyl and chloromethyl isomers show similar isomer distributions. Further, the GLC analyses of the mixtures of chloromethyl monochloro esters, formed by two pathways, indicate the identity of the mixtures. The final identification was made with GLC – MS.

The mass spectra of chloromethyl esters differ somewhat from the spectra of methyl esters. The molecular ion peaks of parent esters are low and as expected, $M^{\cdot+}$ is not shown by any of the monochloro isomers. α-Cleavages give intense $M-ClCH_2O^{\cdot}$ and $M-ClCH_2OCO^{\cdot}$ ions in all parent esters and in short-chain chloro esters. The 2-chloro isomers can easily be identified and distinguished from the other isomers on the basis of the McLafferty rearrangement (fragment ion $C_3H_4Cl_2O_2^{\cdot+}$ at *m/z* 142). The corresponding ion at *m/z* 108 is the base peak in C_6-C_{11} parent esters and nearly in all 4-chloro and ω-chloro isomers. On the other hand, this fragment ion is small in all 3-chloro and 5-chloro esters. The loss of a chlorine atom from the molecular ion is characteristic for 3-chloro isomers and small $M-101$, $M-Cl\cdot-CH_2ClOH$ or $M-HCl-ClCH_2O\cdot$, and $M-102$, $M-CH_2ClOH-HCl$, peaks for ω-chloro isomers. The other isomers, however, are indistinguishable by MS on the basis of their very similar mass spectra, as are also the corresponding methyl esters.[16]

Isomer distribution. The results of the quantitative analyses of monochloro isomers formed in two different chlorination processes are illustrated in Figs. 2 and 3. The quantities of monochloro esters are given relative to the ω-chloro isomers and to the isomers, formed in the chlorinations without benzene (Table 1). The isomer distributions were estimated by GLC without weight response factors, the determination of which would have required laborious syntheses of model samples. However, owing to the use of a glass capillary column with temperature programming, the differences between factors of monochloro esters were supposed to be negligible.[2]

The chlorinations of chloromethyl esters and methyl esters[1-3] gave nearly the same isomer distributions under the same reaction conditions, without solvent. The amounts of 2-chloro and ω-chloro isomers were, however, smaller in the reactions of the chloromethyl esters, particularly, with increase in chain length.

From Figs. 2 and 3 it can be seen that benzene strongly affects the selectivity of the reagent. In the chlorination without solvent the reactivities of C_2-H up to $C_{\omega-1}-H$ increase as usual with increasing distance from the deactivating chloromethoxyl group the main reaction product appearing to be in general the (ω-1)-chloro isomer. With an increase in chain length, however, the selectivity of the substitution of (ω-1)-hydrogen decreases and the amounts of the mid-chain isomers become relatively more abundant.

When the reaction was carried out in benzene, the main product varied with increasing the chain length from the (ω-1)-chloro to the (ω-4)-chloro isomer. As with the chlorinations of alkane carbochlorides,[12-14] the most striking difference between the chlorination methods given in this work is the small amounts of ω-chloro isomers formed in benzene, compared with the 1.1 – 4.3 times greater amounts without solvent (Table 1). The quantities of 2-chloro and 3-chloro isomers were smaller in benzene and the proportions of the mid-chain isomers, as a consequence, higher.

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Table 1. The relative quantities[a] of monochloro esters formed in the chlorinations of aliphatic n-C_3-C_{12} chloromethyl esters.

Chain length	Method[b]	Isomeric monochloro esters										
		2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl	9-Cl	10-Cl	11-Cl	12-Cl
C_3	A	0.2	1.0									
	B	0.4	1.0									
	B/A	1.5	0.9									
C_4	A	0.1	1.2	1.0								
	B	0.1	2.7	1.0								
	B/A	0.8	1.4	0.6								
C_5	A	0.1	1.0	1.9	1.0							
	B	0.1	2.1	5.8	1.0							
	B/A	0.6	0.9	1.4	0.4							
C_6	A	0.1	0.9	1.7	2.1	1.0						
	B	0.1	1.3	6.9	7.9	1.0						
	B/A	0.2	0.5	1.4	1.3	0.3						
C_7	A	0.1	0.8	1.5	1.9	2.0	1.0					
	B	0.1	1.1	6.3	8.7	7.8	1.0					
	B/A	0.2	0.4	1.2	1.4	1.1	0.3					
C_8	A	0.1	0.8	1.4	1.9	2.0	2.0	1.0				
	B	0.1	1.3	6.0	9.8	11.5	8.9	1.0				
	B/A	0.1	0.4	1.0	1.3	1.4	1.1	0.2				
C_9	A	0.1	0.9	1.6	1.8	1.9	2.0	2.1	1.0			
	B	0.1	1.3	4.8	8.1	10.8	11.5	8.0	1.0			
	B/A	0.1	0.3	0.8	1.1	1.4	1.4	1.0	0.2			
C_{10}	A	0.1	1.1	1.9	2.2	2.3	2.3	2.4	2.4	1.0		
	B	0.1	1.1	5.3	8.5	12.7	14.9	13.9	8.5	1.0		
	B/A	0.1	0.2	0.7	1.0	1.3	1.5	1.4	0.8	0.2		
C_{11}	A	0.1	1.2	2.2	2.5	2.6	2.6	2.7	2.7	2.7	1.0	
	B	0.1	1.1	4.9	9.2	12.5	13.8	14.1	12.2	8.0	1.0	
	B/A	0.1	0.2	0.6	1.0	1.3	1.4	1.4	1.2	0.8	0.3	
C_{12}	A	0.1	1.8	3.3	3.9	3.9	4.0	4.2	4.2	4.1	3.9	1.0
	B	0.1	1.0	5.7	10.6	14.0	15.0	15.4	14.9	13.2	9.1	1.0
	B/A	0.1	0.2	0.6	0.9	1.2	1.3	1.3	1.2	1.1	0.8	0.3

[a] Relative to the ω-chloro isomers (= 1.0) and isomers formed in the chlorination of the neat substrates (B/A).
[b] Chlorination in the absence (A) and in the presence (B) of benzene.

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Short Communications

Separation of the Different Classes of Conjugates Formed by Metabolism of Benzo[*a*]pyrene in the Northern Pike *(Esox lucius)* *

LENNART BALK,** ANDERS KNALL and JOSEPH W. DePIERRE

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

There is now a growing awareness concerning the fate of polycyclic hydrocarbons and the many other xenobiotics with which we pollute our environment. Many of these substances find their way sooner or later into our aquatic environment. For instance, polycyclic hydrocarbons, including benzo[*a*]pyrene, reach our rivers, lakes and seas in the form of spillages of crude and refined petroleum products, in industrial and domestic effluents, in run-off water from the land, and by dry or wet precipitation from the atmosphere. (The recent article by Neff[1] provides a good review of polycyclic hydrocarbons in the aquatic environment.) It seems likely that the growing frequency of tumors in fish that live in polluted waters is closely related to increasing aquatic pollution.[2]

In our laboratory we have recently begun intensive investigations on the fate of xenobiotics in the Northern pike (*Esox lucius*). First, we have characterized different drug-metabolizing systems using subcellular fractions from the liver of this fish.[3] Subsequently, we have complemented these studies with an investigation on the bioaccumulation of benzo[*a*]pyrene in Northern pike from the surrounding water.[4] The factor by which benzo[*a*]pyrene is concentrated from the water varies widely for different organs, but the highest concentrations are recovered in the liver–bile–intestine and the kidney–urine systems.

The present experiments represent therefore a continuation of our earlier studies. Here we have attempted to use a published procedure – namely, chromatography on a column of alumina oxide[5] – to separate the different classes of conjugates (sulfates, glucuronides, glutathione conjugates) formed during the metabolism of benzo[*a*]pyrene in the Northern pike. Such separation is an important first step in characterizing all the different products resulting from *in vivo* metabolism of benzo[*a*]pyrene and other xenobiotics.

The fish used in this study were Northern pike purchased from a local hatchery in the larval stage. The fish were maintained in 100-l glass aquaria with continuously circulating top water (*circa* 1 l/min) and fed commercial trout pellets. The fish were starved during the period of exposure, so that possible adsorption to food and feces should not disturb the distribution of benzo[*a*]pyrene between the water and the fish. However, a starvation period of 4.5 days is not uncommon for the pike in its natural environment. The fish were used when they were between 20–30 g in weight and approximately 10 cm long. Both sexes were used without discrimination. The fish were subjected to a continuous 12 h light–12 h dark cycle. At no time did any fish show symptoms of illness or bad health.

The solution of [G-^{3}H]-benzo[*a*]pyrene (purchased from the Radiochemical Centre, Amersham, England) was evaporated to dryness and the benzo[*a*]pyrene subsequently dissolved in hexane and purified with 8 extractions using alcoholic NaOH.[6] Benzo[*a*]pyrene purified according to this method is about 99.85 % pure. The benzo[*a*]pyrene was then dissolved in 25 μl acetone and added to 10-l aquaria with continuous stirring to obtain a homogeneous solution-suspension containing 87 ±10 ng/l (which corresponds to mildly polluted water[1]).

If the entire amount of benzo[*a*]pyrene added to one aquarium was taken up by one fish, this would give a dose of approximately 50 μg/kg body weight. Judging from experiments with mammals[7] this dose is far too low to induce benzo[*a*]pyrene monooxygenase activity. However, a dose-response study for induction with 3-methylcholanthrene has not yet been performed in fish.

One fish was placed in each aquarium and exposed for 4.5 days. After immobilization with the

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** To whom correspondence should be addressed.

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anesthetic MS-222 (tricaine methanesulfonate) the livers and gall bladders (containing bile) were removed from 3 fish and pooled. These organs were homogenized separately in 70% ethanol using a glass-glass homogenizer and approximately 15 up-and-down hand-driven strokes. The sample was then centrifuged at 2250 g for 15 min in a desk centrifuge and the clear supernatant was collected using a Pasteur pipette. The pellet was resuspended in 70% ethanol, homogenized, and centrifuged a total of three additional times and the four different supernatants were pooled.

The separation of benzo[*a*]pyrene itself, metabolites and water-soluble conjugates was accomplished by chromatography on a column of alumina oxide (150 × 15 mm; Aluminium oxid 90 aktiv neutral (*aktivitätsstufe* I) grain size 0.063–0.200 mm (70–230 mesh ASTM) essentially according to the published procedure for separation of the water-soluble metabolites of benzo[*a*]pyrene produced by cultured human colon.[5] The column was eluted with 150 ml absolute ethanol, 150 ml water, 150 ml 0.05 M ammonium phosphate, pH 3.0 and 150 ml 25% formic acid, in that order, at a flow rate of 2 ml/min and 5 ml fractions were collected. 0.2 ml aliquots of each fraction were submitted to scintillation counting and the external standard procedure was used to correct for quenching. Approximately 85% of the total radioactivity applied to the column was recovered and we are presently trying to determine what happens with the other 15%.

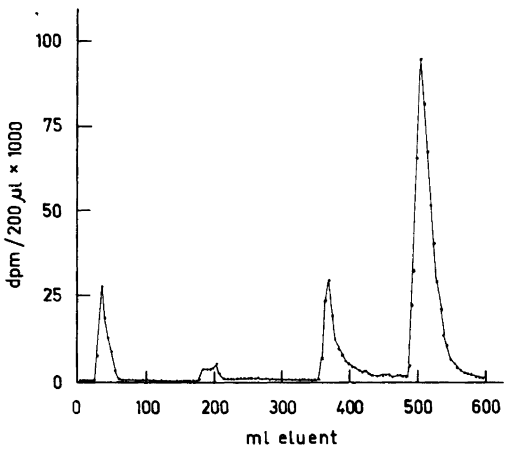

Fig. 1. Separation of benzo[*a*]pyrene and its metabolites recovered from the liver on an alumina oxide column. The experimental procedure is discussed in the text. The presumptive identity of the peaks is, from left to right, (*e.g.*, in order of elution from the column), benzo[*a*]pyrene + unconjugated metabolites, sulfate conjugates, glucuronides and glutathione conjugates.

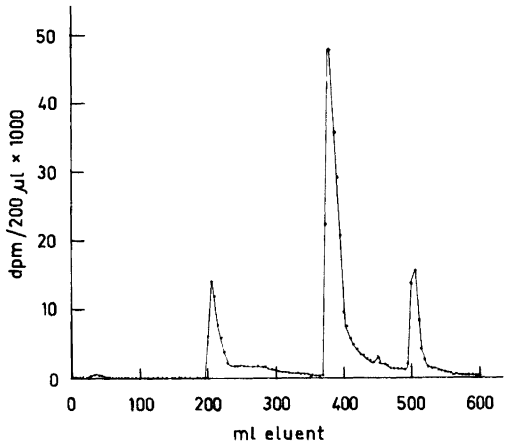

Fig. 2. Separation of benzo[*a*]pyrene and its metabolites recovered in the bile on an alumina oxide column. See legend to Fig. 1.

Figs. 1 and 2 illustrate the elution profiles obtained from liver and bile, respectively. According to the earlier study,[5] the peaks contaim, in order of elution from the column, benzo[*a*]pyrene and unconjugated metabolites; sulfate conjugates; glucuronides; and glutathione conjugates. It is not yet clear where mercapturic acids and amino acid conjugates would be eluted from such a column, a question which we are presently investigating. We are also in the process of reconfirming the identity of the sulfate, glucuronide, and glutathione conjugate peaks.

With these reservations in mind, certain observations can be made about the formation of benzo[*a*]pyrene conjugates in the liver and secretion of such conjugates into the bile of the Northern pike. It is immediately apparent that the conjugate pattern from these two body compartments is quite different. About 75% of the conjugates present in the liver are glutathione conjugates, most of the remaining are glucuronides, and only very small amounts of sulfate conjugates are recovered from this organ.

On the other hand about 60% of the conjugates recovered from bile are glucuronides, while the remaining 40% is approximately equally divided between sulfate and glutathione conjugates. In addition the bile contains virtually no benzo[*a*]pyrene and conjugated metabolites, while the liver apparently contains significant amounts of both the parent hydrocarbon and of unconjugated metabolites. These findings suggest that glucuronides and sulfates of benzo[*a*]pyrene are

selectively secreted into the bile and, consequently, that glutathione conjugates must be selectively secreted into the blood and excreted, either in unchanged form or after conversion to mercapturic acids, in the urine. For the reasons discussed above, these conclusions are at present preliminary and our investigations continue.

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Reaction of Chemical Probes with Phosphatidylethanolamine of Liver Microsomes *

CONNY VALTERSSON

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The two main lipids of the microsomal membranes are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The latter makes up about 30% of the total. Distribution of phospholipids within the membrane has a great significance since many important properties such as permeability, stability and enzyme activity are often dependent on the presence of specific lipids at defined locations.[1] In previous investigations it was found that the microsomal PE is to a large extent localized on the outer, cytoplasmic surface of the microsomes.[2] These experiments, however, were performed using phospholipase A_2 (PLase A_2) which may perturbate membranes. Therefore, it is possible that the phospholipases not only hydrolyze lipids available on the outer surface of the vesicles but also induce and increase mobility of lipids, thereby secondarily causing molecular rearrangements. We have tested a number of amino-reacting reagents on intact microsomal vesicles in order to avoid this possible problem and to obtain more information on the structure and composition of these membranes.

The reagents used were 1,5-difluoro-2,4-dinitrobenzene (DFDNB), 1-fluoro-2,4-dinitrobenzene (FDNB), fluorescamine (FA), methyl acetimidate (MA), dimethyl 3,3-dithiobispropionimidate (DTBP) and isoethionylacetimidate (IA). The concentrations of the individual probes were analyzed by constructing a concentration curve reaching the plateau values. After the reaction with isolated microsomes, the lipids were extracted with chloroform – methanol (2:1). Following partition, separation of the reacted PE from the non-reacted part was accomplished by thin layer chromatography on silica gel plates using in the first direction chloroform – methanol – water (65:24:4 v/v), and chloroform – acetone – methanol – acetic acid – water (50:20:10:10:5 v/v) in the second direction. After chromatography, the plates were stained with iodine and scraped; then the amount of phosphate was determined.

Depending on the properties of the probes, different reaction patterns with PE were obtained (Table 1). DFDNB is a bifunctional probe which penetrates membranes easily and has previously been used in a number of studies. At a plateau concentration all PE of the microsomes is reacted, which is consistent with earlier studies.[3] The monofunctional variant of this reagent, FDNB, also freely penetrates membranes and, again, reacts with all PE upon incubation. Three other non-charged reagents were also tested for interaction with microsomes.[4] FA is insoluble in water, as opposed to MA and DTBP; FA and MA are monofunctional but DTBP is a bifunctional reagent. It appears that these reagents penetrate most membranes, and, consequently, they should react with all species of microsomal PE. However, at plateau concentrations, of all three reagents, only 70% of the PE is reacted, indicating that a part of the lipid is in a compartment which is not available for the probes. To further investigate this question, two concentrations of Triton X-100 were applied: 0.05% which increases membrane permeability for macromolecules and 0.25% which solubilizes some components. Complete reaction was obtained with MA in the presence of 0.05% Triton, while the higher Triton concentration was necessary for the reaction of FA with all PE. On the other hand, interaction with DTBP could not be increased even in the presence of high Triton concentration. It appears that a part of the PE is compartmentalized in the membrane and not easily available; the reaction of the probe with this part of the lipid depends on the properties of the probe itself. The charged monofunctional probe, IA, as opposed to

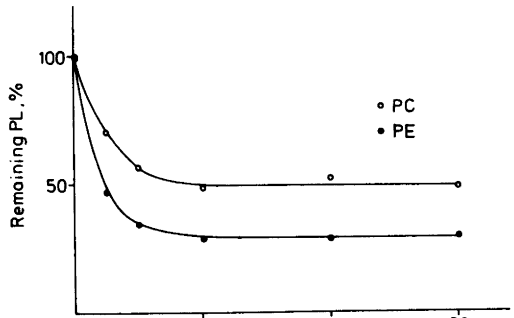

Fig. 1. PLase A_2 treatment of microsomes. Liver microsomes were incubated with purified PLase A_2 (0,5 IU/mg) in the presence of 1 mM $CaCl_2$ and 40 mg bovine serum albumin/ml. Incubations were conducted at 0 °C. The values give the phospholipid remaining in the membranes after centrifugation.

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Table 1. Reaction of various amino-reacting probes with microsomal PE. Isolated washed total liver microsomes were incubated in a medium containing 0.1 M phosphate buffer, pH 8.0, 0.25 M sucrose, microsomes and the probe given below. When indicated, Triton X-100 was included in the incubation medium. The incubation was performed at 20 °C for 2 h for DFDNB and FDNB, 1 h for MA and IA and 2 min at 0 °C for FA. The trichloroacid (5%) precipitated microsomes were extracted with chloroform –methanol 2:1 and the reacted and unreacted PE were separated by thin layer chromatography. Abbreviations see text. The data are given as mean values ±S.E.M. ($n=5$).

Probe	Concentration μmol/mg protein	Total PE reacted, % None	0.05 % Triton	0.25 % Triton
DFDNB	0.05	33±3		
	0.2	98±5		
	0.3	99±2		
FDNB	0.25	47±4		
	0.75	97±6		
	1.5	98±3		
FA	0.5	48±6		
	1.0	68±8		
	2.0	70±3	75±4	96±7
MA	25	42±2		
	75	67±8		
	125	68±6	92±11	
DTBP	25	33±3		
	50	67±5		
	100	70±4		75±7
IA	10	6±1		
	25	13±2		
	50	14±1	16±1	51±4

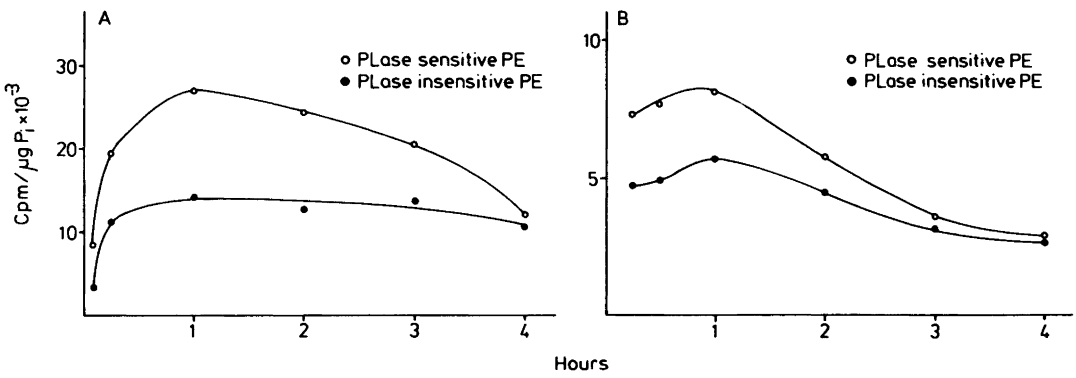

Fig. 2. PLase A_2 treatment of *in vivo* labeled microsomes. a, Rats were injected with 7.4 MBq [^{3}H]ethanolamine into the portal vein at various time-points before decapitation. The isolated liver microsomes were subjected to PLase A_2 treatment (0,5 IU/mg, 0 °C, 15 min) and the specific radioactivity was determined in the PE isolated from the pellet after ultracentrifugation. b, Rats were injected into the portal vein with 7.4 MBq [^{3}H]glycerol and the incubations and measurements were performed as in a.

the other probes, does react badly with microsomal PE, and only a partial interaction takes place even in the presence of high detergent concentrations.

When intact microsomes were treated with PLase A_2, the PE could again be divided into two pools (Fig. 1). About 70% of the PE could be removed from the microsomal vesicles. Half of the largest component, PC, was untouched during the hydrolysis.

The metabolic activity of the phospholipid in the PLare A_2 sensitive and insensitive compartments is obviously different (Fig. 2). When liver microsomes were prepared from rats *in vivo* labeled with [^{3}H]ethanolamine, the specific activity of the PE in the pool sensitive to PLase A_2 was about double that of the lipid in the other pool. The value in the PLase A_2-sensitive pool was calculated from the difference between the PE from non-treated microsomes and the PE remaining after enzyme hydrolysis. Equilibration occurred only after 4 h. The situation is quite similar when the *in vivo* labeling is performed with [^{3}H]glycerol.

The experiments with the mono- and bifunctional probes are in agreement with the PLase A_2 experiments. Separate from the problem of outside −inside localization, there is obviously a compartmentalization of the PE in the membranes. One is available for probes and hydrolytic enzymes; this part also possesses a high turnover in comparison with the remaining PE. The second pool is probably present for functional reasons and does not only reflect the bilayer distribution. It may be associated with proteins buried in the inner compartments, or the lipid may take part in the formation of inverted micelles.

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Synthesis of Chloromethoxybenzenes by Catalytic Decarbonylation of Phenoxyacetyl Chlorides with Chlorotris(triphenylphosphine)rhodium(I)

TORE BENNECHE and KJELL UNDHEIM

Department of Chemistry, University of Oslo, Oslo 3, Norway

Thiophenols are selectively chloromethylated on the sulfur atom whereas phenols react with the chloromethylating reagent preferentially in the nucleus. *O*-Chloromethylation of phenols can be achieved, however, by a somewhat inconvenient and laborious method which involves preparation of sodium phenoxymethane sulfonates under drastic conditions with subsequent chlorination and desulfonylation.[1] Anisole and anisoles carrying electron attracting groups, *e.g.* 4-chloroanisole, can be selectively chlorinated in the methoxy function under free radial conditions.[2,3] Decarbonylation of phenoxyacetyl chlorides under Friedel-Crafts conditions appears to be limited to phenoxyacetyl chlorides having at least one electron attracting substituent in the 2- or 4-position.[4] Thermal decarbonylation has been reported for phenoxyacetyl chlorides with two or more halogen atoms in the nucleus.[5]

We needed ready access to chloromethyl aryl ethers and as a method for their preparation we decided to study decarbonylation reaction of acid chlorides, in particular since certain transition metal compounds are known to promote decarbonylation of acyl halides, *e.g.* aromatic acyl halides.[6-8] Thus, when phenoxyacetyl chlorides were heated with chlorotris(triphenylphosphine)rhodium(I) as catalyst they were readily decarbonylated to the corresponding chloromethyl ethers. Whereas aromatic acyl halides in general have to be heated above 200 °C for the decarbonylative halogenation, the aryloxyacetyl chlorides were readily decarbonylated in the range 150–180 °C at atmospheric pressure; decrease in pressure also decreased the temperature required and also the rate of decarbonylation (Table 1).

The reaction is simple to carry out, the product being distilled directly from the reaction mixture.

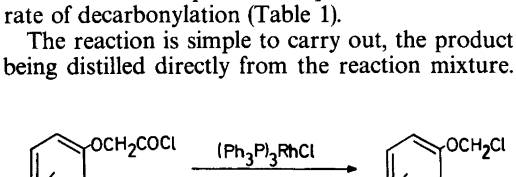

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Table 1. Decarbonylation of phenoxyacetyl chlorides.

	Substituent R	Temp. (°C)	Time (min)	Yield[a] (%)
2*a*	H	170	30	78
2*a*	H	130[b]	360	75
2*b*	2-Me	160	30	75
2*c*	2-MeO	170	30	79
2*d*	4-Cl	170	30	83
2*e*	2,4-Cl_2[c]	180	5	75
2*f*	4-MeCO[d]	150	20	71

Reactants *1* 28 mmol and catalyst 0.36 mol.%. [a] Isolated. [b] At 30 mm Hg. [c] Thermal decarbonylation requires reflux temperature (*i.e. ca.* 280 °C) for 0.5–3 h. [d] 3.8 mmol.

Moreover, the method can be used for aryl ethers containing both electron-donating and -attracting substituents (Table 1). Since the boiling point of the product lies well below that of the acid chloride reactant, the latter will not co-distill with the product.

2-Nitrophenoxyacetyl chloride could not be decarbonylated by this method because of extensive decomposition of the acetyl chloride at elevated temperature.

When the compounds of Table 1 were heated without the catalyst in the same temperature range, no significant decarbonylation was observed.

Experimental. Phenoxyacetyl chlorides 1 were prepared as described in the literature: *1a* b.p. 110–112 °C/10 mmHg;[9] *1b* b.p. 120–121 °C/10 mmHg;[10] *1c* b.p. 76–79 °C/0.02 mmHg;[11] *1d* b.p. 78 °C/0.05 mmHg;[12] *1e* b.p. 102–104 °C/0.5 mmHg;[12] *1f* b.p. 126–128 °C/0.01 mmHg.[13] ^{1}H NMR ($CDCl_3$): δ for CH_2 were in the above order 4.88, 4.90, 4.89, 4.82, 4.90, 4.97.

General procedure for decarbonylation of phenoxyacetyl chlorides. Chlorotris(triphenylphosphine)-rhodium(I) (100 mg) was added to the freshly distilled phenoxyacetyl chloride (28 mmol) and the mixture heated until evolution of carbon monoxide had ceased; the conditions are given in Table 1. The chloromethyl phenyl ethers were distilled off from the reaction mixture at reduced pressure: *2a* b.p. 71–72 °C/10 mmHg;[14] *2b* 96–98 °C/10 mmHg; *2c* 60–62 °C/0.02 mmHg;[1] *2d* 39–41 °C/0.01 mmHg;[14] *2e* 57–62 °C/0.01 mmHg;[1] *2f* 89–91 °C/0.01 mmHg. ^{1}H NMR ($CDCl_3$): δ for CH_2 were in the above order 5.82, 5.77, 5.86, 5.80, 5.80, 5.85.

Decarbonylation of phenoxyacetyl chlorides at reduced pressure. Chlorotris(triphenylphosphine)-rhodium(I) (100 mg) was added to freshly distilled phenoxyacetyl chloride (28 mmol) and the mixture

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heated at 130 °C and at 30 mmHg. The decarbonylated product was distilled off as formed; the reaction required *ca.* 6 h to go to completion.

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Dolichol Distribution and Biosynthesis in Hepatocytes *

T. EKSTRÖM [a] and T. CHOJNACKI [b]

[a] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden and [b] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Dolichol is present in hepatocytes in various amounts depending on the species.[1] In rat liver there is about 40 μg dolichol per gram wet weight of tissue but only a small part of the polyprenol is phosphorylated, that is, in an active form. This α-saturated polyprenol is an obligatory intermediate in several steps of glycoprotein synthesis and consequently the amount of lipid has considerable importance in regulating cell function.[2] Dolichol does not represent a designation for a single type of compound, rather a family of compounds with different numbers of isoprene residues. From liver to liver the number of isoprene residues ranges from 16 to 23. In order to obtain information about the mechanism of biosynthesis and the possible function of the different dolichols, experiments were performed by using isolated hepatocytes possessing the same, or nearly the same, properties as those in the intact liver.

Labeling of hepatocyte dolichol is a difficult problem mainly because of the lack of available precursors and of the metabolic pathway related to cholesterol. Since all the double bonds, with the exception of the two at the Ω-end, are in *cis* configuration, it would be ideal to have mevalonate of the *S*-type which is, however, not available commercially. The first part of dolichol biosynthesis follows the route common to cholesterol, which is disadvantageous from the point of view of labeling, since the rate of cholesterol biosynthesis is at least a hundred times faster. Hepatocytes were isolated by perfusion first with an EGTA-containing medium followed by collagenase which yielded cells with over 90% viability. The cells were suspended in Krebs-Hensleit buffer and incubated for 5 h with [^{3}H]-(*R,S*)-mevalonate having high specific activity (Table 1). In order to calculate specific radioactivity, we have measured the amount of dolichol by using high pressure liquid chromatography (HPLC).[3] The dolichol content was about 40 μg in 1×10^8 cells and 70% consists of polyprenols with 18 and 19 isoprene residues. Smaller amounts were also present with 17, 20 and 21 residues. The procedure employed made it possible to label all of the dolichols that were isolated. The highest specific activity was obtained in the shortest dolichol with 16 residues, and the specific radioactivity decreased gradually reaching the lowest level in the longest dolichol. This finding would agree with a gradual addition of isopentenyl residues to the growing polyprenol chain occurring probably in the outer mitochondrial membrane.

In spite of the fact that during biosynthesis the dolichol product is in the pyrophosphate form, the large majority of the newly synthesized lipids are in dephosphorylated form and are stored either as free alcohols or esterified with a fatty acid. Consequently the amount of available active dolichol monophosphate is regulated by a CTP specific kinase.[4] By using [^{32}P]CTP and an excess of exogenous dolichol(C55), we have studied the subcellular localization of the dolichol kinase by isolating subfractions from rat liver (Table 2). As opposed to Golgi membranes, outer and inner

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Table 1. Labeling of dolichol in hepatocytes with [^{3}H]mevalonate. Hepatocytes at a number of 350×10^6 were incubated with 9.25 MBq [^{3}H](*R,S*)-mevalonate for 5 h in 25 ml of Krebs-Hensleit buffer at 37 °C. After incubation the suspension was subjected to alkaline hydrolysis and dolichol was extracted with diethyl ether. The extract was placed on Al_2O_3 column and eluted with increasing concentration of diethyl ether in hexane. Dolichol measurements were made on HPLC.

Dolichol	Amount after purification nmol 1×10^8 cells	% of total	Radioactivity cpm/1×10^8 cells	cpm/nmol dolichol
C80	0.25	2.0	509	2.027
C85	1.52	12.2	1.952	1.282
C90	4.73	38.2	2.444	517
C95	4.35	34.9	1.123	258
C100	1.58	12.7	352	222

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Table 2. Phosphorylation of dolichol with [^{32}P]CTP. Subcellular fractions were incubated in a 500 μl mixture containing 27 mM Tris-HCl, pH 7.0, 0.5 mM EDTA, 20 mM UTP, 5 mM β-mercaptoethanol, 30 mM $CaCl_2$, 185 kBq [γ-^{32}P]CTP, 40 μM unlabeled CTP, 50 μl dolichol (1 mg/ml) in 0.5% Triton X-100 and 1 – 7 mg protein. Incubation was at 30 °C for 5 min. Dolichol phosphate was isolated by SiO_2-column and thin layer chromatography.

Fraction	Dolichol monophosphate pmol/(mg protein)
Microsomes	11.0
Microsomes, trypsin-treated	0.4
Golgi membranes	1.2
Outer mitochondrial membranes	0.2
Inner mitochondrial membranes	0.3

mitochondrial membranes, the microsomes possessed a high capacity for dolichol phosphorylation. When microsomes were treated with trypsin before measurement of the kinase activity, dolichol phosphorylation was eliminated completely. This indicates that this enzyme is associated with the cytoplasmic surface of the microsomes.

The functional importance of different chain lengths in dolichols is not known and therefore attempts were made to enrich microsomal membranes in a well-defined polyprenol. Egg lecithin and dolichol were subjected to intensive sonication which resulted in liposome formation. After a 90 min incubation about 5% of the dolichol entered into the hepatocyte and was present in all fractions (Table 3). The highest concentration appeared in microsomes and interestingly some of the lipids were distributed in the soluble cytoplasm. A portion of the dolichol, about 10%, was phosphorylated, indicating the functional importance of CTP-mediated microsomal dolichol kinase.

When the isolated microsomes, after preincubation with dolichol(C95), were incubated *in vitro* with nucleotide activated sugars, the newly incorporated lipid intermediate proved to be effective in accepting the various sugars. Glycosylation was most effective with mannose followed by glucose, with the lowest incorporation obtained with acetylglucosamine. In a moderate degree, glycosylation of endogenous proteins was also increased.

These experiments demonstrate that the hepatocyte system is suitable for investigation of dolichol biosynthesis by using labeled mevalonate as a precursor. One of the key enzymes in regulation of the amount of dolichol monophosphate is the CTP specific kinase, which is located exclusively on the outer surface of microsomal membranes. An effective way to study the specific role of dolichol in microsomes is to incubate the hepatocytes with the dolichol containing liposomes. This results in the uptake of dolichol, transport in the cytoplasm, and phosphorylation to an active intermediate.

Table 3. Incubation of hepatocytes with liposomal [^{3}H]dolichol (C95). Hepatocytes at a number of 300 $\times 10^6$ were incubated for 90 min in 25 ml of Krebs-Hensleit buffer at 37 °C with egg lecithin liposomes containing 0.7 μmol [^{3}H]dolichol (C95). After incubation fractionation was performed and lipids from the fractions were extracted with chloroform – methanol (2:1). Dolichol and dolichol monophosphate were isolated by chromatography on DEAE-Sephadex column.

Fraction	Dolichol pmol per mg protein	Dolichol monophosphate
300 *g* pellet	51.9	5.1
Mitochondria	46.0	5.9
10 000 *g* pellet	139.4	10.5
Microsomes	197.4	27.7
Supernatant	51.8	2.6

Acknowledgements. This work was supported by the Swedish Medical Research Council and the Magnus Bergvall Foundation.

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Bromine Oxidation of Methyl 2-Acetamido-2-deoxy-α-D-glucopyranoside

ROLF ANDERSSON,[a] IBRAHIM GOUDA,[a] OLLE LARM,[a] and BENGT LINDBERG[b]

[a] Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden and [b] Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

On oxidation of glycopyranosides with bromine water at pH 7,[1] the secondary alcohol groups are preferentially oxidized. When the (C)−H of the secondary alcohol group and a bulky substituent (the aglycon or a hydroxyl group) are in *syn*-diaxial relationship, the reaction is considerably hindered. For certain glycosides, the reaction therefore shows high regioselectivity; thus, methyl α-D-galactopyranoside reacts mainly in the 4-position. The reaction offers a convenient synthesis of unprotected glycosiduloses, a class of substances not easily available by other methods. A limitation is that the uloses are further oxidized and the yields, even under optimal conditions, are only moderate.

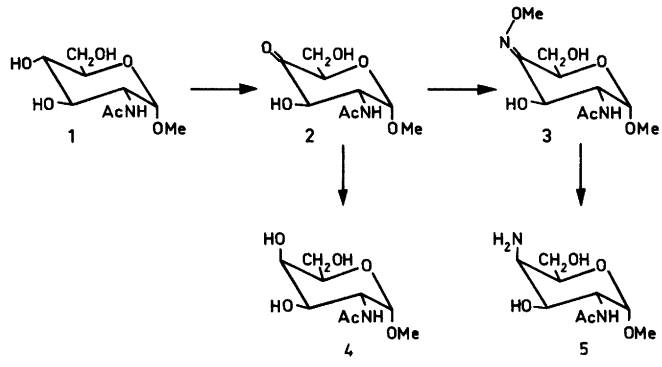
Acetamido groups should become *N*-brominated during the reaction but this reaction is reversible and the acetamido groups should be regenerated during the work-up. The oxidation of acetamido-deoxyglycosides should therefore be more regiospecific than for ordinary glycosides. We now report the oxidation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside and the transformation of the resulting 4-ulose into the corresponding 2-acetamido-2-deoxy-D-*galacto* and 2-acetamido-4-amino-2,4-dideoxy-D-*galacto* derivatives.

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (*1*) was treated with bromine (3.7 mol) in water at pH 7 and room temperature. The resulting methyl 2-acetamido-2-deoxy-α-D-*xylo*-hexopyranosid-4-ulose (*2*) was not isolated but converted into the more stable *O*-methyloxime (*3*). The chromatographically homogeneous oxime was isolated by chromatography on silica gel. The compound resisted all attempts at crystallization. NMR spectra and GLC of its TMS derivative indicated that only one of the two possible isomers had been formed. The same observation was made by Schnarr and Szarek on preparing *O*-methyloximes of methyl pentosid-4-uloses.[2] These authors deduced the configurations of their *O*-methyloximes from the differences in chemical shifts in the ^{13}C NMR spectra of the C-3 and C-5 signals when going from the ulose to the *O*-methyloxime. The free 4-ulose (*2*) was prepared by treating *3* with an acidic cation exchange resin. The same upfield shift (1.6 ppm) was, however, observed for the C-3 and C-5 signals on going from *2* to *3* and no conclusion regarding the configuration of *3* could be drawn from this experiment. In the ^1H NMR spectrum of *3*, the shift of H-5 (δ 5.03), was 0.8 ppm downfield of that of H-3 (δ ~4.26), indicating that the *O*-methyloxime *3* has the *Z* configuration.[3,4] The same shift difference was observed in dimethylsulfoxide-d_6.

The 4-ulose (*2*) was recovered by treating the oxime (*3*) with acidic cation exchange resin and then hydrogenated over palladium on charcoal to give methyl 2-acetamido-2-deoxy-α-D-*galacto*-pyranoside (*4*) in 59 % yield. Only traces of the corresponding D-*gluco* derivative were found, when the trimethylsilylated product was examined by GLC.

Catalytic hydrogenation of the oxime (*3*) yielded methyl 2-acetamido-4-amino-2,4-dideoxy-α-D-galactopyranoside (*5*, 75 %), identified by its ^1H and ^{13}C NMR spectra. No signals for the D-*gluco*-isomer were observed in these spectra. The compound resisted all attempts at crystallization.

The ^1H and ^{13}C NMR spectra of *2*, *3* and *5* (see



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the experimental part) were in agreement with the postulated structures. Signals in the ^1H NMR spectrum were assigned by homonuclear decoupling experiments and those in the ^{13}C NMR spectra by comparison with related substances and by using the deuterium-induced differential isotope shift technique.[5]

The transformation of the D-glucosamine derivative (*1*) into the considerably more expensive D-galactosamine derivative (*4*), by the route described above, would be of practical value if the yields could be improved.

The route to aminodeoxyglycosides *via* glycosiduloses, as exemplified above, is short and should, despite the moderate yields, be a useful alternative to other methods. One such synthesis has been previously described.[6]

Experimental. 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 or dimethylsulfoxide-d_6 at 30 °C, using a JEOL FX 90Q instrument. Differential ^{13}C spectra were measured using a coaxial, dual cell from Wilmad Glass Co. As references, external TMS (^{13}C) and internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^1H) were used. 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-101, were used.

Methyl 2-acetamido-2-deoxy-α-D-xylo-hexopyranosid-4-ulose O-*methyloxime (3).* A solution of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (*1*, 1 g) in 0.13 M bromine water was kept at room temperature and the pH maintained at 7.0 by titration with 1 M sodium hydroxide. After 4 h, when all bromine was consumed, the pH was adjusted to 5.0, the solution concentrated to 50 ml and methoxylamine hydrochloride (2.5 g) added. The mixture was kept at 50 °C and the pH maintained at 4.0 by titration with M sodium hydroxide. After 2.5 h, the pH was raised to 7.0 and the solution concentrated to dryness. Extraction of the residue with chloroform (4 × 25 ml), concentration and chromatography on a silica gel column (50 × 1.5 cm), irrigated with chloroform–ethanol, 9:1, and monitored by TLC, yielded 3 (0.34 g), $[\alpha]_{578}^{25}$ +103° (*c* 0.6, chloroform). ^1H NMR (D_2O) δ, Hz: 5.08, $J_{1,2}$ 2.3, (H-1); 4.28–4.24 (H-2, H-3); 5.03, $J_{5,6}$ 3.4, $J_{5,6'}$ 7.0 (H-5); 3.78, $J_{6,6'}$ 12.0, (H-6); 4.01 (H-6'); 2.04 ($CH_3C=O$); 3.47 (CH_3-O-C); 3.91 (CH_3-O-N). ^{13}C NMR (differential isotope shifts in parentheses), δ: 96.6 (0.00, C-1); 55.1 (0.14, C-2); 69.7 (0.11, C-3); 154.8 (0.06, C-4); 71.3 (0.06, C-5); 61.5 (0.12, C-6); 175.4 (0.09, $CH_3C=O$); 23.0 ($CH_3C=O$); 57.6 (0.06, CH_3-O-C); 63.3 (0.05, CH_3-O-N).

Methyl 2-acetamido-2-deoxy-α-D-xylo-hexopyranosid-4-ulose (2). A solution of *3* (224 mg) in water (25 ml) was stirred with Dowex 50 (H^+, 1 g) at room temperature for 3 h and filtered. Evaporation with toluene and drying overnight in a desiccator (phosphorus II oxide) yielded 2 (180 mg). ^1H NMR (dimethylsulfoxide-d_6); δ, Hz: 4.77, $J_{1,2}$ 2.8 (H-1); 3.96–4.30 (H-2, H-3, H-5); 3.54–3.82 (H-6, H-6'), 8.17, $J_{2,NH}$ 7.7 (NH); 3.37 (CH_3O); 1.85 ($CH_3C=O$). ^{13}C NMR (differential isotope shifts in parentheses); δ: 97.8 (0.00, C-1); 56.3 (0.14, C-2); 72.3 (0.11, C-3); 204.0 (0.03, C-4); 73.9 (0.00, C-5); 59.0 (0.11, C-6); 55.2 (CH_3-O-C); 22.4 ($CH_3C=O$), and 169.2 ($CH_3C=O$).

Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (4). The 4-ulose, 2 (200 mg), in methanol (25 ml) containing triethylamine (3 ml), was hydrogenated at atmospheric pressure and room temperature overnight, using 10 % palladium on charcoal (200 mg) as catalyst. The mixture was filtered and the solution concentrated. The residue was crystallized from ethanol–light petroleum (1:1), yielding the title compound (105 mg), m.p. 212–213 °C (decomp.), $[\alpha]_{578}^{25}$ +172° (*c* 1.06, methanol). It was indistinguishable from an authentic sample (NMR).

Methyl 2-acetamido-4-amino-2,4-dideoxy-α-D-galactopyranoside (5). The oxime 3 (70 mg) was hydrogenated as above and the product fractionated on a Dowex 50 (H^+) column (15 × 1 cm) which was irrigated first with water (300 ml) and then with 2 % aqueous ammonia (300 ml). The fractionation was monitored by TLC and the title compound was obtained as a syrup (46 mg), $[\alpha]_{578}^{25}$ +170° (*c* 1.8, methanol). ^1H NMR, δ, Hz: 4.81, $J_{1,2}$ 2.3 (H-1); 4.14, $J_{2,3}$ 11.1 (H-2); 3.95, $J_{3,4}$ 3.6 (H-3); 3.29, $J_{4,5}$ 1.4 (H-4); 3.94, $J_{5,6}$ 4.9, $J_{5,6'}$ 6.7 (H-5); 3.76 (H-6, H-6'); 2.05 ($CH_3C=O$); 3.40 (CH_3-O-C). ^{13}C NMR, δ: 99.5 (0.00, C-1); 50.9 (0.14, C-2); 69.7 (0.12, C-3); 53.0 (0.25, C-4); 71.3 (0.02, C-5); 63.1 (0.13, C-6); 176.0 (0.09, $CH_3C=O$); 23.3 (0.13, $CH_3C=O$); 56.5 (0.07, CH_3-O-C).

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Oxidation with Concurrent Solvolysis of 4-Alkylthio-pyrimidines

ANSGAR HEIM PEDERSEN and KJELL UNDHEIM

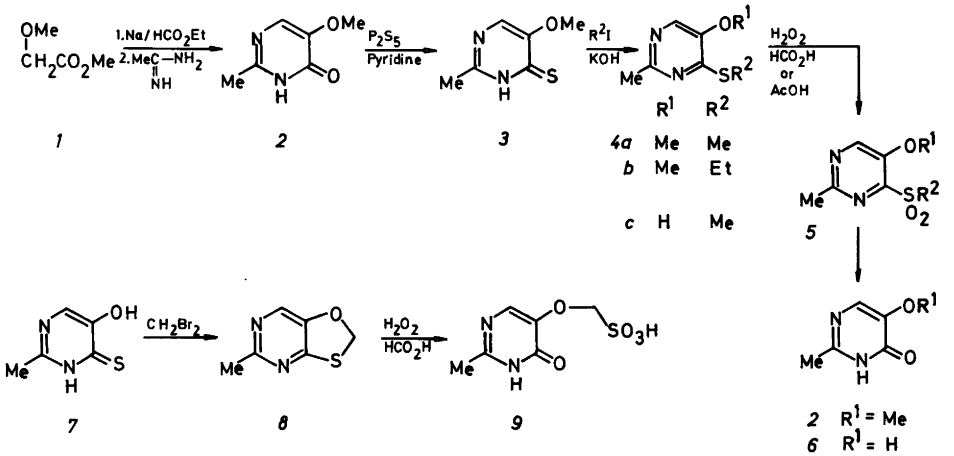
Department of Chemistry, University of Oslo, Oslo 3, Norway

Sulfonyl and sulfinyl substituents in active azine positions are readily displaced by nucleophiles.[1] The oxides of heterocyclic sulfides are generally formed by oxidation reactions of the parent sulfide. Whereas the rate of oxidation of the sulfide is enhanced by high electron availability on the thioether sulfur atom, substitution of the oxidized sulfide is facilitated by electron deficiency in its environment. We herein report on cases where the rate of solvolysis of the oxidized thioether function is faster than its formation under the conditions chosen for the experiments.

The thioethers for the oxidation studies were prepared as shown in Scheme 1. Formylation of methyl methoxyacetate by means of sodium and ethyl formate, and then subsequent condensation of the product with acetamidine furnished the 5-methoxy-2-methyl-4-pyrimidinone *2*. The latter was converted into the thiolactam *3* by means of phosphorus pentasulfide, and *3* was subsequently *S*-alkylated (*4a*, *4b*). The 5-hydroxy analogue *4c* was similarly prepared. In *4*, the electron deficient pyrimidine ring will decrease the rate of oxidation as compared with its benzene analogue. This is to some extent compensated for by the electron donating properties of the 2-methyl group and the 5-substituent. Even so, the rate of oxidation using peracetic acid, which was generated *in situ* from acetic acid and hydrogen peroxide, was such that at least one week at room temperature was required before all the thioether had reacted. The stronger electron donating power of the hydroxy group over the methoxy group did not markedly affect the rate. With the more powerful performic acid, which was generated *in situ* as above, all the thioether had reacted after 2 days. The product isolated from the reaction has been identified as the lactams *2* [2] and *6*.[3] Oxidized sulfides in the 2-position are less reactive,[4] however, and did not suffer solvolysis when oxidized under the above conditions.[5]

Irrespective of whether the solvolysis occurs at the oxidation level of a sulfoxide or sulfone, the resultant acid is expected to be oxidized further to a sulfonic acid.[6,7] This was confirmed through the preparation of the bicyclic analogue 5-methyl[1,3]-oxathiolo[4,5-*d*]pyrimidine *8* from the 5-hydroxy-4-pyrimidinethione *7* and dibromomethane, and by the subsequent oxidation of *8* as above; the product was the postulated sulfonic acid *9*.

Experimental. *5-Methoxy-2-methyl-4-pyrimidinone* [2] 2. Methyl methoxyacetate (104.1 g, 1.0 mol) was added dropwise with stirring at 0 °C to a mixture of sodium (23.0 g, 1.0 mol) and ethyl formate (74.1 g, 1.0 mol) in dry diethyl ether (800 ml). The mixture was stirred at room temperature for 5 h before acetamidine hydrochloride (94.5 g, 1.0 mol) in methanol (600 ml) was added, and the ether was subsequently distilled off. The residual mixture was then heated under reflux for 17 h, the solvent distilled off at reduced pressure, water (500 ml) added, and the resultant solution adjusted to pH 5 with concentrated hydrochloric acid which



Scheme 1.

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precipitated the product; yield 49.5 g (35 %), m.p. 212 – 215 °C ($CHCl_3$).

5-Methoxy-2-methyl-4-pyrimidinethione 3. A mixture of 5-methoxy-2-methyl-4-pyrimidinone (12.0 g, 0.086 mol) and phosphorus pentasulfide (19.1 g, 0.086 mol) in dry pyridine (330 ml) was heated under reflux and stirring for 3 h. The cold reaction mixture was poured into water (400 ml) and the resultant mixture was concentrated to *ca.* 50 ml at reduced pressure. Most of the thione crystallized out (10.4 g) during this operation. Another crop was obtained by extracting the filtrate with chloroform (10 × 15 ml); total yield 11.8 g (88 %), m.p. 206 – 208 °C (MeOH). Anal. $C_6H_8N_2OS$: C, H. ^{1}H NMR (TFA): δ 2.87 (2-Me), 4.06 (OMe), 7.49 (H-6). MS [70 eV; m/z (% rel. int.)]: 156 (100, M), 155 (22), 123 (28), 122 (22), 93 (22), 87 (21).

5-Methoxy-2-methyl-4-methylthiopyrimidine 4a. Methyl iodide (7.1 g, 0.050 mol) was added dropwise to a solution prepared from 5-methoxy-2-methyl-4-pyrimidinethione (6.0 g, 0.038 mol) in 2 M potassium hydroxide (300 ml). The mixture was stirred for 2 h at room temperature before extraction with ether (3 × 100 ml). The ether solution was washed, dried ($MgSO_4$), the ether distilled off, and the white solid residue recrystallized from water; yield 5.3 g (82 %), m.p. 90 °C. Anal. $C_7H_{10}N_2OS$: C, H. ^{1}H NMR (TFA): δ 2.73 (SMe), 2.85 (2-Me), 4.07 (OMe), 7.73 (H-6). MS [70 eV; m/z (% rel. int.)]: 170 (100, M), 155 (47), 137 (27), 123 (27), 82 (17).

4-Ethylthio-5-methoxy-2-methylpyrimidine 4b was prepared as *4a* above. The product was purified by sublimation at 30 °C/0.01 mmHg; yield 80 %, m.p. 53 – 54 °C. Anal. $C_8H_{12}N_2OS$: C, H. ^{1}H NMR (TFA): δ 1.47 and 3.39 (*S*-Et), 2.83 (2-Me), 4.05 (OMe), 7.69 (H-6). MS [70 eV; *m/z* (% rel. int.)]: 184 (100, M) 169 (50), 156 (28), 151 (63), 123 (41), 82 (21).

5-Hydroxy-2-methyl-4-methylthiopyrimidine[8] 4c was prepared as above.

5-Methoxy-2-methyl-4-pyrimidinone[2] 2 *and 5-hydroxy-2-methyl-4-pyrimidinone*[3] 6. General procedure: The 4-alkylthiopyrimidine *4* (0.015 mol) was added to an ice-cold solution prepared from 35 % hydrogen peroxide (6.3 g, 0.065 mol) and formic acid (60 ml). The mixture was stirred at room temperature for 2 d before most of the solvent was removed at reduced pressure. Water was added to the residue and the solvents again removed at reduced pressure. The residual material was dissolved in water and the pH adjusted to *ca.* 5 by addition of sodium carbonate; the lactams *2* and *6* were precipitated in 50 – 75 % yields.

5-Methyl[1,3]oxathiolo[4,5-d]pyrimidine 8. Dibromomethane (1.20 g, 0.007 mol) in DMF (10 ml) was added to a mixture of 5-hydroxy-2-methyl-4-pyrimidinethione[9] (1.00 g, 0.007 mol) and sodium carbonate (0.74 g, 0.007 mol) in DMF (50 ml). The mixture was stirred at 85 °C for 4 h before the solvent was distilled off at reduced pressure. Water (40 ml) was added to the residue, the mixture extracted with ether (5 × 25 ml), the dried ($MgSO_4$) solution evaporated and the residual oil left in the cold where it slowly crystallized and was further purified by sublimation at 20 °C/0.01 mmHg; yield 0.9 g (83 %), m.p. 42 – 43 °C. Anal. $C_6H_6N_2OS$: C, H. ^{1}H NMR (TFA): δ 2.82 (5-Me), 6.20 (CH_2), 7.75 (H-7). MS [70 eV; *m/z* (% rel. int.)]: 154 (100, M), 153 (20), 113 (22), 109 (58), 108 (52), 82 (23), 80 (63).

(2-Methyl-4-oxo-5-pyrimidinyloxy)methanesulfonic acid 9. 35 % hydrogen peroxide solution in formic acid and 5-methyl[1,3]oxathiolo[4,5-*d*]pyrimidine were reacted together as described above in the general oxidation procedure for the synthesis of *2* and *6*. After the addition of water to the partially evaporated reaction mixture, and the subsequent evaporation, the semisolid residue was crystallized from ethanol – water (2:1) in large white crystals; yield 45 %, m.p. 158 °C (decomp.). ^{1}H NMR (TFA): 2.93 (2-Me), 5.36 (CH_2), 8.12 (H-6). MS [70 eV; *m/z* (% rel. int.)]: 220 (4, M), 139 (33), 138 (33), 126 (92), 108 (19), 64 (100, SO_2). High resolution MS: Found M: 220.0148. Calc. for $C_6H_8N_2O_5S$: 220.0152.

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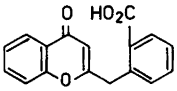
Synthesis of Benzylchromones and Benzoxanthones Related to Natural Products

ANDERS KJÆR and DANA KJÆR

Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark

An array of procedures is available for the synthesis of naturally occurring chromones and flavones. None of them have been applied, however, to the synthesis of the homoflavone carboxylic acid skeleton *1*.



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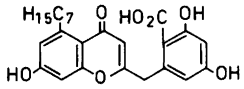
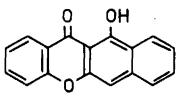


2

We were attracted to this system because of its presence in the unique, naturally occurring homoflavone, siphulin *2*, a constituent of the North Scandinavican lichen *Siphula ceratites* (Wahlenberg) Fr., isolated and identified by Bruun several years ago.[1] Apart from being the only homoflavone of natural derivation, siphulin, an obvious polyketide, carries distinction as the sole chromone carboxylic acid among natural products. On cyclization, the 2-(*o*-carboxybenzyl)-chromone system *1* could conceivably provide an entrance into the tetracyclic



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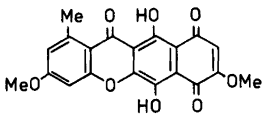

4

class of 11-hydroxy-12*H*-benzo[*b*]xanthen-12-ones *3* with the biochrome bikaverin *4*, a long-standing interest of ours,[2] as a conspicuous member. Both siphulin *2* and bikaverin *4* possess structural features of potential biological interest. With a view to studying the structure-activity relationships within the two classes of compounds, efficient synthetic routes, leading, at will, to 2-(*o*-carboxybenzyl)chromones of the siphulin type, or 11-hydroxy-12*H*-benzo[*b*]xanthen-12-ones, structurally related to bikaverin, were explored.

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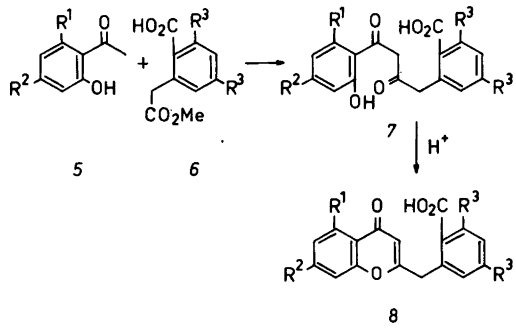


5 *6* *7* *8*

We observed that enolates of *o*-hydroxyacetophenones *5*, generated in tetrahydrofuran by means of sodium hydride, reacted smoothly with methyl *o*-carboxyphenylacetates *6* to give enolised 1,3-diketones *7*, which, in turn, were easily converted by acid into the desired, colourless homoflavone acids *8* in satisfactory yields (Table 1).

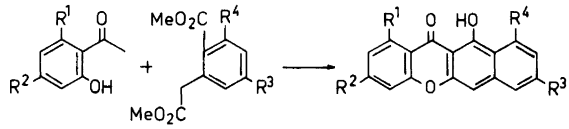
2-Hydroxy-4-methoxy-6-heptylacetophenone *5* ($R^1 = C_7H_{15}$, $R^2 = OMe$) was produced by subjecting sphaeropherol (5-heptylresorcinol), in its turn arising from alkaline hydrolysis and decarboxylation (see Experimental) of methyl 2,4-dihydroxy-6-heptyl benzoate,[3] to Houben-Hoesch acetylation, followed by selective methylation. 3,5-Dimethoxyhomophthalic acid was conveniently prepared by metalation and carbonization of di-*O*-methyl orsellinic acid, according to Hauser,[4] and was converted into its monomethyl ester *6* ($R^3 = OMe$) on brief exposure to methanolic HCl. Attempts to demethylate siphulin tri-*O*-methyl ether (*8*, $R^1 = C_7H_{15}$; $R_2 = R_3 = OMe$), under various conditions, were accompanied by extensive decarboxylation.

Substituting the acid esters *6* with the dimethyl homophthalates *9* in the Claisen-type condensation resulted in a surprisingly efficient, one step synthesis

Table 1. 2-(*o*-Carboxybenzyl)-chromones *8*,[a] synthesized from *5* and *6*.

R^1	R^2	R^3	M.p. °C	Yield %[b]
H	H	H	209	55
H	OMe	H	245	55
Me	OMe	H	244	52
Me	OMe	OMe	205	68
C_7H_{15}	OMe	OMe[c]	167–168[c]	77

[a] All compounds were recrystallized from ethanol and gave analytical figures within ±0.3% of the calculated values; their ^1H and mass spectra were in agreement with structure *8*. [b] The yields refer to analytically pure products. [c] Siphulin trimethyl ether; Lit.[1] m.p. 166–167 °C.



5 9 10

of the yellow 11-hydroxy-12*H*-benzo[*b*]xanthen-12-ones *10*, a number of which were produced by varying the *o*-hydroxyacetophenone and dimethyl homophthalate reactants (Table 2). The fully substituted derivative *10* ($R^1 = Me$, $R^2 = R^3 = R^4 = OMe$) has been further elaborated into bikaverin. This conversion, and several other aspects of the work outlined above, will be described in a forthcoming publication.

Experimental. Melting points (uncorr.) are determined in capillary tubes in a heated block. ^1H NMR spectra are recorded in $CDCl_3$ solutions at 90 MHz on a Bruker HX-90E instrument.

Sphaeropherol (5-heptylresorcinol). Methyl 2,4-dihydroxy-6-heptylbenzoate [3] (10.6 g) was dissolved in dimethyl sulphoxide (120 ml). After adding a solution of potassium hydroxide (12.1 g) in water (25 ml), the mixture was heated, in an argon stream, to 115 °C for 2.5 h. After cooling, the mixture was poured onto ice and conc. hydrochloric acid. The reaction product was extracted with ether, the ether solution was washed with sodium bicarbonate solution and water and dried. On evaporation a brownish solid remained, which was recrystallized first from light petroleum and then from aqueous acetic acid to give pure spaeropherol monohydrate (7.3 g, 81%), m.p. 56 °C (Lit.[1] m.p. 54–55 °C).

(2,4-Dihydroxy-6-heptylacetophenone) and an isomer. A suspension of anhydr. zinc chloride (2.0 g) in anhydr. ether (20 ml), containing sphaeropherol (3.15 g), was saturated at 0 °C with anhydr. hydrogen chloride. Acetonitrile (0.9 g) was added, and a slow stream of hydrogen chloride was maintained for 4 h. The reaction mixture was kept at 0 °C for 3 days, at the end of which the precipitate (2.5 g) was filtered off and subjected to hydrolysis on boiling with sulphuric acid (5 ml 5 N in 200 ml of water) for 1 h. Extraction with ether and recrystallization of the product from light petroleum (with a few drops of ether) gave an analytical specimen of 2,4-dihydroxy-6-heptylacetophenone (*5*, $R^1 = C_7H_{15}$, $R^2 = OH$), m.p. 61–62 °C, anal. $C_{15}H_{22}O_3$: C, H. ^1H NMR: δ 0.88 (3H, t, *J* 6 Hz), 1.1–1.8 (10 H, m), 2.62 (3H, s), 2.80 (2H, t, *J* 6 Hz), 6.22 (2H, s), 6.34 (1H, s; exch. w. D_2O), 11.5 (1H, s; exch. w. D_2O).

The ether filtrate was washed with water (2 × 10 ml) and evaporated to give 0.4 g of unreacted sphaeropherol. On saturating the aqueous phase with dichloromethane, a solid separated (0.95 g). It was hydrolyzed as described above, yielding an additional crop of 2,4-dihydroxy-6-heptylacetophenone (0.23 g) (bringing the total yield, based on reacted spaeropherol, to 57%), and another product (0.17 g), m.p. 46–47 °C, anal. $C_{15}H_{22}O_3$: C, H, after separation on silica gel plates with hexane – diethyl ether – acetic acid (30:20:1) as an eluant. The ^1H NMR spectrum revealed its identity as *2,6-dihydroxy-4-heptyl-acetophenone*, supposedly owing its unparalleled formation in a Houben-Hoesch synthesis to the bulkiness of the alkyl grouping of the substrate: δ 0.85 (3H, t, *J* 6 Hz), 1.1–1.8 (10 H, m), 2.44 (2H, t *J* 6 Hz), 2.67 (3H, s), 6.16 (2H, s), 9.52 (2H, s (br); exch. w. D_2O).

2-Hydroxy-4-methoxy-6-heptylacetone. To a solution of 2,4-dihydroxy-6-heptylacetophenone (1.5 g) in acetone (8 ml), covered with argon, were added, portionwise, a total of 0.85 g of dimethyl sulphate and 5.3 ml of 10% sodium hydroxide while the solution was maintained at 45 °C. After mixing the reagents, the solution was kept at 45 °C for 4 h and then poured onto ice and conc. hydrochloric acid. The oily material was extracted with ether and purified by flash chromatography on silica gel with hexane – ethyl acetate – acetic acid (85:15:1) as the mobile phase. The monomethyl ether (1.10 g, 70%), previously described by Bruun as an oily degradation product of siphulin,[1] was characterized by its ^1H NMR spectrum: δ 0.84 (3H, t, *J* 7 Hz), 1.1–1.8 (10H, m), 2.61 (3H, s), 2.82 (2H, t, *J* 7 Hz), 3.76 (3H, s), 6.22 (2H, s), 12.1 (1H, s; exch. w. D_2O).

Dimethyl 3-methoxy-homophthalate. 3-Hydroxy-homophthalic acid [7] (5 g), dissolved in anhydr. acetone, was stirred with silver oxide (prepared from 12 g of silver nitrate) and methyl iodide (25 ml) at 25 °C for 48 h. After filtration and evaporation, the dimethyl 3-methoxy homophthalate distilled as a colourless oil (5.4 g, 90%), b.p. 140–143 °C (1.7 mmHg) (Lit.[8]: b.p. 117–124 °C (0.3 mmHg)).

Methyl 2-carboxy-3,5-dimethoxyphenylacetate. 3,5-Dimethoxyhomophthalic acid (1 g) was dissolved in methanol (8 ml), containing 0.4 ml of methanol, saturated with hydrogen chloride. After 1 h at 22 °C, the solution was evaporated and the acid ester was recrystallized from ethyl acetate (1.24 g, 91%), m.p. 128–129 °C, anal. $C_{12}H_{14}O_6$: C, H.

Dimethyl 3,5-dimethoxyhomophthalate. 3,5-Dimethoxyhomophthalic acid [4] (1.9 g) was dissolved in methanol (10 ml) to which methanol (10 ml), saturated with hydrogen chloride, was added. The

Table 2. 11-Hydroxy-12*H*-benzo[*b*]xanthen-12-ones *10*,[a] synthesized from *5* and *9*.

R^1	R^2	R^3	R^4	M.p. °C	Yield %[b]
H	H	H	H	203 – 205[c]	70
H	OMe	H	H	183 – 184	63
Me	OMe	H	H	209 – 211	52
H	OMe	H	OMe	245 – 247(d.)	38
Me	OMe	H	OMe	252 – 254(d.)	42
Me	OMe	OMe	OMe	260 – 262(d.)	23

[a] All compounds were recrystallized from dichloromethane-hexane and gave analytical figures within ±0.2% of the calculated values; all exhibited ^{1}H and mass spectra in agreement with structure *10*. [b] The yields refer to analytically pure products. [c] Identical with a specimen (m.p. 198 – 203 °C), prepared, in about 1% yield, by a Nencki type of reaction;[5] described also (m.p. 205 – 209 °C) as the product of a photoinduced cyclization of unknown generality.[6]

solution was refluxed for 2 h, evaporated to dryness, and the residue taken up in ether. The solution was washed with sodium bicarbonate solution and water, dried and evaporated. The ester solidified on standing, m.p. 39 – 41 °C (previously reported as an oil[9,10]).

General procedure for the synthesis of 2-(o-carboxybenzyl)-chromones (Table 1) and 11-hydroxy-12H-benzo-[b]*xanthen-12-ones (Table 2).* To a stirred suspension of sodium hydride (40 mM) in anhydrous tetrahydrofuran, kept in a slow stream of argon, a solution of the *o*-hydroxyacetophenone (10 mM) in tetrahydrofuran (5 ml) was slowly added, followed by a solution of the mono- or diester (10 mM) in tetrahydrofuran (5 – 10 ml). The mixture was refluxed for 5 h, cooled and poured into 6 M hydrochloric acid (50 ml). After standing overnight, the products were collected by filtration and/or extracted with ether or chloroform. The acid derivatives, *8*, were freed of non-acid contaminants by distribution between sodium bicarbonate and ether (or chloroform), and were isolated by acidification of the aqueous phase, and reextraction. All reaction products were produced in analytically pure form by repeated recrystallizations (Tables 1 and 2), in a few cases preceded by chromatographic purification.

Acknowledgement. This paper is submitted in honour of Professor Holger Erdtman on the occasion of his 80th birthday in appreciation of his contributions to organic chemistry.

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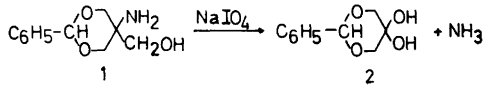
The Preparation of 5-Oxo-2-phenyl-1,3-dioxane

HELMUT VORBRÜGGEN*

Woodward Research Institute, CH-4000 Basle 7, Switzerland

On repeating the preparation of the synthetically useful benzal-derivative of 1,3-dihydroxyacetone the 5-oxophenyl-1,3-dioxane *2* by $NaIO_4$-cleavage [1] of 5-hydroxymethyl-5-amino-2-phenyl-1,3-dioxane *1* according to Marei and Raphael [2] we encountered difficulties and obtained varying yields of impure *2*.

On studying the reaction we found that the pH of the solution increased to 9.5 during the $NaIO_4$-oxidation due to the liberated NH_3. Since *2* seemed to be alkali-labile, we added an equivalent amount of KH_2PO_4 to the solution of *1* to neutralize the NH_3 and then obtained *2* in more than 90% yield. Control of the pH might be important in similar oxidations of 1,2-aminoalcohols with $NaIO_4$ liberating NH_3 or amines.



1 2

5-Oxo-2-phenyl-1,3-dioxane (2). The amino-alcohol *1* (105 g; 0.5 mol) was dissolved at 30 °C in H_2O (250 ml) and methanol (250 ml) and cooled to 5 °C with stirring. After adding solid KH_2PO_4 (68 g; 0.5 mol) a solution of $NaIO_4$ (107 g; 0.5 mol) in H_2O (1 l, dissolved at 30 °C) was added dropwise during 45 min maintaining a temperature of +10 °C −15 °C inside the flask, whereupon a colorless precipitate formed. After warming to room temperature during 1 h the partly crystalline mixture was extracted with ethyl acetate (4 × 750 ml) and the extracts dried with Na_2SO_4 (300 g). Concentrating the extracts to 500 ml gave a first crop (70.0 g) of colorless crystalline hydrate *2*, m.p. 84 − 85 °C. On concentrating the mother liquors to 100 ml and finally to 30 ml while adding each time about 0.5 ml of water, two further crops of slightly yellowish hydrate *2* (23.3 g), m.p. 78 − 80 °C were obtained. Combined yield of *2* 92.3 g = 95%.

Crude yellowish hydrate *2* (50 g) can be readily recrystallized from ethyl acetate (250 ml) − water (5 ml) to give after cooling to − 18 °C and washing with ice-cold ethyl acetate colorless *2* (43.8 g); m.p. 85 − 86 °C.

* Present address: Schering A.G., D-1000 Berlin 65, Germany.

Acknowledgement. This paper is submitted in honour of Professor Holger Erdtman on the occasion of his 80th birthday in appreciation of his contributions to organic chemistry.

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The Hydroxylation of Thianthrene Cation Radical in Buffered Acetonitrile. The Final Word on the Mechanism?

OLE HAMMERICH[a] and VERNON D. PARKER[b]

[a] Department of General and Organic Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark and [b] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The kinetics of the hydroxylation of thianthrene cation radical were examined in acetonitrile (AN) and in AN containing 2,6-lutidine (L), trifluoroacetic acid (TFA) and $CF_3CO_2^-LH^+$. The analysis of reaction orders and deuterium kinetic isotope effects resulted in consistency with mechanism (i)–(iv) and three different rate laws depending upon the relative magnitudes of k_{iii}, k_{-iii} and k_{iv}. Only a

$$Th^{\cdot+} + H_2O \overset{K_i}{\rightleftarrows} Th^{\cdot}-\overset{+}{O}H_2 \quad \text{(i)}$$

$$Th^{\cdot}-\overset{+}{O}H_2 + B \overset{K_{ii}}{\rightleftarrows} Th^{\cdot}-OH + BH^+ \quad \text{(ii)}$$

$$Th^{\cdot}-OH + Th^{\cdot+} \underset{k_{-iii}}{\overset{k_{iii}}{\rightleftarrows}} Th^+-OH + Th \quad \text{(iii)}$$

$$Th^+-OH + B \overset{k_{iv}}{\rightarrow} ThO + BH^+ \quad \text{(iv)}$$

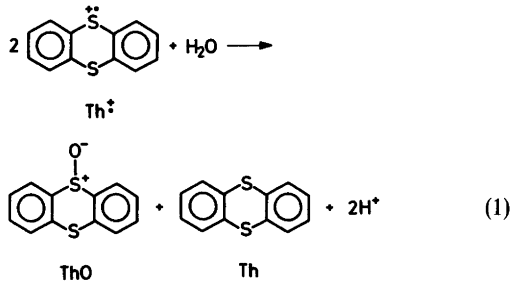
very small inhibition was observed in the presence of TFA indicating that the acid is not significantly dissociated under the experimental conditions. When present, $CF_3CO_2^-$ was observed to be the strongest base (B) in the system and effectively participated in reaction (ii). The reaction order in water was observed to be as high as 4.5 in neutral AN and to depend upon $[H_2O]$. The high and varying reaction order in water was explained by the effect of hydration equilibrium (v) on the hydronium ion activity. As the water concentration in AN increases H_3O^+ becomes less active and

$$H_3O^+ + n\,H_2O \rightleftarrows H_3O^+(H_2O)_n \quad \text{(v)}$$

participates less effectively in back reaction (ii). In the absence of water, either pyridine or trifluoroacetate ion participates in reaction (vi) which is reversible during cyclic voltammetry at 100 mV/s.

$$Th + N \rightleftarrows Th^+ - N^{(+)} + 2e^- \quad \text{(vi)}$$

The reactions of thianthrene cation radical ($Th^{\cdot+}$) with nucleophiles have been the subject of numerous investigations.[1-44] The reaction of $Th^{\cdot+}$ with water (1) was among the first ion radical reactions to be investigated by kinetic techniques.[1,2] The initial



(1)

investigations led to the conclusion that the dication (Th^{2+}) formed in disproportionation reaction (2) was the intermediate which reacts with water (3) giving rise to rate law (4). This proposal was challenged on the basis of voltammetric experi-

$$2\,Th^{\cdot+} \overset{K_2}{\rightleftarrows} Th^{2+} + Th \quad \text{(2)}$$

$$Th^{2+} + H_2O \overset{k_3}{\rightarrow} ThO + 2\,H^+ \quad \text{(3)}$$

$$\text{Rate} = 2\,k_3K_2[Th^{\cdot+}]^2[H_2O]/[Th] \quad \text{(4)}$$

ments and it was suggested that $Th^{\cdot+}$ was the species undergoing reaction with water.[3] An alternative mechanism was suggested which involved steps (5)–(7)

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$$Th^{\cdot+} + H_2O \overset{K_5}{\rightleftharpoons} Th^{\cdot} - OH + H^+ \quad (5)$$

$$Th^{\cdot} - OH + Th^{\cdot+} \overset{K_6}{\rightleftharpoons} Th^{+} - OH + Th \quad (6)$$

$$Th^{+} - OH \overset{k_7}{\rightarrow} ThO + H^+ \quad (7)$$

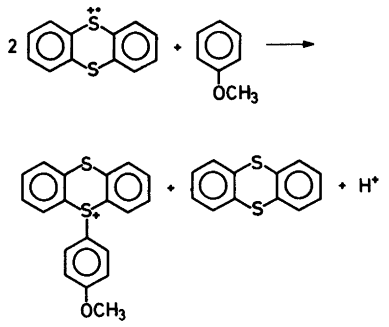
This mechanism had previously been proposed for the conversion of phenoxathiin cation radical to the oxide.[4] It was pointed out that it is difficult to distinguish between the two mechanisms by kinetic measurements.[3] The latter led to attempts to accurately determine the value of K_2 in order to test the feasibility of the disproportionation mechanism.[5,6] The experimental quantities necessary to determine K_2 are the reversible potentials for reactions (8) and (9), *i.e.* E_8^{rev} and E_9^{rev}. The difficulty in obtaining these data lies in the very high reac-

$$Th - e^- \overset{E_8^{rev}}{\rightleftharpoons} Th^{\cdot+} \quad (8)$$

$$Th^{\cdot+} - e^- \overset{E_9^{rev}}{\rightleftharpoons} Th^{2+} \quad (9)$$

tivity of Th^{2+}. In the initial attempts to determine E_9^{rev}, measurements were carried out in trifluoroacetic acid (TFA) containing H_2SO_4.[5] In this solvent system the difference in reversible potentials for reactions (8) and (9) was observed to be of the order of 780 mV which corresponds to K_2 equal to about 10^{-13}. This prohibitively small value made the disproportionation mechanism appear to be highly unlikely, providing that K_2 is the same in acetonitrile (AN) as in the acidic solvent. However, when it was later discovered that the reversible potential for reaction (9) could be determined in AN by conducting measurements on solutions containing suspended Al_2O_3 it became apparent that K_2 is strongly solvent dependent and the value in AN was observed to be 2.3×10^{-9}.[6] Having a reliable value of K_2, along with the reasonable assumption that back reaction (2) is diffusion controlled, is sufficient to place a maximum of about 2.3 M^{-1} s^{-1} for the observed rate constant for mechanism (2)–(3) using the reasoning developed in related work.[7] The value observed [1,2] was of the order of 0.2 M^{-1} s^{-1}, a fact which is consistent with the disproportionation mechanism. Thus, the combination of kinetic and thermodynamic data was not sufficient to distinguish between the two mechanisms in question.

The stalemate encountered in the attempts to elucidate the mechanism of the hydroxylation of $Th^{\cdot+}$ led to the examination of a related reaction, that of $Th^{\cdot+}$ with anisole which has the stoichiometry depicted by eqn. (10).[8] A kinetic study of this reaction had also resulted in the proposal of a



(10)

disproportionation mechanism [9] with a rate law of the same form as (4). However, a more detailed analysis showed that the reaction orders in substrate (Th) and anisole (AnH) are not −1 and 1 as predicted by rapid disproportionation equilibrium followed by rate determining reaction of the dication with anisole but rather more complex with an observed rate constant at a particular [AnH] described by eqn. (11)[8] for reactions conducted in the presence

$$1/k_{obs} = A[Th]/[AnH] + B/[AnH] \quad (11)$$

of excess Th and AnH. The important feature of eqn. (11) is that plots of $1/k_{obs}$ *vs.* the thianthrene concentration are predicted to have intercepts, the magnitude of which are dependent upon [AnH]. On the other hand analysis of the disproportionation mechanism under conditions where (2) cannot be considered to be in equilibrium resulted in expression (12) for the observed rate constant. This relationship predicts an intercept independent upon

$$1/k_{obs} = A'[Th]/[AnH] + B' \quad (12)$$

[AnH]. Thus, under the reaction conditions used, the anisylation of $Th^{\cdot+}$ was shown to take place by a mechanism giving rise to rate law (13), in which k_{obs} is defined in eqn. (11). This rate law is incon-

$$\text{Rate} = k_{obs}[Th^{\cdot+}]^2 = k_{app}[Th^{\cdot+}]^2[AnH]/(\textit{constant} + [Th]) \quad (13)$$

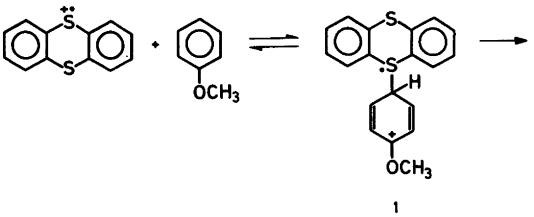
sistent with the disproportionation mechanism. Mechanism (14)–(16) was proposed to account for the kinetic data. The essential features of this

$$Th^{\cdot+} + AnH \overset{K_{14}}{\rightleftharpoons} (Th/AnH)^{\cdot+} \quad (14)$$

$$(Th/AnH)^{\cdot+} + Th^{\cdot+} \underset{k_{-15}}{\overset{k_{15}}{\rightleftharpoons}} (Th/AnH)^{2+} + Th \quad (15)$$

$$(Th/AnH)^{2+} \xrightarrow{k_{16}} Th^{+} - An + H^{+} \quad (16)$$

mechanism are that covalent bond formation occurs in the dication–anisole complex (16) and that the initial reversible interaction of $Th^{\cdot+}$ and AnH (14) results in a π complex. The overall scheme was called the "complexation mechanism".[8] The reasoning behind the formulation of the mechanism in this manner rather than in the way proposed earlier[3] for the hydroxylation of $Th^{\cdot+}$ as in eqns. (5)–(7) was that *1*, resulting from the reaction of $Th^{\cdot+}$ and anisole would be expected to undergo irreversible proton loss (17) before involvement of the second



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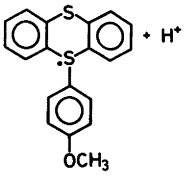


2

(17)

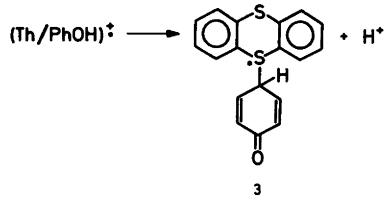
$Th^{\cdot+}$ moiety. Mechanism (14)–(16) results in rate law (18), which is of the same form as (13).

$$\text{Rate} = 2k_{16}K_{14}K_{15}[Th^{\cdot+}]^2[AnH]/(k_{16}/k_{-15} + + [Th]) \quad (18)$$

The reaction of $Th^{\cdot+}$ with phenol (PhOH) in either acetonitrile or dichloromethane was observed to follow rate law (19).[10] It was suggested that the

$$\text{Rate} = k_{app}[Th^{\cdot+}][PhOH] \quad (19)$$

reason for the difference in mechanism with phenol and anisole as the nucleophiles is due to the facile deprotonation of the phenol containing complex accompanied by irreversible covalent bond formation (20), a pathway not available to the



(20)

3

anisole complex. The proton transfer from oxygen (20) is suppressed in the presence of TFA and kinetic studies in CH_2Cl_2–TFA resulted in a rate expression identical in form to that when anisole is the nucleophile.[10] This provides very strong support that neither *1*, *2* nor *3* are intermediates in the reactions second order in $Th^{\cdot+}$ and emphasizes the need to invoke the complexation mechanism to explain the kinetic data.

The hydroxylation of $Th^{\cdot+}$ was reexamined by Evans and Blount[11] using stopped flow kinetic techniques. The reaction was observed to be second order in cation radical, third order in water, and inhibited by acid. The kinetic observations led to the proposal of mechanism (21)–(23) and rate law (24) for the reaction in acetonitrile.

$$Th^{\cdot+} + H_2O \overset{K_{21}}{\rightleftharpoons} Th^{\cdot} - \overset{+}{O}H_2 \quad (21)$$

$$Th^{\cdot} - \overset{+}{O}H_2 + H_2O \overset{K_{22}}{\rightleftharpoons} Th^{\cdot} - OH + H_3O^{+} \quad (22)$$

$$Th^{\cdot} - OH + Th^{\cdot} - \overset{+}{O}H_2 \xrightarrow{k_{23}} \text{products} \quad (23)$$

$$\text{Rate} = 2k_{23}K_{21}^2K_{22}[Th^{\cdot+}]^2[H_2O]^3/[H_3O^{+}] \quad (24)$$

More recently[12] we have shown that the kinetic analysis used by Evans and Blount[11] involved the improper use of the equilibrium approximation and that mechanism (21)–(23) results in reaction orders in water of either 1 or 2 depending upon the magnitude of K_{21}. Furthermore, kinetic studies[12] of the hydroxylation of $Th^{\cdot+}$ in CH_2Cl_2–TFA resulted in a rate law which is consistent with either the complexation mechanism analogous to (14)–(16) or with a modification of mechanism (5)–(7). The fact that the reaction is first order in water suggests

the complexation mechanism since proton loss before the rate determining step would most likely require a molecule of water acting as a base and hence would result in a reaction order of 2 in water.

Another aspect of the study by Evans and Blount[11] which we find highly questionable is the interpretation of experiments carried out in which pyridine was added to acetonitrile-trifluoroacetic anhydride (TFAn) solutions of $Th^{\cdot+}$ and Th. The addition of TFAn to acetonitrile-supporting electrolyte solutions has been shown to be a highly effective means of removing residual water by virtue of reaction (25).[13] However, such solutions are then several mM in TFA.

$$TFAn + H_2O \rightarrow 2\ TFA \qquad (25)$$

Evans and Blount[11] disregarded this and interpreted cyclic voltammograms conducted on solutions of Th (1 mM) in acetonitrile-TFAn (4 %) to to which pyridine (Pyr) had been added in concentrations ranging from 0.9 to 1.6 mM as resulting from the reaction of pyridine with $Th^{\cdot+}$. It is almost inconceivable that there could have been any pyridine present in the solutions due to protonation by TFA. The voltammetric experiments resulted in the proposal that the potential for reaction (26) is of the order of 400 mV positive of that for reaction (8) which leads to a value of about 10^{-7} for K_{27}.

$$Th^{\cdot} - Pyr^{+} - e^{-} \rightleftarrows Th^{+} - Pyr^{+} \qquad (26)$$

$$Th^{\cdot} - Pyr^{+} + Th^{\cdot+} \overset{K_{27}}{\rightleftarrows} Th^{+} - Pyr^{+} + Th \qquad (27)$$

But kinetic experiments resulted in apparent second order rate constants of the order of $2 \times 10^6\ M^{-1}\ s^{-1}$ at pyridine concentrations of about 5 mM. The mechanism giving rise to these kinetics was proposed to be (28)–(30). The maximum possible value of k_{29} can be estimated to be $10^3\ M^{-1}\ s^{-1}$ assuming

$$Th^{\cdot+} + Pyr \overset{K_{28}}{\rightleftarrows} Th^{\cdot} - Pyr^{+} \qquad (28)$$

$$Th^{\cdot} - Pyr^{+} + Th^{\cdot+} \overset{k_{29}}{\rightarrow} Th^{+} - Pyr^{+} + Th \qquad (29)$$

$$Th^{+} - Pyr^{+} + H_2O \overset{\text{fast}}{\longrightarrow} Th^{+} - OH + PyrH^{+} \qquad (30)$$

that the voltammetric peak assignment was correct and that back reaction (27) is diffusion controlled. Thus, the observed rate constants are more than 10^3 times greater than the equilibrium data suggest is possible. Furthermore, it clearly is not justifiable to consider reaction (29) as being irreversible when the reverse reaction is expected to approach the diffusion controlled limit.

In our opinion it is ironic that work by Evans and Blount[11] which appears to have resulted in the postulation of a reaction mechanism for the hydroxylation of $Th^{\cdot+}$ which is inconsistent with the experimentally derived rate law and in erroneous conclusions regarding the mechanism of hydroxylation in the presence of pyridine has been highly praised in recent review articles.[14,15] Hanson[14] states that this work[11] is an elegant demonstration of both the value of electrochemical techniques in the investigation of ion radicals and the subtleties of the reactivity of $Th^{\cdot+}$. Shine[15] concludes that the complete and complex nature of the reaction was elucidated by Evans and Blount.[11]

The objective of the work described in this paper was to attempt to remove the uncertainties regarding the mechanism of the hydroxylation of $Th^{\cdot+}$ in acetonitrile by carrying out experiments in buffered media in which the nature of the bases in proton transfer equilibria are better defined and to use kinetic isotope effects as a mechanistic probe. Preliminary results of this investigation have been reported.[16]

RESULTS

Kinetic measurements. The kinetics of the reactions of $Th^{\cdot+}$ were studied by derivative cyclic voltammetry (DCV)[45] and the data were treated according to recently described procedures.[46] The analysis is based upon eqn. (31) which indicates a direct proportionality between the apparent rate

$$k_{app} \sim v_{\frac{1}{2}}/C_A^z C_X^x \qquad (31)$$

constant k_{app} and $v_{\frac{1}{2}}$, the voltage sweep rate necessary for the derivative peak ratio to equal 0.500. The equation refers to reactions of intermediate B generated in reaction (32) and reacting by an unknown mechanism in which X is a reactant and

$$A \pm e^{-} \rightleftarrows B \qquad (32)$$

assumes constant temperature.[46] In eqn. (31) C refers to concentrations, x to the reaction order in X and z is defined by eqn. (33) which gives the

Table 1. Derivative cyclic voltammetry kinetics of the hydroxylation of thianthrene cation radical in acetonitrile.[a]

Run	C_A/mM	C_X/M	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_X^4$	$v_{\frac{1}{2}}/C_X^3$
1	1.00	0.56	4.10	41.7	23.3
2	1.00	0.83	24.6	51.8	43.0
3	1.00	1.11	41.8	27.5	30.6
4	1.00	1.39	81.0	21.7	30.2
5	1.00	1.67	133.8	17.2	28.7
6	0.50	0.56	4.07	41.4	23.2
7	1.00	0.56	4.92	50.0	28.0
8	2.00	0.56	3.24	33.0	18.5
9	2.00	0.83	17.0	35.8	29.7
10	2.00	1.11	43.1	28.4	31.5
11	2.00	1.39	73.9	19.8	27.5
12	2.00	1.67	83.1	10.7	17.8

[a] C_A is the substrate concentration and C_X is the water concentration. All measurements were at 18.2 °C. $E_{sw}-E_{rev}=300$ mV.

$$R_{A/B}=1+z \quad (v_{\frac{1}{2}}/C_A^z=\text{constant}) \qquad (33)$$

combined reaction order in A and B ($R_{A/B}$) assuming only C_A is changed in the series of experiments used to determine $R_{A/B}$. For example, z is 0 for a first order reaction of B following charge transfer (32), 1 for a second order reaction of B, 0 when the reaction order in B is 2 and that for A is -1, *etc.*[46] Throughout this paper, A refers to Th, B to $Th^{\cdot+}$, and X to H_2O or D_2O.

Hydroxylation of $Th^{\cdot+}$ in neutral acetonitrile. The data summarized in Table 1 show the dependence of $v_{\frac{1}{2}}$ on C_A and C_X. The last two columns are tests for reaction orders of 4 and 3, respectively, in H_2O. It is clear from the data that the reaction order in water is dependent upon both C_A and C_X. An increasing trend in $v_{\frac{1}{2}}/C_X^x$ as C_X is increased is indicative that the value of x tested is too low. For example, the best fit for runs 1 and 2 is when x is equal to about 4.5. On the other hand for runs 3–5, $x=3$ gives a constant value of $v_{\frac{1}{2}}/C_X^x$ which indicates that under these conditions the reaction is very nearly third order in water. At a higher value of C_A, data for runs 8–9 indicate that under these conditions x is very close to 4 but the data for runs 10–11 indicate that x is closer to 3. The best fit for the water concentration interval represented by runs 11–12 is for x close to 1. The reaction order in water is obviously a complex function of the reaction conditions.

Runs 6–8 show that $v_{\frac{1}{2}}$ increases in going from C_A equal to 0.5 to 1.0 mM but then declines upon a further doubling in C_A. This indicates that a value of $R_{A/B}$ cannot be assigned which gives a good fit of the data at all three concentrations, the value of z must be positive to be consistent with runs 6 and 7 and negative for runs 7 and 8.

A further complication was observed when DCV experiments were carried out at lower water concentrations. The data in Table 2 show the effect of v on the derivative peak ratio, R_I'. A well-behaved reaction gives data according to relationship (33).[47] In this case no such relationship was found and R_I' was observed to be a minimum at about 0.3 V/s

$$\ln R_I' = m \ln(1/v) + c \qquad (33)$$

Table 2. Evidence for the reversibility of the hydroxylation of thianthrene cation radical in acetonitrile.[a]

v/V s^{-1}	R_I'[b]
0.100	0.817 (0.004)
0.200	0.816 (0.004)
0.300	0.809 (0.003)
0.400	0.818 (0.004)
0.800	0.832 (0.007)
1.000	0.840 (0.005)
10.0	0.947 (0.010)

[a] Measurements on a 1.0 mM solution of thianthrene in the presence of water (278 mM) at 19.3 °C. $E_{sw}-E_{rev}=300$ mV. [b] The ratio of first derivative peaks on the reverse and forward scans of a cyclic voltammogram. The numbers in parentheses refer to standard deviations in five measurements.

Table 3. Deuterium kinetic isotope studies of the reaction of thianthrene cation radical with water in acetonitrile.[a]

Run	X	C_X/M	$v_{\frac{1}{2}}$/V s^{-1}	k_H/k_D
13	H_2O	1.39	37.3	8.9
14	D_2O	1.39	4.2	
15	H_2O	2.09	91.8	9.5
16	D_2O	2.09	9.64	
17	H_2O	2.78	176.8	12.1
18	D_2O	2.78	14.6	

[a] Measurements at 19.4 °C. $E_{sw}-E_{rev}$ = 200 mV. C_A = 1.0 mM.

and to increase with further decreases in v. A similar phenomenon was observed in related studies and found to be due to an overall reversible reaction.[48] This implies that reaction (1) is reversible under the reaction conditions.

The data in Table 3 show that the hydroxylation of $Th^{\cdot+}$ in neutral acetonitrile is subject to an appreciable deuterium kinetic isotope effect ranging from 8.9 at C_X equal to 1.39 M to 12.1 at a water concentration of 2.78 M.

Measurements at a water concentration of 1.39 M resulted in data which gave a constant value of $v_{\frac{1}{2}}/C_A^z$ with $z=0.3$ while in the presence of D_2O (2.78 M) the relationship was satisfied with $z=0.6$ as shown in Table 4. These data indicate that the reaction order in cation radical is greater than 1 and that the reaction is probably inhibited by Th.

Hydroxylation of $Th^{\cdot+}$ in acetonitrile containing TFA. Data from runs 24–26 (Table 5) show that the reaction order in water in this medium follows the same general pattern as in neutral acetonitrile. Runs 24 and 25 give data consistent with $x=4$ while that for runs 25 and 26 taken together is more consistent with $x=3$. Thus, it appears that the reaction order in water is a continually changing function depending strongly on C_X. Comparing $v_{\frac{1}{2}}$ for runs 24–26 with those for runs 1, 3 and 5 (Table 1) results in the conclusion that TFA at a concentration of 9.9 mM has very little effect on the apparent rate of the reaction. The concentration of TFA was successively doubled in runs 26 to 29 with the result that about a 10% decrease in the apparent rate constant was observed with each concentration change.

Hydroxylation of $Th^{\cdot+}$ in acetonitrile containing 2,6-lutidine. The data in Table 6 show that in the presence of 2,6-lutidine (L) (2.2 mM) and water (1.11 M) the apparent rate constant decreases significantly with increases in C_A. When $z=-0.6$,

Table 4. Reaction order analysis of the reaction of the thianthrene cation radical with water in acetonitrile.[a]

Run	X	C_X/M	C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A^{0.3}$	$v_{\frac{1}{2}}/C_A^{0.6}$
19	H_2O	1.39	0.50	28.9	283	–
20	H_2O	1.39	1.00	38.1	303	–
21	H_2O	1.39	2.00	45.1	291	–
22	D_2O	2.78	1.00	14.6	–	921
23	D_2O	2.78	2.00	22.9	–	953

[a] Measurements at 19.4 °C. $E_{sw}-E_{rev}$ = 200 mV.

Table 5. The effect of trifluoroacetic acid on the kinetics of the hydroxylation of thianthrene cation radical in acetonitrile.[a]

Run	C_{TFA}/mM	C_X/M	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_X^4$	$v_{\frac{1}{2}}/C_X^3$
24	9.9	0.56	3.54	36.0	20.2
25	9.9	1.11	51.0	33.6	37.3
26	9.9	1.67	150.4	19.3	32.3
27	19.8	1.67	123.3	–	–
28	39.6	1.67	111.8	–	–
29	79.2	1.67	96.7	–	–

[a] Substrate concentration was 1.00 mM and the temperature was 18.2 °C. $E_{sw}-E_{rev}$ = 300 mV.

Table 6. Reaction order analysis for the hydroxylation of thianthrene cation radical in acetonitrile containing 2,6-lutidine.[a]

Run	C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A^{-0.6}$
30	0.25	20.2	0.139
31	0.50	14.0	0.146
32	1.00	9.13	0.145

[a] In solvent containing water (1.11 M) and 2,6-lutidine (2.2 mM) at 18.2 °C. $E_{sw}-E_{rev}=300$ mV.

Table 7. The reaction order in water during hydroxylation of thianthrene cation radical in acetonitrile containing 2,6-lutidine.[a]

Run	C_L/mM	C_X/M	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_X$
33	10.8	0.28	7.71	27.5
34	10.8	0.56	7.91	14.1
35	10.8	1.11	13.5	12.2
36	2.2	0.28	4.69	16.8
37	2.2	0.56	6.67	11.9
38	2.2	0.83	9.70	11.7
39	2.2	1.11	13.6	12.3
40	2.2	1.67	21.7	13.0

[a] Substrate concentration was 0.50 mM and the measurements were at 18.2 °C. $E_{sw}-E_{rev}=300$ mV.

$v_{\frac{1}{2}}/C_A^z$ was very nearly constant. A comparison of the data for run 32 with that for run 3 (Table 1) shows that $v_{\frac{1}{2}}$ is about a factor of 4 lower in the presence of L. With the exception of that from run 33 the data in Table 7 show that the reaction order in water is very nearly 1 in the presence of L either at a concentration of 2.2 or 10.8 mM.

Hydroxylation of Th$^{\cdot+}$ in buffered acetonitrile. Runs 41–43 demonstrate the effect of substrate concentration on the apparent rate constant for reactions carried out in a buffer solution consisting of $LH^+CF_3CO_2^-$ (3.3 mM) and L (7.5 mM) in solvent containing water (1.11 M). The best data fit was obtained for $v_{\frac{1}{2}}/C_A^z$ when $z=0.75$ (Table 8). In buffer solution containing excess TFA, $v_{\frac{1}{2}}/C_{CF_3CO_2^-}$ was observed to be constant indicating a reaction order of 1 in the anion (Table 9). The data in Table 10 show that either in the presence of H_2O or D_2O $v_{\frac{1}{2}}$ increases nearly linearly with increasing $CF_3CO_2^-$ concentration in buffer with excess L present but decreases when excess TFA is present. A kinetic isotope effect of the order of 2 was observed in the basic buffer (runs 48–51).

The data in Table 11 show the effect on k_H/k_D of successive additions of L to a solution containing TFA originally present at a concentration of 21.8 mM. Before adding L, k_H/k_D was observed to be equal to 3.5 and approached 1 as the concentration of $LH^+CF_3CO_2^-$ was increased by addition of L.

The effect of substrate concentration on the apparent rate constants and the deuterium kinetic isotope effect is demonstrated by the data in Table 12. In the presence of either nucleophile $v_{\frac{1}{2}}/C_A^z$ was very nearly constant when z was 0.83 indicating an apparent value of $R_{A/B}$ of about 1.83. In all cases, the value of k_H/k_D was only slightly greater than 1.

Voltammetric study of the hydroxylation of Th$^{\cdot+}$ in acetonitrile in the presence of pyridine and TFAn. A cyclic voltammogram for the oxidation of Th in acetonitrile containing Bu_4NBF_4 (0.2 M) at a voltage sweep rate of 100 mV/s measured in the

Table 9. The reaction order in trifluoroacetate ion under pseudo second order reaction conditions.[a]

Run	C_L/mM	$v_{\frac{1}{2}}$/V s^{-1}	$(v_{\frac{1}{2}}/C_L)\times 10^{-3}$
44	1.7	16.2	9.53
45	3.4	28.8	8.47
46	5.1	43.9	8.61
47	6.8	62.0	9.12

[a] In solvent containing H_2O (1.11 M) and trifluoroacetic acid (16.3 mM) at 19.4 °C. $E_{sw}-E_{rev}=200$ mV.

Table 8. Reaction order analysis of the hydroxylation of thianthrene cation radical in buffered acetonitrile.[a]

Run	C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$(v_{\frac{1}{2}}/C_A)\times 10^{-3}$	$(v_{\frac{1}{2}}/C_A^{0.75})\times 10^{-3}$
41	0.25	12.5	50.0	6.29
42	0.50	21.3	42.6	6.37
43	1.00	33.6	33.6	5.98

[a] In solvent containing water (1.11 M), 2,6-lutidine (10.8 mM) and trifluoroacetic acid (3.3 mM) at 18.6 °C. $E_{sw}-E_{rev}=200$ mV.

Table 10. Kinetic evidence for the catalysis of the hydroxylation of thianthrene cation radical by trifluoroacetate ion.[a]

Run	X[b]	C_{TFA}/mM	$v_{\frac{1}{2}}$/V s^{-1}	$(v_{\frac{1}{2}}/C_{TFA})\times 10^{-3}$	k_H/k_D
48	H_2O	3.3	83.6	25.3	2.0
49	D_2O	3.3	41.7	12.6	
50	H_2O	6.6	134.4	20.4	2.3
51	D_2O	6.6	58.5	8.9	
52	D_2O	9.9	81.4	8.2	
53	D_2O	13.2	79.2	6.0	
54	D_2O	16.5	67.3	4.1	

[a] In solvent containing 2,6-lutidine (10.8 mM) and thianthrene (0.5 mM). $E_{sw}-E_{rev}=200$ mV. Measurements at 19.4 °C. [b] At a concentration of 1.11 M.

Table 11. Effect of trifluoroacetate ion on the magnitude of the deuterium kinetic isotope effect.[a]

Run	X[b]	C_L/mM	$v_{\frac{1}{2}}$/V s^{-1}	k_H/k_D
55	H_2O	0	20.0	3.5
56	D_2O	0	5.64	
57	H_2O	1.15	47.6	1.7
58	D_2O	1.15	28.0	
59	H_2O	2.3	77.9	1.3
60	D_2O	2.3	58.2	
61	H_2O	4.6	150	1.2
62	D_2O	4.6	125	

[a] In solvent containing trifluoroacetic acid (21.8 mM) and thianthrene (0.5 mM) at 19.4 °C. $E_{sw}-E_{rev}=200$ mV. [b] At a concentration of 1.11 M.

presence of Al_2O_3 is illustrated in Fig. 1a. The voltammogram measured under the same conditions after the addition of pyridine (50 mM) is shown in Fig. 1b. The effect of the addition of pyridine was to bring about an approximate doubling of the oxidation peak current and the complete elimination of current for the reverse process. This behaviour is typical for a rapid ECE type process. The voltammogram illustrated in Fig. 1c is for the same solution after the addition of TFAn (6%). The oxidation peak current in this case was nearly identical to that in 1b but the process appears as a quasi-reversible charge transfer indicating complete chemical reversibility of the processes taking place.

DISCUSSION

Kinetic data for the hydroxylation of thianthrene cation radical are now available under a wide variety of conditions; (i) in acetonitrile or dichloromethane as solvent, (ii) in the presence of trifluoroacetic acid, (iii) in the presence of pyridine and 2,6-lutidine, (iv) in acidic acetonitrile buffers and (v) in basic acetonitrile buffers. Thus, it is not satisfactory to demonstrate a mechanism consistent with data obtained only under one set of experimental conditions. It is highly desirable to

Table 12. A kinetic isotope effect study under pseudo second order reaction conditions.[a]

Run	X[b]	C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$(v_{\frac{1}{2}}/C_A^{0.83})\times 10^{-4}$	k_H/k_D
63	H_2O	0.25	32.5	3.17	1.03
64	D_2O	0.25	31.4	3.07	
65	H_2O	0.50	(70.0)	(3.84)	1.17
66	D_2O	0.50	59.6	3.27	
67	H_2O	1.00	102.9	3.18	1.02
68	D_2O	1.00	101.0	3.12	

[a] In solvent containing trifluoroacetic acid (16.3 mM) and 2,6-lutidine (3.4 mM) at 19.4 °C. $E_{sw}-E_{rev}=200$ mV. [b] At a concentration of 1.11 M.

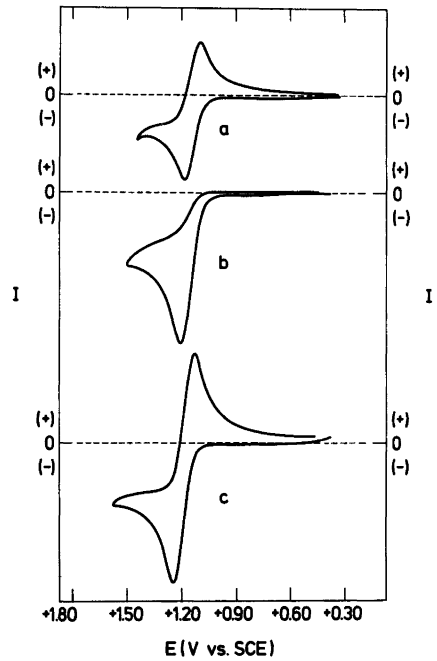


Fig. 1. Cyclic voltammograms of thianthrene in acetonitrile containing Bu_4NBF_4 (0.2 M). a, In the presence of neutral Al_2O_3, b, Solution a plus pyridine (50 mM) and c, Solution b plus TFAn (6 %). Sweep-rate: 100 mV s^{-1}, $t = 22$ °C.

have a mechanistic framework unifying the results from all of the diverse experiments.

The most pertinent results that require explanation are the following: (1) The reaction order in water for reactions conducted in either neutral acetonitrile or in the presence of TFA is varying in the range of 2 – 5 and strongly depends upon the reaction conditions. (2) The reaction order in water is very close to 1 in acetonitrile containing 2,6-lutidine. (3) The reaction order in water is 1 for reactions carried out in CH_2Cl_2 – TFA.[12] (4) The apparent second order rate constant in acetonitrile is inversely proportional to the concentration of strong mineral acid[11] but only slightly affected by TFA. (5) The deuterium kinetic isotope effect for reactions of $Th^{\cdot+}$ with H_2O or D_2O varies from greater than 10 to about 1 and can be related to the reaction order, $R_{A/B}$.[16] (6) In the presence of pyridine and TFAn, cyclic voltammetry shows that Th is involved in a $2e^-$ oxidation process of the ECE type that is chemically reversible, *i.e.* the CV peak current ratio is approximately unity, in contrast to earlier results which indicated rapid and irreversible hydroxylations under the same conditions.[11]

In order to explain the high reaction orders in water when the reaction is carried out in neutral acetonitrile or in the presence of TFA it is tempting to postulate a series of hydration equilibria (34) – (36) which could explain why the reaction order is higher at low water concentrations. A certain degree

$$Th^{\cdot+} + H_2O \overset{K_{34}}{\rightleftharpoons} Th^{\cdot+}(H_2O) \quad (34)$$

$$Th^{\cdot+}(H_2O) + H_2O \overset{K_{35}}{\rightleftharpoons} Th^{\cdot+}(H_2O)_2 \quad (35)$$

$$Th^{\cdot+} + n\,H_2O \overset{K_{36}}{\rightleftharpoons} Th^{\cdot+}(H_2O)_n \quad (36)$$

of hydration, for example a hydration number n as in eqn. (36), might be necessary before bond formation (37) takes place. If the magnitude of the hydration equilibrium constants were such that as

$$Th^{\cdot+}(H_2O)_n \rightleftarrows Th^{\cdot} - OH + H_3O^+ + (n-2)\,H_2O \quad (37)$$

the water concentration is increased the equilibria, one at a time, would be displaced to the right and the reaction order in water could conceivably be n at low concentration when all equilibria are displaced to the left and then decrease by one unit at a time until all equilibria lie to the right and the rate would become independent of $[H_2O]$. The data in Tables 1 – 5 could be incorporated into a mechanism involving the hydration equilibria. However, the hydration equilibria explanation is doomed by the data in Table 7. The presence of 2,6-lutidine in concentrations as low as 2.2 mM has a profound influence on the water reaction order. Under these conditions, the hydroxylation of $Th^{\cdot+}$ is clearly first order in H_2O. It is inconceivable that the low concentrations of 2,6-lutidine could completely inhibit the hydration equilibria (34) – (36).

The effect of the base, 2,6-lutidine, on the reaction kinetics suggests that the complexity of the water reaction order is simply a consequence of acid – base equilibria. In general terms, where the nature of bases B are not specified, the mechanism of the hydroxylation can be formulated as in eqns. (38) – (41). When B is water, for every mol of ThO

$$Th^{\cdot+} + H_2O \overset{K_{38}}{\rightleftharpoons} Th^{\cdot} - \overset{+}{O}H_2 \quad (38)$$

$$Th^{\cdot} - \overset{+}{O}H_2 + B \overset{K_{39}}{\rightleftharpoons} Th^{\cdot} - OH + BH^+ \quad (39)$$

$$Th^{\cdot} - OH + Th^{\cdot +} \underset{k_{-40}}{\overset{k_{40}}{\rightleftharpoons}} Th^{+} - OH + Th \quad (40)$$

$$Th^{+} - OH + B \xrightarrow{k_{41}} ThO + BH^{+} \quad (41)$$

produced, two mol of H_3O^+ are formed which can show their inhibiting influence *via* reaction (39). Under conditions where electron transfer reaction (40) is rate determining[11] a reaction order of 2 in water is predicted by this mechanism in the absence of other complications. However, the observed order is higher and in this work we have shown that it is varying as well. The higher order can be accounted for by equilibrium (42) if we assume that H_3O^+ is a more effective participant

$$H_3O^+ + n\,H_2O \overset{K_{42}}{\rightleftharpoons} H_3O^+(H_2O)_n \quad (42)$$

in reverse reaction (39) than is $H_3O^+(H_2O)_n$.

Mechanism (38)–(41) accounts for the fact that the reaction order in water is 1 in the presence of 2,6-lutidine. In this case B/BH^+ is L/LH^+ and the only participation of water in the hydroxylation is as the nucleophile in reaction (38). Equilibrium (42) is insignificant under these conditions due to very low H_3O^+ concentrations.

The fact that TFA only very slightly inhibits the rate of hydroxylation of $Th^{\cdot +}$ (Table 5) is indicative that equilibrium (43) is displaced to the left and significant concentrations of H_3O^+ do not

$$TFA + H_2O \overset{K_{43}}{\rightleftharpoons} CF_3CO_2^- + H_3O^+ \quad (43)$$

result from the presence of TFA in the solvent system. On the other hand, strong mineral acids do inhibit the reaction and this must be due to H_3O^+ when they are present.

Mechanism (38)–(41) does not provide an explanation of the kinetics of the hydroxylation in CH_2Cl_2–TFA.[12] The most compelling evidence that a different mechanism is involved in the latter solvent system is that the reaction is first order in H_2O. Water must be the strongest base in this solvent and steps (38) and (39) require that the minimum reaction order in H_2O is 2 providing that K_{38} and K_{39} are small. Furthermore, if equilibrium (42) is responsible for the high reaction orders in acetonitrile it would be expected to be as, if not more, important in CH_2Cl_2–TFA.

We have proposed that the mechanism of hydroxylation of $Th^{\cdot +}$ in CH_2Cl_2 is the complexation sequence (44)–(46).[12] As pointed out in the previous paragraph, the mechanism is different in the two solvents, acetonitrile and dichloromethane. The difference in mechanism is readily accounted for

$$Th^{\cdot +} + H_2O \overset{K_{44}}{\rightleftharpoons} (Th/H_2O)^{\cdot +} \quad (44)$$

$$(Th/H_2O)^{\cdot +} + Th^{\cdot +} \underset{k_{-45}}{\overset{k_{45}}{\rightleftharpoons}} (Th/H_2O)^{2+} + Th \quad (45)$$

$$(Th/H_2O)^{2+} \xrightarrow{k_{46}} Th^{+} - \overset{+}{O}H_2 \quad (46)$$

by considering the expected effect of solvent polarity on equilibrium (38). Forward reaction (38) involves the formation of a covalent bond between S and O accompanied by the concentration of charge on the oxygen atom. Since the charge is dispersed in $Th^{\cdot +}$, K_{38} is expected to be strongly solvent dependent and greater the more polar the solvent. Thus, in non-polar CH_2Cl_2, equilibrium (38) may be insignificant compared to (44). Since reaction (44) is simply the formation of a complex it is likely that it precedes equilibrium (38) in the more polar solvent acetonitrile as well. In dichloromethane reactions (45) and (46) can take place leading eventually to ThO. In order for mechanism (44)–(46) to be favourable, $(Th/H_2O)^{\cdot +}$ must be significantly more easily oxidized than $Th^{\cdot +}$ so that the magnitude of K_{45} is appreciably greater than K_2 in this solvent system. The effect of complex formation is expected to be in that direction since charge repulsion in the dication is partially relieved. A related phenomenon is observed for disproportionation equilibria of anion radicals which are profoundly affected by association of dianions with counter ions.[49]

Since TFA does not inhibit the hydroxylation of $Th^{\cdot +}$ in acetonitrile, we concluded that K_{43} is not of sufficient magnitude for equilibrium (43) to produce significant concentrations of H_3O^+. The effect of solvent polarity on equilibrium (43) is expected to be a displacement to the left as the solvent becomes less polar. On this basis we must conclude that equilibrium (43) is even less important in CH_2Cl_2 than in acetonitrile.

The inhibition of the hydroxylation by TFA in dichloromethane is difficult to explain in quantitative terms. The effect of TFA could either be the deactivation of water or the stabilization of $Th^{\cdot +}$ by specific solvation. In this respect it is informative to recall that reversible cyclic voltammograms have been reported in H_2O–TFA (50 vol.-%). In this

system there is a very high mol fraction of water and the role of TFA is not likely to be deactivation of water.[19]

In neutral acetonitrile a large deuterium kinetic isotope effect ($k_H/k_D = 9-12$) was observed (Table 3) under conditions where $R_{A/B}$ was about 1.3 in the presence of H_2O and 1.6 in the presence of D_2O (Table 4). Three rate laws consistent with mechanism (38)–(41) have been shown to be of importance depending upon the relative magnitude of k_{40}, k_{-40} and k_{41}.[16] The rate laws (47)–(49) must be consistent with the observed reaction orders as well

$$\text{Rate} = 2\,k_{41}K_{38}K_{39}K_{40}[\text{Th}^{\cdot+}]^2[\text{H}_2\text{O}][\text{B}]^2/[\text{BH}^+][\text{Th}] \quad (47)$$

$$\text{Rate} = 2\,k_{40}K_{38}K_{39}[\text{Th}^{\cdot+}]^2[\text{H}_2\text{O}][\text{B}]/[\text{BH}^+] \quad (48)$$

$$\text{Rate} = 2\,k_{40}k_{41}K_{38}K_{39}[\text{Th}^{\cdot+}]^2[\text{H}_2\text{O}][\text{B}]^2/[\text{BH}^+](k_{41}[\text{B}]+k_{-40}[\text{Th}]) \quad (49)$$

as the kinetic isotope effects. In neutral or acidic acetonitrile B is most likely water. The large value of k_H/k_D suggests that proton transfers (39) and (41) both contribute to the rate and rate law (49) provides for the non-integral reaction orders. When the nucleophile is H_2O, $R_{A/B}$ is about 1.3 which indicates that the limiting case where reaction (40) can be considered to be in equilibrium and $R_{A/B}$ is 1 is approached. In acidic buffer (Table 12) $R_{A/B}$ was observed to be equal to 1.83 which indicates that this case approximates the condition where the electron transfer reaction (40) is rate determining and that rate law (48) applies. A low value of k_H/k_D was observed in this case, 1.08 ± 0.08. This is also consistent with rate law (48) since in this case the proton transfer takes place in an equilibrium step (39).[50] In basic buffer (Table 8) $R_{A/B}$ is about 1.7 which indicates that the rates of back reaction (40) and reaction (41) are of comparable magnitude and that rate law (49) is approximated by the data measured under these conditions. In this case k_H/k_D is intermediate in value and equal to about 2.2 (Table 10). The observed kinetic isotope effect is once again consistent with the reaction orders and rate law (49) and arises from proton transfer step (41).

It is of interest to observe the effect of trifluoroacetate ion on k_H/k_D in acidic buffer (Table 11). In the absence of $CF_3CO_2^-LH^+$ the observed value was 3.5. As the concentration of the anion is increased k_H/k_D decreases and approaches a value consistent with only an equilibrium isotope effect. This implies that the rate of reaction (41) increases with increasing trifluoroacetate ion and at concentrations of between about 2.3 and 4.6 mM becomes great enough so that it no longer contributes to controlling the rate of the overall reaction. Under conditions where rate law (48) apply (Table 9) the reaction is first order in trifluoroacetate ion. This implies that $CF_3CO_2^-$ is the only base participating in equilibrium (39).

Reactions carried out in the presence of 2,6-lutidine (Tables 6 and 7) are significantly slower than those in either neutral or acidic buffer. The reason for this is apparent from the data in Table 6. Under these conditions $R_{A/B}$ is of the order of 0.4 which indicates that an inhibitor is formed during the reaction. This implies that LH^+ participates in back reaction (39). However, when both 2,6-lutidine and $CF_3CO_2^-LH^+$ are present, $R_{A/B}$ is of the order of 1.75. This means that trifluoroacetate is a more effective base in reaction (39) than is L. It is possible that there is some inhibition by LH^+ in this case as well.

The cyclic voltammograms (Fig. 1) measured for the oxidation of Th in the presence of pyridine indicate that reactions (28) to (30) are fast and irreversible when water is present. When water is effectively removed by reaction with TFAn (25) and the TFA effectively removed by having neutral alumina present in the cell only reactions (28) and (29) take place and are reversible. This clearly shows that the oxidation peak attributed to $\text{Th}^{\cdot}-\text{Pyr}^+$ by Evans and Blount[11] was due to some other process. Apparently under the conditions of their work there was no pyridine present due to protonation by TFA. The peak potential for reaction (50) is slightly positive for the reversible

$$\text{Th} + \text{Pyr} \underset{\longleftarrow}{\overset{E_{50}}{\longrightarrow}} \text{Th}^+ - \text{Pyr}^+ + 2\,\text{e}^- \quad (50)$$

one electron oxidation of Th (8). This implies that the reversible potential for reaction (26) is somewhat but not very much more positive than E_{50} since the latter would fall half-way in between that for the two one electron redox processes. Thus, we can make a reliable estimate of K_{27} to be of the order of 10^{-1}, *i.e.* the interpretation by Evans and Blount[11] of the voltammogram for the oxidation of Th in the presence of pyridine and TFAn led to an error of about 10^6 in K_{27}. Therefore, the mechanism for the hydroxylation of $\text{Th}^{\cdot+}$ in the presence of pyridine proposed by Evans and

Blount[11] requires modification. It is very likely that the kinetic data described in Table II of Ref. 11 refer to the hydroxylation of $Th^{\cdot+}$ in the presence of $CF_3CO_2^-$ $PyrH^+$ and that data are not available for the reaction in the presence of pyridine. Our determination of K_{27} to be 10^{-1} calls for reaction (27) to be very rapid and would be expected to be in equilibrium so that we predict that the reaction follows rate law (51).

$$\text{Rate} = 2\,k_{30}K_{27}K_{28}[Th^{\cdot+}]^2[Pyr][H_2O]/[Th] \quad (51)$$

We have observed quasi-reversible cyclic voltammograms for the oxidation of Th in the presence of $CF_3CO_2^-$ and TFAn, conditions where essentially no water is present, and find that the overall two electron process to give voltammograms very nearly identical to Fig. 1c. This indicates that reaction (52) is reversible under these conditions. This then

$$Th + CF_3CO_2^- \rightleftarrows Th^+ - OCOCF_3 + 2e^- \quad (52)$$

suggests that the mechanism of the hydroxylation of $Th^{\cdot+}$ in the buffers could be described by reactions (53)–(56) and rate law (57). One fact that argues

$$Th^{\cdot+} + CF_3CO_2^- \overset{K_{53}}{\rightleftarrows} Th^{\cdot} - OCOCF_3 \quad (53)$$

$$Th^{\cdot} - OCOCF_3 + Th^{\cdot+} \overset{K_{54}}{\rightleftarrows} Th^+ - OCOCF_3 + Th \quad (54)$$

$$Th^+ - OCOCF_3 + H_2O \underset{k_{-55}}{\overset{k_{55}}{\rightleftarrows}} Th^+ - OH + TFA \quad (55)$$

$$Th^+ - OH + CF_3CO_2^- \xrightarrow{k_{56}} ThO + TFA \quad (56)$$

$$\text{Rate} = 2\,k_{56}K_{53}K_{54}K_{55}[Th^{\cdot+}]^2[CF_3CO_2^-]^2 [H_2O]/[Th][TFA] \quad (57)$$

strongly against this mechanism is that under conditions where electron transfer reaction (54) is rate determining there is no possibility of a deuterium kinetic isotope effect. It therefore appears unlikely that nucleophilic attack by trifluoroacetate (53) significantly contributes to the rate of hydroxylation of $Th^{\cdot+}$.

In conclusion, we point out that in order to establish a mechanism for a reaction as complicated as the hydroxylation of thianthrene cation radical, it is necessary to obtain experimental evidence of various types over a wide range of reaction conditions. In this case, the kinetic isotope effect data was an indispensible aid in the interpretation of kinetic results.

EXPERIMENTAL

The instrumentation, electrodes, cells, data handling procedures and solvent and supporting electrolyte purification were the same as described recently.[51] Thianthrene (Fluka, *purum*), trifluoroacetic acid (Fluka, *purum*), trifluoroacetic anhydride (Fluka, *purum*) and 2,6-lutidine (Fluka, *purum*) were used as received.

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Ring-expansion of Some Sulfur-containing Heterocyclic Compounds with Dimethyl Acetylenedicarboxylate

BO LAMM* and CARL-JOHAN AURELL

Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

A number of *S*-heterocyclic 7-, 8- and 9-membered ring compounds belonging to the benzo[*b*]thiepin, benzo[*b*]thiocin, and benzo[*b*]thionin systems have been prepared through [2+2]cycloaddition of dimethyl acetylenedicarboxylate to enamines, β-keto-ester anions and one β-diketone anion. In the addition to the 5-membered ring compound, 3-hydroxy-2-methoxycarbonylbenzo[*b*]thiophene, a fluorescent by-product has been identified as an α-pyrone besides the main product, a benzo[*b*]thiepin.

In a research program concerning medium-size ring compounds containing sulfur, we have studied the [2+2]-cycloaddition of nucleophilic compounds to dimethyl acetylenedicarboxylate (DMAD), followed by electrocyclic ring opening. This paper describes the access to the benzo[*b*]thiepin, the benzo[*b*]thiocin, and the benzo[*b*]thionin systems *via* such reactions. Compounds belonging to these systems have a seven-, and eight-, or a nine-membered ring containing one sulfur atom and are thus representatives of rather unusual structure types.

Cycloaddition to enamines. Dutch workers have described the cycloaddition of DMAD to 3-(1-pyrrolidino)benzo[*b*]thiophene, followed by ring opening.[1] This leads to a benzo[*b*]thiepin. The next larger ring system is obtained in a similar addition to the pyrrolidine enamine of thiochroman-3-one.[2] A plan to perform the analogous reaction sequence starting with the readily available[3] thiochroman-4-one initially met with difficulty, since the corresponding enamine could not be prepared through azeotropic water removal.[4] The method using a stoichiometric amount of titanium tetrachloride as condensation agent[5] was successful, however. The pyrrolidine enamine reacts with DMAD in tetrahydrofuran solution, and the cyclobutene adduct is stable, at room temperature for at least two weeks. It can be obtained as a viscous liquid in a purity sufficient for spectral characterization through chromatography on neutral alumina. On the contrary, chromatography on silica gel causes hydrolysis to a β-keto ester.

The initial adduct can be thermally rearranged to an eight-membered ring compound by heating at 150 °C for 1 h. The latter compound easily crystallizes. Since silica gel caused formation of the corresponding eight-membered ring β-keto ester, we suspected the cyclobutene–butadiene interconversion to be acid-catalyzed. However, treatment of the initial adduct in tetrahydrofuran solution either with acetic acid or trifluoroacetic acid, with or without water present, gave none of the eight-membered ring compounds; heating alone appears to be the best method. The catalytic action of silica gel is evidently due to other factors than its acidity.

The syntheses are illustrated in Scheme 1. The β-keto ester is drawn as its enol form, which is supported by ^{1}H NMR.

The analogous expansion from a seven- to a nine-membered ring has also been carried out. From the easily available[6] 5-oxo-2,3,4,5-tetrahydrobenzo[*b*]thiepin, the pyrrolidine enamine was prepared.[5] Addition of DMAD in tetrahydrofuran solution gave an 84 % yield of the nine-membered ring compound, 2,3-dihydro-5,6-bis-methoxycarbonyl-7-(1-pyrrolidinyl)benzo[*b*]thionin. In this case, the electrocyclic ring opening is fast enough at

* Author to whom correspondence should be addressed. Present address: AB Hässle, S-431 83 Mölndal, Sweden.

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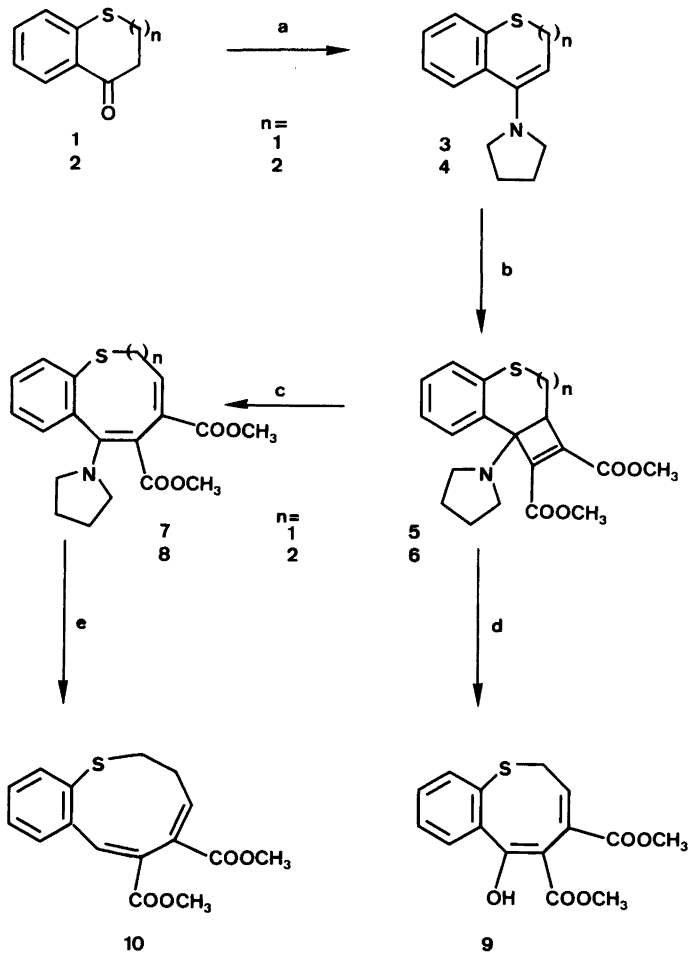


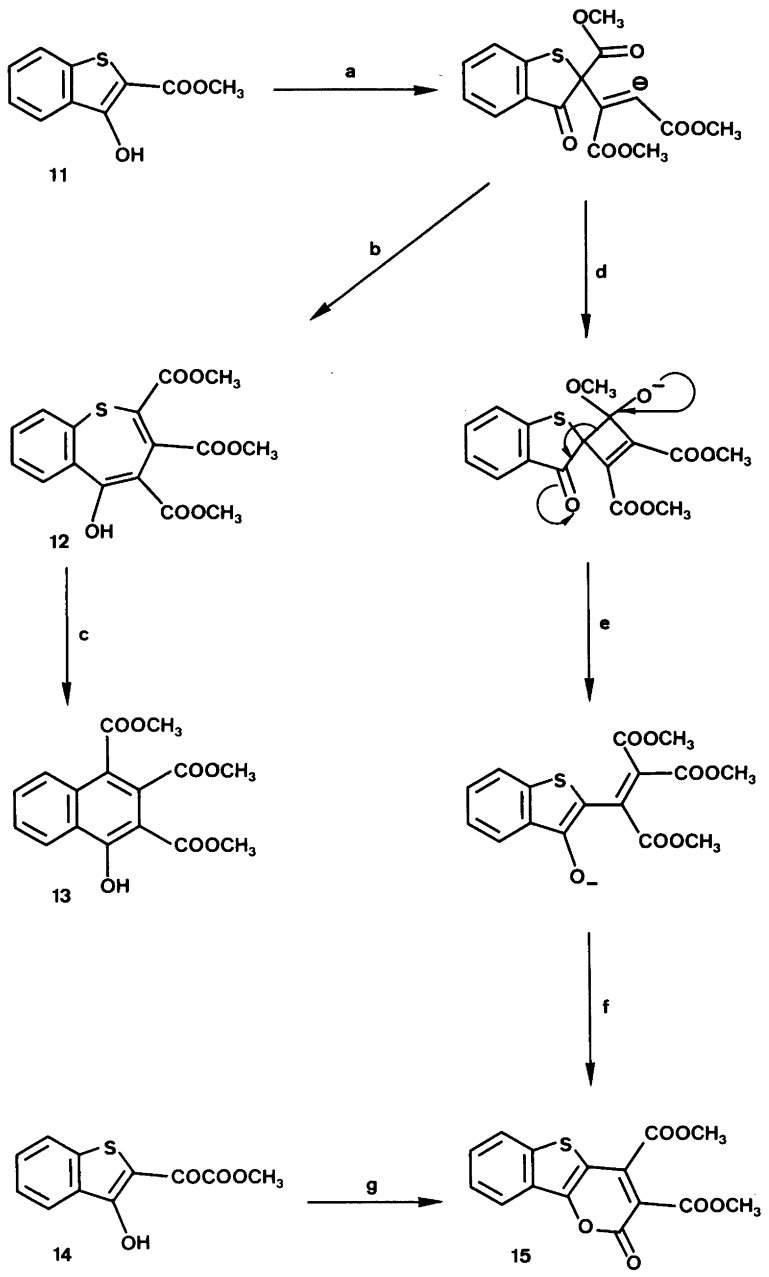
Scheme 1. Cycloaddition to enamines. Conditions: *a* pyrrolidine, $TiCl_4$, *b* dimethyl acetylenedicarboxylate, *c* n=1; 150 °C, 1 h, n=2; room temp., *d* silica gel chromatography; *e* B_2H_6.

or below room temperature to prevent the observation of the initial cyclobutene adduct.

Upon treatment with diborane, cyclic enamine esters undergo reductive C–N bond cleavage to yield unsaturated esters.[7] This reaction, when applied to the above enamine ester, gave the expected 2,3-dihydro-5,6-bis-methoxycarbonylbenzo[*b*]thionin in 72% yield. See Scheme 1.

Cycloaddition to β-ketoester anions. DMAD undergoes cycloaddition to the conjugate bases of carbocyclic β-keto esters[8] and their *N*-heterocyclic analogues.[9] We have now extended this reaction to a five- and a six-membered ring S-heterocyclic β-keto ester, respectively, and the expected seven- and eight-membered rings have been obtained.

In the reaction of the five-membered ring compound, 3-hydroxy-2-methoxycarbonylbenzo[*b*]-thiophene, a by-product was formed in *ca.* 1% yield. It was detected because of its fluorescence, which also aided in the separation from the main product, 5-hydroxy-2,3,4-tris-methoxycarbonylbenzo[*b*]thiepin. High-resolution MS gave for the by-product the molecular formula $C_{15}H_{10}O_6S$. The ^1H NMR spectrum shows four aromatic H atoms and two CH_3O groups. Of two reasonable isomeric structures accounting for these data, one is denoted *15* and is shown in Scheme 2. Its rational name is 3,4-bis-methoxycarbonyl-2-oxo-2*H*[1]-



Scheme 2. Reactions of 3-hydroxy-2-methoxycarbonylbenzo[*b*]thiophene. Conditions: *a*, *b* NaH, then dimethyl acetylenedicarboxylate followed by acid work-up, *c* 180 °C, 1 h, *a,d,e,f* possible route to lactone, *g* $ClCOCH_2COOCH_3$, triethylamine.

benzothieno[3,2-*b*]pyran. An analogous lactone has been obtained as one of the products from addition of DMAD to 1,2-dihydro-2-ethoxycarbonyl-1-methylindol-3-one.[9] A mechanism involving a spirocyclic intermediate has been suggested,[9] for the formation of the lactone, and the same mechanism may be applied to our case; see Scheme 2.

It is also possible to envisage a route leading to 2,3-bis-methoxycarbonyl-4-oxo-4*H*-[1]benzothieno[3,2-*b*]pyran. However, this isomer is an α,β-unsaturated ketone (a γ-pyrone) and would not be expected to be fluorescent, as was pointed out by Dr. H.-D. Becker. In fact, a compound containing a 2,3-dicarboxyl-substituted γ-pyrone ring annealed to a benzene ring, 2-ethoxycarbonyl-4-oxochromen-3-carboxylic acid, is known,[10] and no mention of fluorescence is made. An independent synthesis of *15* from a glyoxylic ester was carried out (Scheme 2), and the properties of the material thus obtained were identical with those of the by-product. Structure *15* is therefore ascertained.

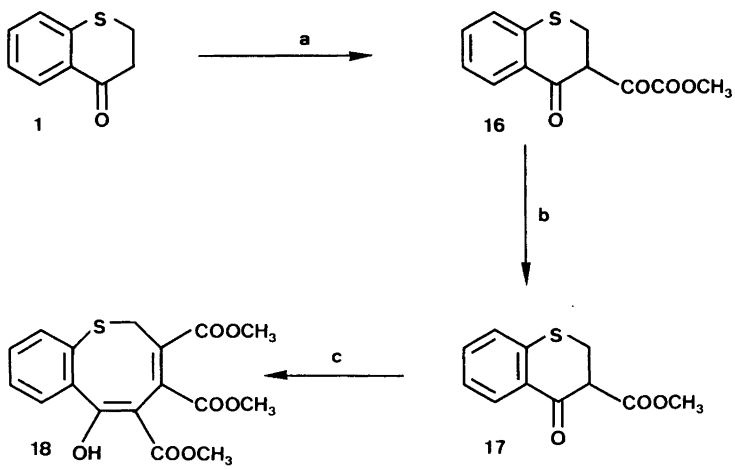
The benzo[*b*]thiepin compound is colourless and stable at room temperature. Like known analogues,[1,11] it suffers extrusion of sulfur upon heating, yielding 2,3,4-tris-methoxycarbonyl-1-naphthol.

The cycloaddition of DMAD to the anion of 3-methoxycarbonylthiochroman-4-one is illustrated in Scheme 3 together with the synthesis of the keto ester. The latter was obtained *via* decarbonylation after condensation of thiochroman-4-one with dimethyl oxalate.[12] The attempted preparation of 3-methoxycarbonylthiochroman-4-one according to a different literature procedure[13] failed completely. The latter route involves Dieckmann cyclization of methyl 2-(2-cyanoethyl)thiobenzoate, but we found that this compound upon treatment with base as described[13] instead undergoes retro-cyanoethylation. Methyl 2-mercaptobenzoate was the only aromatic compound obtained. The conclusions in Ref. 13 were based on IR, but not NMR, spectra and must be regarded with the utmost suspicion.

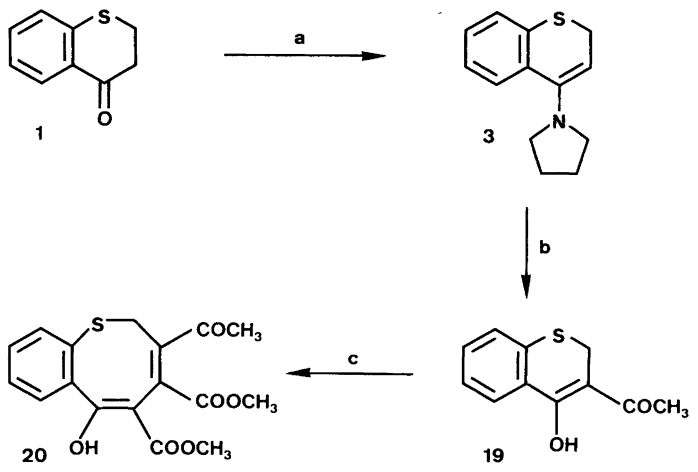
Cycloaddition to a β-diketone anion. Like β-keto esters, β-diketones might be expected to add DMAD *via* their conjugate bases. As one representative example, 3-acetylthiochroman-4-one was chosen. This diketone was easily prepared through acylation of an enamine. According to ^{1}H NMR, it is completely enolic and is more adequately named 3-acetyl-4-hydroxy-2*H*-thiochromene. Its conjugate base, generated with sodium hydride, reacts with DMAD to form, after ring opening, 3-acetyl-6-hydroxy-4,5-bis-methoxycarbonyl-2*H*-benzo[*b*]-thiocin. The synthesis is illustrated in Scheme 4.

EXPERIMENTAL

General comments. Most syntheses were performed at least twice, and the yields were reproducible. Reactions involving air- or moisture-sensitive compounds were routinely performed under nitrogen using magnetic stirring. The term "usual work-up" means distribution of the reaction mixture



Scheme 3. Synthesis of 6-hydroxy-3,4,5-tris-methoxycarbonyl-2*H*-benzo[*b*]thiocin. Conditions: *a* dimethyl oxalate, CH_3ONa, *b* 180 °C, 30 min, *c* dimethyl acetylenedicarboxylate.



Scheme 4. Synthesis of 3-acetyl-6-hydroxy-4,5-bis-methoxycarbonyl-2*H*-benzo[*b*]thiocin. Conditions: *a* pyrrolidine, $TiCl_4$, *b* acetyl bromide, *c* NaH, then dimethyl acetylenedicarboxylate followed by acid work-up.

between water and an appropriate solvent, in most cases, dichloromethane, washing the organic phase with water followed by brine, drying over magnesium sulfate or sodium sulfate and removal of the solvent at aspirator pressure. Solids were recrystallized from solvents specified under each compound. A technique often successful in removing coloured impurities is filtration through a short silica gel column. Solvents used in ester condensation, enamine formation and cycloaddition reactions were dried before use.

Abbreviations: Dimethyl acetylenedicarboxylate DMAD, tetrahydrofuran, THF.

Instruments for spectrometry: Bruker WH 270 FT NMR, and AEI MS 902 mass spectrometer. Melting points have been determined with a Kofler Hot Stage microscope. Elemental analyses were carried out by Novo Microanalytical Laboratory, Bagsvaerd, Denmark.

Thiochroman-4-one (1) and 5-oxo-2,3,4,5-tetrahydrobenzo[b]*thiepin (2)* were prepared according to literature procedures.[3,6]

4-(1-Pyrrolidinyl)-2H-thiochromene (3). To a solution of 16.4 g (0.1 mol) of *1* and 42.5 g (0.6 mol) of pyrrolidine in 50 ml of benzene and 50 ml of hexane, 10 g (52 mmol) of titanium tetrachloride was added dropwise at −10 °C. The resulting light brown suspension was stirred for 24 h at room temperature, filtered, and evaporated. The residue, a semi-crystalline mass, was used directly in the following steps. Yield 17.4 g (80 %), ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.80–1.92 (4 H, m), 2.86–2.96 (4 H, m), 3.27 (2 H, d, *J* 6 Hz), 5.17 (1 H, t, *J* 6 Hz), 7.07–7.48 (4 H, m).

2,3-Dihydro-5-(1-pyrrolidinyl)benzo[b]*thiepin (4).* A mixture of 60 g (0.34 mol) of *2*, 78.5 g (1.1 mol) of pyrrolidine, 100 ml of benzene, and 100 ml of hexane was cooled to −10 °C. At this temperature, 32.5 g (0.17 mol) of titanium tetrachloride was added dropwise. After 48 h, the reaction mixture was filtered and the liquid part evaporated at reduced pressure. The residue, a yellowish-red oil, was pure enough for the following steps. Yield 63 g (81 %), ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.82–1.87 (4 H, m), 1.99–2.05 (2 H, m), 2.90–2.95 (4 H, m), 3.24–3.29 (2 H, m), 4.97 (1 H, t, *J* 8 Hz), 7.17–7.57 (4 H, m).

7,8-Bis-methoxycarbonyl-6-(1-pyrrolidinyl)-3-thia-4,5-benzobicyclo[4.2.0]*octa-4,7-diene (5).* To a solution of 21.7 g (0.1 mol) of *3* in 70 ml of THF was added during 1 h a solution of 14.2 g (0.1 mol) of THF was added during 1 h a solution of 14.2 g (0.1 mol) of DMAD in 20 ml of THF. The addition took place at 0 °C, and the mixture was left for 48 h at room temperature. After evaporation of the solvent at reduced pressure, the resulting dark oil was chromatographed on neutral alumina with dichloromethane as the eluent. The main fraction was evaporated to give a reddish oil which was essentially pure according to NMR. Keeping at room temperature for two weeks provoked no change. The yield was 22.3 g (62 %). ^{1}H NMR (270 MHz, $CDCl_3$); δ 1.78–1.83 (4 H, m), 2.53–2.62 (2 H, m), 2.73 (1 H, dd, *J* 13 and 3 Hz), 2.72–2.80 (2.80 (2 H, m), 3.21 (1 H, dd, *J* 13 and 3 Hz), 3.63 (3 H, s), 3.73 (3 H, t, *J* 3 Hz), 3.81 (3 H, s), 7.06–7.24 (3 H, m), 8.11–8.16 (1 H, m).

4,5-Bis-methoxycarbonyl-6-(1-pyrrolidinyl)-2H-benzo [b]*thiocin (7).* The purified oil *5*, 22.3 g, was

kept at 150 °C for 1 h and allowed to cool. Crystals were obtained through trituration with ether. They were separated and dissolved in dichloromethane. Filtration through a short silica gel column removed some impurities, and upon evaporation of the eluate, colourless crystals, 18 g (50 % from 3), were obtained, m.p. 143–145 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.77–2.00 (4 H, m), 2.93–3.03 (2 H, m), 3.20–3.40 (2 H, m), 3.39 (1 H, dd, *J* 10 and 7 Hz), 3.61 (3 H, s), 3.62 (3 H, s), 3.88 (1 H, t, *J* 10 Hz), 6.26 (1 H, dd, *J* 10 and 7 Hz), 7.09–7.27 (4 H, m). Anal. $C_{19}H_{21}NO_4S$: C, H, S.

2,3-Dihydro-5,6-bis-methoxycarbonyl-7-(1-pyrrolidinyl)-benzo[b]*thionin* (8). To a solution of 21 g (90 mmol) of *4* in 175 ml of THF was added a solution of 21 g (90 mmol) of *4* in 175 ml of THF was added a solution of 14.2 g (0.1 mol) of DMAD in 15 ml of THF at 0 °C during 1 h. After 36 h, the solution was evaporated to give a greenish, crystalline residue. After the same purification as for 7, colourless crystals were obtained, 28.4 g (84 %), m.p. 170–172 °C, ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.81–1.95 (4 H, m), 2.49–2.63 (2 H, m), 2.93–3.23 (4 H, m), 3.39 (3 H, s), 3.37–3.51 (2 H, m), 3.64 (3 H, s), 6.78 (1 H, dd, *J* 11.5 and 4 Hz), 7.08–7.12 (1 H, m), 7.21–7.25 (2 H, m), 7.51–7.55 (1 H, m). Anal. $C_{20}H_{23}NO_4S$: C, H, S.

*6-Hydroxy-4,5-bis-methoxycarbonyl-2*H*-benzo*[b]*thiocin* (9). To a solution of 21.7 g (0.1 mol) of *3* in 70 ml of toluene was added a solution of 14.2 g (0.1 mol) of DMAD in 30 ml of toluene at 0 °C during 3 h. After 48 h at room temperature, 25 ml of acetic acid was added, and the reaction mixture was left overnight. The solvent was removed at reduced pressure and the residue was dissolved in a mixture of dichloromethane, acetic acid and water. Enough silica gel was added to produce a thick slurry, which was left for 48 h. After filtration and evaporation, the residue was chromatographed (silica gel – dichloromethane) to give 11.3 g (40 %) of colourless crystals, m.p. 100–101 °C, ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.78 (1 H, dd, *J* 17.2 and 6.9 Hz), 3.19 (1 H, dd, *J* 17.2 and 6.9 Hz), 3.66 (3 H, s), 3.70 (3 H, s), 4.34 (1 H, t, *J* 6.9 Hz), 7.52–7.61 (3 H, m), 7.89 (1 H, s), 8.52–8.56 (1 H, m). Anal. $C_{15}H_{14}O_5S$: C, H, S.

2,3-Dihydro-5,6-bis-methoxycarbonylbenzo[b]*thionin* (10). A solution of 11.2 g (30 mmol) of *8* in 100 ml of THF was cooled to 0 °C. Gaseous diborane (100 mmol), generated *ex situ*,[14] was introduced. After 20 h, the reaction mixture was evaporated to dryness. Ethyl acetate, 50 ml, was added. The solution was filtered, evaporated and chromatographed on silica gel, first with dichloromethane, later with dichloromethane – methyl acetate 9:1 as the eluent. After evaporation of the main fraction and trituration with ether, 6.8 g (72 %) of colourless crystals were obtained, m.p. 132–134 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.62–3.01 (4 H, m), 3.64 (3 H, s), 3.84 (3 H, s), 6.75 (1 H, t, *J* 8 Hz), 6.91–6.97 (1 H, m), 7.20–7.26 (2 H, m), 7.55–7.59 (1 H, m), 8.13 (1 H, s). Anal. $C_{16}H_{16}O_4S$: C, H, S.

3-Hydroxy-2-methoxycarbonylbenzo[b]*thiophene* (11) was prepared according to the literature.[15]

5-Hydroxy-2,3,4-tris-methoxycarbonylbenzo[b]*thiepin* (12). Sodium hydride dispersion, 2.1 g (45 mmol NaH), was washed with hexane and suspended in 50 ml of toluene, and 8 g (40 mmol) of *11* was added. After the hydrogen evolution had ceased, 5.7 g (40 mmol) of DMAD in 20 ml of toluene was added during 1 h at 0 °C. After 3 days at room temperature, acetic acid was carefully added until the suspension had dissolved. Usual work-up gave a partially crystallized mass. The crystals, consisting of starting material, 1.4 g, were removed and the remainder chromatographed on silica gel with toluene as the eluent. The main fraction was contaminated with a strongly fluorescent (yellowish-green) compound, which could be removed through its very low solubility in methanol. The main product was obtained as colourless crystals, 3.7 g (41 %), m.p. 123–125 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.68 (3 H, s), 3.80 (3 H, s), 3.82 (3 H, s), 7.42–7.46 (3 H, m), 7.80–7.84 (1 H, m), 13.69 (1 H, s). Anal. $C_{16}H_{14}O_7S$: C, H, S.

2,3,4-Tris-methoxycarbonyl-1-naphthol (13). In a sealed glass ampoule, 0.3 g of 12 was heated at 180 °C for 1 h. A yellowish, crystalline mass was formed upon cooling. Recrystallization gave a quantitative yield of colourless crystals, m.p. 94–96 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.90 (3 H, s), 3.95 (3 H, s), 3.96 (3 H, s), 7.59–7.75 (2 H, m), 8.13–8.18 (1 H, m), 8.45–8.50 (1 H, m), 12.40 (1 H, s). MS [IP 34 eV; *m/e* (% rel. int.)]: 318 (38.8), 287 (31), 286 (100), 241 (8.3), 228 (25), 227 (10.8), 198 (19.2), 171 (11.1), 170 (70.4). Mol. wt., obs. 318.0723, calc. for $C_{16}H_{14}O_7$ 318.0740.

3-Hydroxy-2-methoxalylbenzo[b]*thiophene* (14). To a solution of 7.5 g (50 mmol) of 3-hydroxybenzo[*b*]thiophene[16] and 8.3 g (70 mmol) of dimethyl oxalate in 100 ml of methanol was added a solution of 50 mmol of sodium methoxide (from 1.15 g of sodium) in 100 ml of methanol. The mixture turned dark red within seconds. After a 10 min reflux period, a solution of 5 ml of conc. hydrochloric acid in 50 ml of methanol was added to the cold reaction mixture. A brick-red precipitate formed, which was filtered off and recrystallized from 250 ml of methanol – acetone 5:1. The hot solution was filtered to remove a dark-coloured, amorphous impurity. Orange, slender needles were obtained, m.p. 137–138 °C, 4.15 g (35 %). ^{1}H NMR (270 MHz, $CDCl_3$): δ 4.02 (3 H, s), 7.38–8.04 (4 H, m), 12.67 (1 H, s). Anal. $C_{11}H_8O_4S$: C, H, S.

*3,4-Bis-methoxycarbonyl-2-oxo-2*H*-[1]benzothieno*[3,2-b]*-pyran* (15). *By-product in the synthesis*

of 12: The fluorescent material virtually insoluble in methanol amounted to 100 mg, m.p. 226–227 °C. Attempted ^{13}C NMR failed because of insufficient solubility in a number of solvents including DMSO-d_6. ^1H NMR (270 MHz, $CDCl_3$): δ 3.98 (3 H, s), 4.04 (3 H, s), 7.46–7.61 (2 H, m), 7.83–7.86 (1 H, m), 8.06–8.09 (1 H, m). MS [IP 50 eV; *m/e* (% rel. int.)]: 318 (100), 290 (28.3), 287 (22.6), 259 (14.3), 173 (23), 144 (13.6), 104 (16). Mol. wt., obs. 318.0167, calc. for $C_{15}H_{10}O_6S$ 318.0198.

Independent synthesis of 15 *from* 14: A solution of 4 g (17 mmol) of *14* and 2 g (20 mmol) of triethylamine in the minimum amount of benzene was treated with small portions of methyl chloroformyl acetate (prepared from the potassium salt of malonic acid monomethyl ester and thionyl chloride[17]) until a 20 % excess had been added. Crystals were immediately formed in the reaction mixture. The solvent was removed at reduced pressure and the triethylamine hydrochloride was washed away with water. The remainder, yellow crystals, amounted to 3 g (56 %). The ^1H NMR spectrum was identical with that of the by-product described above. M.p. and mixed m.p. 226–227 °C.

3-Methoxalylthiochroman-4-one (16) and *3-methoxycarbonylthiochroman-4-one* (17) were prepared according to the literature.[12]

6-Hydroxy-3,4,5-tris-methoxycarbonyl-2H-benzo[b]thiocin (18). Sodium hydride dispersion, 1.7 g (35 mmol NaH), was washed with hexane and suspended in 50 ml of toluene. To this mixture was added 6.44 g (29 mmol) of *17* in 25 ml of toluene. After the hydrogen evolution had ceased, 4.12 g (29 mmol) of DMAD in 15 ml of toluene was added at 0 °C during 1 h. After 24 h at room temperature, acetic acid, 10 ml, was carefully added, followed by 60 ml of 2 M hydrochloric acid and 100 ml of toluene. After usual work-up, the crude product was recrystallized from methanol to yield 7.81 g (74 %) of colourless crystals, m.p. 146–150 °C with decomposition. ^1H NMR (270 MHz, $CDCl_3$): δ 3.55 (1 H, d, *J* 11.2 Hz), 3.63 (3 H, s), 3.71 (3 H, s), 3.79 (3 H, s), 4.20 (1 H, d, *J* 11.2 Hz), 7.25–7.30 (4 H, m), 12.94 (1 H, s). Anal. $C_{17}H_{16}O_7S$: C, H, S.

3-Acetyl-4-hydroxy-2H-thiochromene (19). To a solution of 34.7 g (0.16 mol) of *3* and 16.2 g (0.16 mol) of triethylamine in 100 ml of chloroform, a solution of 19.7 g (0.16 mol) of acetyl bromide in 50 ml of chloroform was added during 30 min. The mixture was refluxed for 2 h. Water, 60 ml, and conc. hydrochloric acid, 60 ml, were added, and refluxing was continued for another 2 h. The cooled organic phase was washed with water and extracted 3 times with 1 M potassium hydroxide. The alkaline solution was acidified with hydrochloric acid and extracted with chloroform. After drying, filtration and evaporation of the solvent, the remainder was recrystallized from methanol to yield 13.2 g (40 %) of yellow needles, m.p. 82–84 °C. ^1H NMR (270 MHz, $CDCl_3$): δ 2.26 (3 H, s), 3.72 (2 H, s), 7.18–7.28 (3 H, m), 7.94–7.97 (1 H, m), 16.52 (1 H, s). Anal. $C_{11}H_{10}O_2S$: C, H, S.

3-Acetyl-6-hydroxy-4,5-bis-methoxycarbonyl-2H-benzo[b]thiocin (20). Sodium hydride dispersion, 3.1 g (60 mmol NaH), was washed with hexane and suspended in 80 ml of toluene. Of *19*, 10.3 g (50 mmol) was added in portions. When the hydrogen evolution had ceased, 7.1 g (50 mmol) of DMAD was added at 0 °C during 2 h. After 24 h at room temperature, 20 ml of acetic acid was carefully added, followed by 100 ml of 2 M hydrochloric acid and 200 ml of toluene. After the usual work-up, recrystallization from methanol gave 13.2 g (76 %) of colourless crystals, m.p. 124–128 °C. ^1H NMR (270 MHz, $CDCl_3$): δ 2.07 (3 H, s), 3.36 (1 H, d, *J* 11 Hz), 3.64 (3 H, s), 3.78 (3 H, s), 4.21 (1 H, d, *J* 11 Hz), 7.31–7.35 (4 H, m), 12.87 (1 H, s). Anal. $C_{17}H_{16}O_6S$: C, H, S.

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Tobacco Chemistry. 56.* The Stereochemistries of the Tobacco Diterpenoids: The (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)- and (1*S*,2*E*,4*R*,6*E*,8*S*,11*E*)-2,6,11-Cembratriene-4,8-diols. Acid-induced Transformations of Cembratrienediols

INGER WAHLBERG,[a] DAN BEHR,[a] ANN-MARIE EKLUND,[a] TOSHIAKI NISHIDA,[a] CURT R. ENZELL[a] and JAN-ERIC BERG[b]

[a] Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62 Stockholm, Sweden and [b] Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The stereochemistries of the (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)- and (1*S*,2*E*,4*R*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diols (*1*,*2*) have been determined by X-ray analyses.

Acid-induced transformations of the 4,8-diols (*1*, *2*) and 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 (*3*,*4*) have been studied. The results show that the 4*S*,6*R*- and 4*R*,6*R*-diols (*3*, *4*) are interconvertible with the 4*S*,8*S*- and 4*S*,8*R*-diols (*1*, *7*) and with the 4*R*,8*S*-diol (*2*), respectively. The 4,6-diols (*3*,*4*) also undergo epimerization reactions and a fragmentation reaction yielding a *seco*-aldehyde (*6*).

The pioneer studies on the tobacco cembranoids carried out in the 1960:s led to the isolation of the two major components,[2] which were later fully identified as 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 (*3*, *4*).[1,3,4] A series of minor components, which included *inter alia* two diastereoisomers of 2,6,11-cembratriene-4,8-diol (*1*, *2*), was also found to be present.[5] Although results obtained later have shown that the two 4,8-diols (*1*, *2*) have (1*S*,2*E*,4*S*,11*E*)- and (1*S*,2*E*,-4*R*,11*E*)-configurations, respectively,[4] the geometries of their 6,7 double bonds and their chiralities at C-8 have remained unknown.

Our recent isolation of these two 4,8-diols (*1*, *2*) from a wax extract of green leaves of Greek tobacco has encouraged studies on their stereostructures and their generation *via* acid-induced rearrangements of the 4,6-diols *3* and *4*.

RESULTS

Stereochemistry. A detailed analysis of the ^{1}H NMR spectra using spin decoupling and spin simulation techniques revealed that the 6,7 as well as the 2,3 double bonds in the two 4,8-diols (*1*, *2*) have *E*-geometries ($J_{6,7}=16.1$ Hz, $J_{2,3}=16.0$ Hz for *1* and $J_{6,7}=16.4$ Hz, $J_{2,3}=15.7$ Hz for *2*). However, since conclusive evidence for the C-8 configurations was not obtainable from the ^{1}H and ^{13}C NMR spectra, X-ray analyses were carried out on the 4*S*,8-diol (*1*) and the epoxide (*5*) derived from the 4*R*,8-diol (*2*).

Diol *1* forms orthorhombic crystals of the monoclinic space group $P2_1$. The crystal data, obtained on a Philips PW 1100 diffractometer, were: $a=9.492$, $b=11.305$ and $c=10.577$ Å, $\beta=119.18°$, $Z=2$. The present *R*-value including thermal parameters for all non-hydrogen atoms is 0.183; location of the hydrogen atoms and further refinement being under way.[6] A stereoscopic view, which summarizes the *X*-ray results and demonstrates that diol *1* is (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol, is shown in Fig. 1.

Epoxide *5* crystallizes in the orthorhombic space group $P2_1$ with $a=12.479$, $b=8.483$, $c=9.580$ Å, $\beta=110.92°$, $Z=2$. The structure, shown in Fig. 2,

*For part 55 see Ref. 1.

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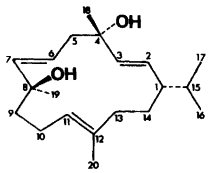

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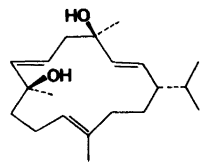

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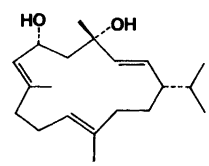

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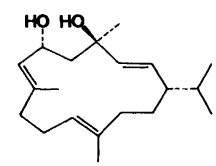

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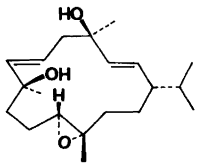

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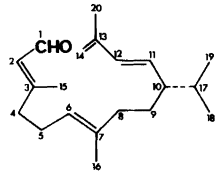

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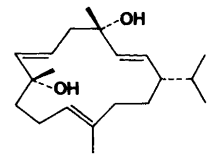

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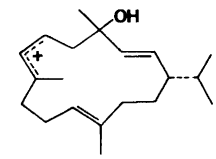


8a 4*R*
8b 4*S*

has so far been refined to an *R*-value of 0.093 with anisotropic thermal parameters assigned for all non-hydrogen atoms.[6] The analysis shows that epoxide *5* is (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol and hence that diol *2* has an *S*-configuration at C-8.

Acid-induced transformations. A plausible biogenetic route to the 4*S*,8*S*- and 4*R*,8*S*-diols (*1*, *2*) would involve allylic rearrangements of the 4*S*,6*R*- and 4*R*,6*R*-diols (*3*, *4*), respectively. Roberts and

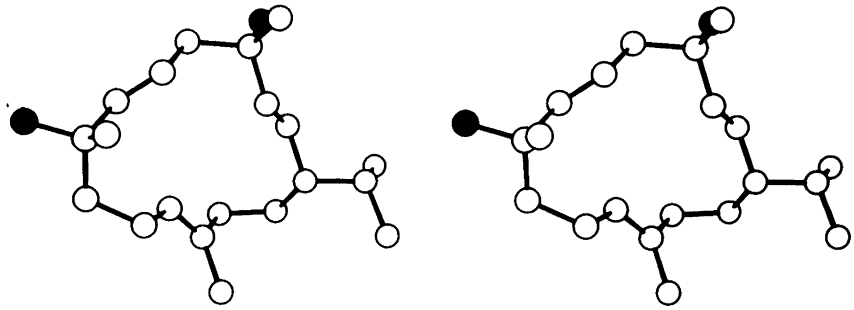


Fig. 1. Stereoscopic view of (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*1*).

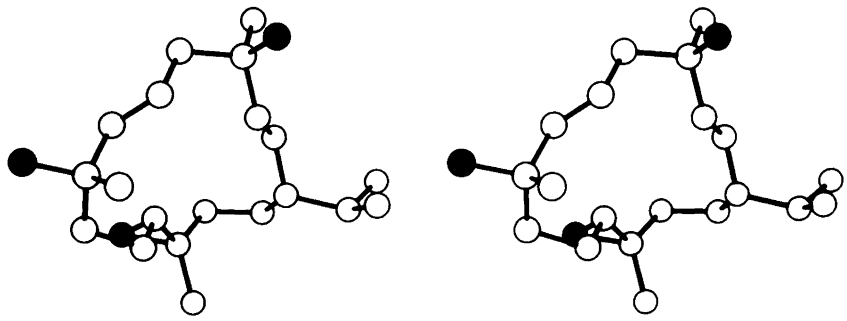


Fig. 2. Stereoscopic view of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*5*).

Table 1. Carbon-13 chemical shifts and assignments for compounds *1*, *2*, *6* and *7*.[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	47.4	128.2	138.4	73.1[c]	47.5	123.2	140.4	73.4[c]	42.3	22.8	126.5	132.6	37.5	27.9	33.1	20.6	19.6	29.8	28.2	14.6
2	47.2	130.0	137.4	72.7[c]	47.1	123.6	140.3	73.3[c]	42.9	22.7	126.9	132.2	37.2	27.4	32.8	20.3	19.7	27.3[d]	27.4[d]	14.4
6	193.4	128.4	166.8	41.6	26.7	124.0	137.6	38.7	31.8	50.3	135.5	133.7	143.3	114.8	17.7	16.2	33.3	21.2	19.6	19.0
7	47.3[b]	127.2	138.8	73.4[c]	48.0[b]	121.7	140.8	73.3[c]	42.1	22.2	126.9	132.0	37.3	27.7	32.9	20.7	19.6	30.0[d]	29.3[d]	14.3

[a] δ-Values in $CDCl_3$ (*1*, *2* and *7*) or CD_3OD (*6*) relative to TMS. [b,c,d] Assignments may be reversed.

Rowland carried out these conversions synthetically simply by subjecting each of the 4,6-diols (*3*,*4*) to slow chromatography over acidic alumina and obtained a 20 % yield of the corresponding 4,8-diol (*1* and *2*, respectively).[5]

In our hands, treatment of the 4*S*,6*R*-diol (*3*) with dilute sulfuric acid in dioxane – water for 6 h afforded, in addition to starting material (*3*, 24 % isolated yield), five major products, which were isolated. Four of these were identified as the 4*S*,8*S*- and 4*R*,8*S*-diols (*1*, *2*; 15 % and 10 %), the 4*R*, 6*R*-diol (*4*; 12 %) and 10-isopropyl-3,7,13-trimethyl-2,6,11,13-tetradecatetraen-1-al (*6*; 5 %).[7] The fifth product was assigned the structure (1*S*,2*E*,4*S*,6*E*,-8*R*,11*E*)-2,6,11-cembratriene-4,8-diol (*7*; 3 %) on the basis of the following evidence.

The ^{1}H NMR spectrum displayed signals due to two methyl groups on fully substituted, oxygen-carrying carbon atoms and one vinylic methyl group, which were ascribed to H-18, H-19 and H-20, respectively. An analysis of the olefinic region by spin decoupling and spin simulation methods established the presence of the disubstituted 2,3 and 6,7 double bonds and revealed that these have *E*-geometries, $J_{2,3}=15.4$ Hz and $J_{6,7}=14.0$ Hz.

The assignment of an *E*-stereochemistry to the trisubstituted 11,12 double bond rests on a comparison, which showed that the C-9 to C-14 and C-20 signals were present at virtually invariant positions in the ^{13}C NMR spectra of diols *1*, *2* and *7* (*cf.* Table 1). ^{13}C NMR results were also used to determine the stereochemistry at C-4 and C-8. Thus, the chemical shift values of the C-2 and C-18 signals, δ 127.2 and 30.0, were consistent with a 4*S*-configuration, whereas the shieldings of C-6 and C-19, δ 121.7 and 29.3 for diol 7 as against 123.2 – 123.6 and 28.0 – 27.4 for diols *1* and *2*, indicated that the configuration at C-8 in diol 7 is *R*.

Treatment of the 4*R*,6*R*-diol (*4*) with sulfuric acid in dioxane – water for 6 h, yielded in addition to starting material (*4*, 10 %) again five major products: *1*, *2*, *3*, *6* and *7*; 11, 26, 14, 6 and 2 %, respectively.

It is evident, therefore, that treatment of a 4,6-diol (*3*, *4*) with weak acid occurs with participation of several competing reactions. In order to gain some insight into these, each of the 4,6-diols (*3*, *4*) was again treated with sulfuric acid in dioxane – water. Aliquots were taken after various reaction times and analyzed by HPLC. The results obtained are summarized in Table 2.

It follows that in the interconversions of the 4,6-

Table 2. Relative yields, as determined by integration of HPLC traces, of the products obtained by treatment of *1*–*4* with dilute acid for different reaction times.

Reaction	Product (%)					
	1	*2*	*3*	*4*	*6*	*7*
4*S*,6*R*-Diol (*3*); H_2SO_4–dioxane–H_2O; 20 °C						
15 min	4.6	1.0	87	7.6	–	–
30 min	6.2	1.9	83	8.7	–	–
1 h	8.4	2.8	72	14	1.7	1.7
2 h	13	5.8	58	17	3.9	2.6
4 h	21	12	38	15	8.2	4.9
6 h	22	14	31	15	11	6.5
24 h	29	25	5.6	3.7	23	13
48 h	30	17	2.5	1.7	32	17
4*R*,6*R*-Diol (*4*); H_2SO_4–dioxane–H_2O; 20 °C						
15 min	–	6.5	3.9	86	3.6	–
30 min	1.6	10	6.1	77	5.4	–
1 h	2.0	16	12	62	7.7	–
2 h	3.6	24	16	43	14	–
4 h	6.5	31	19	22	21	1.2
6 h	9.2	34	16	13	25	1.9
24 h	26	31	4.6	3.2	29	6.3
48 h	26	20	3.0	1.7	39	11
4*S*,8*S*-Diol (*1*); H_2SO_4–dioxane–H_2O; 20 °C						
6 h	72	12	3.9	2.5	–	10
48 h	39	16	2.5	2.8	12	27
96 h	32	12	6.6	4.1	24	22
4*R*,8*S*-Diol (*2*); H_2SO_4–dioxane–H_2O; 20 °C						
6 h	14	78	3.9	3.2	–	–
48 h	30	29	2.3	2.7	20	15
96 h	21	21	4.5	3.7	35	15

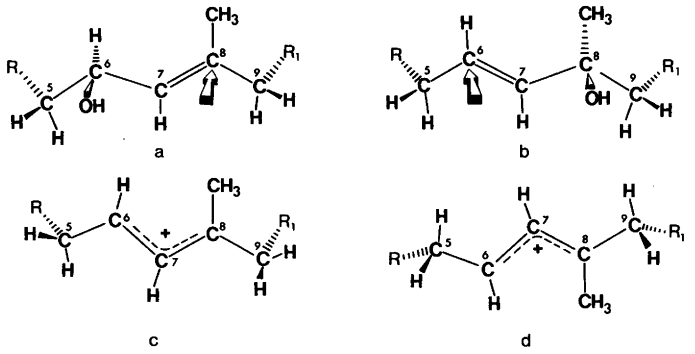
and the 4,8-diols (*e.g.* *3*⇄*1* and *4*⇄*2*) the equilibrium positions favour the formation of the latter. This conclusion is substantiated by the observation that the 4,8-diols *1* and *2* when treated with weak acid give rise to minute quantities of 4,6-diols (*3*, *4*) only (*cf.* Table 2).

Inspection of Dreiding models revealed that an allylic rearrangement of S_N2' type taking place with the well-documented *cis*-stereochemistry[8] in conformer *a* of the 4,6-diols *3* and *4* would explain the formation of the 4*S*,8*S*- and 4*R*,8*S*-diols *1* and *2*, respectively (*cf.* Scheme 1). Conversely, conformer *b* of the 4*S*,8*S*- and 4*R*,8*S*-diols (*1*, *2*), whose existence is corroborated by the results from the X-ray analyses, would be amenable to an S_N2' reaction yielding the 4*S*,6R- and 4*R*,6*R* diols *3* and *4*.

In cannot be excluded, however, that the interconversions of the 4,6- and 4,8-diols (*1*–*4*) may take place by S_N1 types of reactions. Thus, attack of hydroxyl ions on C-8 of carbonium ions *8a* and *8b*, when these exist in conformation *c*, would afforld the 4*S*,8*S*- and 4*R*,8*S*-diols (*1*, *2*), respectively, whereas the 4*S*,6*R*- and 4*R*,6*R*-diols (*3*, *4*) would arise by attachment of hydroxyl ions to C-6.

The generation of the 4*S*,8*R*-diol *7*, which is not consistent with the S_N2' mechanism, may also be rationalized by an S_N1 reaction occurring in conformer *d* of carbonium ion *8a*. For reasons not readily understood, the corresponding reaction is not favoured in the 4*R*-series.

The *seco*-aldehyde (*6*) was first reported as a constituent of tobacco flowers and has been pre-



Scheme 1.

pared from the 4*R*,6*R*-diol (*4*) by the use of *p*-toluenesulphonic acid.[7] It is formed, in our case, by a competing fragmentation reaction, which although occurring in both the 4*S*,6*R*- and 4*R*,6*R*-diols (*3*, *4*) is more effective in the latter (*cf.* Table 2). Attempted acid-induced cyclization of *6* showed that this fragmentation reaction is irreversible, a result which concurs with the observed accumulation of *6* on exposure of the 4,6-diols (*3*, *4*) and also of the 4,8-diols (*1*, *2*; *i.e.* $1 \rightleftarrows 3 \rightarrow 6$) to acid for prolonged reaction times.

Use of BF_3-etherate in toluene as the acidic agent was found to promote the fragmentation reaction and not to involve competing formation of 4,8-diols. Thus, a 10% yield of *6* was obtained from the 4*S*,6*R*-diol (*3*) under these conditions.

It follows from Table 2 that in addition to the allylic rearrangement and fragmentation reactions the 4*S*,6*R*- and 4*R*,6*R*-diols (*3*, *4*) are prone to undergo epimerization of the allylic hydroxyl group at *C*-4 on treatment with weak acid. This process is evidently also part of the reaction sequence, in which the 4*S*,8*S*- and 4*R*,8*S*-diols (*1*, *2*) are interconverted, *i.e.* $1 \rightleftarrows 3 \rightleftarrows 4 \rightleftarrows 2$. Whether a direct epimerization at C-4 occurs in the 4,8-diols *1* and *2* is unclear.

Although oxidative processes are predominant,[9] it seems likely that acid-induced reactions of the types described above, which can be carried out under mild conditions, are involved in the biotransformations of the 4,6-diols *3* and *4*. This view is supported by the fact that the 4,8-diols *1* and *2*,[5] a metabolite derived from *1* [10] and the *seco*-aldehyde *6* [7] are tobacco constituents.

EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos MS50 Stereo DS55SM/DS55S mass spectrometer-computer system and some of the NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 11 were used.

Isolation. An extract (24 g) obtained by immersing green leaves of Greek *Nicotiana tabacum* (Basma Drama) in chloroform was distributed between hexane and methanol–water (80:20). The polar material obtained (16 g) was chromatographed over silica gel using a gradient of hexane–ethyl acetate as eluent to give fractions 1 (1 g), 2 (8 g) and 3 (6 g). Fraction 1 was a complex mixture, which was separated further by chromatography over silica gel and HPLC using columns packed with μ-Porasil and μ-Bondapak/CN to give 12.6 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*1*) and 6.6 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*2*).

(1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-Cembratriene-4,8-diol (*1*) had m.p. 116–119 °C and $[\alpha]_D$ +72° (*c* 0.57, $CHCl_3$) (reported m.p. 118–120 °C; $[\alpha]_D$ +100°);[5] IR ($CHCl_3$) bands at 3600, 3450 and 985 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (d, J = 6.8 Hz)/0.84 (d, J = 6.7 Hz) (H-16/H-17), 1.33 (s, H-18 + H-19), 1.50 (broad s, H-20), 2.29 (dd, J = 8.0 and −13.6 Hz, H-5_A), 2.43 (ddd, J = 1.0, 5.2 and −13.6 Hz, H-5_B), 5.31 (broad t, J = 6 Hz, H-11), 5.32 (dd, J = 6.0 and 16.0 Hz, H-2), 5.39 (d, J = 16.0 Hz, H-3), 5.52 (ddd, J = 5.2, 8.0 and 16.1 Hz, H-6) and 5.69 (dd, J = 1.0 and 16.1 Hz, H-7); MS[*m*/*z*(%)]: 288 (M−18, 3), 270 (9), 255 (5), 245 (6), 227 (13), 187 (6), 159 (12), 135 (19), 121 (17), 107 (41), 93 (28), 81 (56), 71 (36), 55 (30) and 43 (100).

(1*S*,2*E*,4*R*,6*E*,8*S*,11*E*)-2,6,11-Cembratriene-4,8-diol (*2*) had m.p. 146–148 °C and $[\alpha]_D$ +38° (c 0.37, $CHCl_3$) (reported m.p. 150–152 °C and $[\alpha]_D$ +40°);[5]

IR ($CHCl_3$) bands at 3600, 3440 and 985 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (d, J = 6.5 Hz)/0.84 (d, J = 6.2 Hz) (H-16/H-17), 1.37 (s)/1.40 (s) (H-18/H-19), 1.49 (broad s, H-20), 2.20 (dd, J = 7.1 and −13.7 Hz, H-5_A), 2.49 (dd, J = 3.8 and −13.7 Hz, H-5_B), 5.26 (dd, J = 8.6 and 15.7 Hz, H-2), 5.31 (broad t, J = 5 Hz, H-11), 5.47 (d, J = 15.7 Hz, H-3), 5.66 (d, J = 16.4 Hz, H-7) and 5.71 (ddd, J = 3.8, 7.1 and 16.4 Hz, H-6); MS [*m/z* (%)]: 288 (M − 18, 3), 270 (13), 255 (9), 245 (8), 227 (19), 187 (9), 159 (16), 133 (24), 119 (21), 107 (48), 93 (38), 81 (74), 71 (35), 55 (32) and 43 (100).

Preparation of (1S,2E,4R,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (5). To a cooled (0 °C) solution of 12.0 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*2*) and 19.5 mg of sodium acetate in 4 ml of chloroform was added 7.6 mg of *m*-chloroperbenzoic acid. The reaction mixture was kept at 0 °C for 45 min. Work-up and separation by HPLC using a column packed with Spherisorb/CN gave 8.2 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*5*), which had m.p. 128 − 130 °C; $[\alpha]_D$ −12° (*c* 0.97, $CHCl_3$); IR ($CHCl_3$) bands at 3600 and 3450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, J = 7.0 Hz)/0.87 (d, J = 6.9 Hz) (H-16/H-17), 1.19 (s, H-20), 1.36 (s)/1.43 (s) (H-18/H-19), 2.30 (dd, J = 6.3 and −13.0 Hz, H-5_A), 2.47 (dd, J = 6.1 and −13.0 Hz, H-5_B), 2.98 (dd, J = 2.5 and 10.7 Hz, H-11), 5.32 (dd, J = 8.6 and 15.9 Hz, H-2), 5.53 (d, J = 15.9 Hz, H-3), 5.63 (d, J = 15.6 Hz, H-7) and 5.77 (ddd, J = 6.1, 6.3 and 15.6 Hz, H-6); MS [*m/z* (%)]: 304 (M − 18, 2), 286 (13), 268 (11), 243 (11), 225 (12), 215 (5), 201 (5), 185 (7), 173 (12), 159 (15), 145 (25), 133 (23), 119 (28), 105 (32), 95 (35), 81 (43), 69 (26), 55 (32) and 43 (100).

Treatment of (1S,2E,4S,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (3) with acid. I. A solution of 194 mg of *3* in 12 ml of dioxane − water (3:1) and 0.4 ml of dilute H_2SO_4 (5 %) was kept under nitrogen and at room temperature (20 °C) for 5.5 h. The reaction mixture was diluted with water and extracted with diethyl ether. The ether phase was washed with aqueous $NaHCO_3$ and water, dried and concentrated. The residue was separated by HPLC using a column packed with Spherisorb/CN and hexane − ethyl acetate (60:40) as an eluent to give 9.3 mg of 10-isopropyl-3,7,13-trimethyl-2,6,11,13-tetradecatetraen-1-al (*6*),[7] 6.0 mg of (1*S*,2*E*,4*S*,6*E*,8*R*,11*E*)-2,6,11-cembratriene-4,8-diol (*7*), 28.8 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*1*), 18.9 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*2*), 46.6 mg of starting material (*3*) and 24.1 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*4*).

(1*S*,2*E*,4*S*,6*E*,8*R*,11*E*)-2,6,11-Cembratriene-4,8-diol (*7*) was an oil and had $[\alpha]_D$ +54° (*c* 0.33, $CHCl_3$); IR ($CHCl_3$) bands at 3600, 3540 and 985 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (d, J = 6.8 Hz)/0.83 (d, J = 6.8 Hz) (H-16/H-17), 1.29 (s)/1.35 (s) (H-18/H-19), 1.49 (broad s, H-20), 2.27 (dd, J = 7.0 and −13.1 Hz, H-5_A), 2.49 (dd, J = 2.5 and −13.1 Hz, H-5_B), 5.27 (dd, J = 9.0 and 15.4 Hz, H-2), 5.32 (broad t, J = 6 Hz, H-11), 5.44 (d, J = 15.4 Hz, H-3), 5.57 (ddd, J = 2.5, 7.0 and 14.0 Hz, H-6) and 5.64 (d, J = 14.0 Hz, H-7); MS [*m/z* (%)]: 288 (M − 18, 3), 270 (4), 255 (2), 245 (3), 227 (5), 187 (4), 163 (8), 147 (12), 135 (18), 121 (19), 107 (38), 93 (31), 81 (50), 71 (33), 55 (26) and 43 (100).

II. A solution of 200 mg of *3* in 12 ml of dioxane − water (3:1) and 0.4 ml of dilute H_2SO_4 (5 %) was kept under nitrogen and at room temperature. Aliquots containing 1 ml of the reaction mixture were taken after various reaction times, worked up and examined by HPLC using the conditions described above. The results obtained are summarized in Table 2.

Treatment of (1S,2E,4R,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (4) with acid. I. A solution of 109 mg of *4* in 6 ml of dioxane − water (3:1) and 0.2 ml of dilute H_2SO_4 (5 %) was kept under nitrogen and at room temperature for 5.5 h. Work-up and separation by HPLC using a column packed with Spherisorb/CN and hexane − ethyl acetate (60:40) as an eluent afforded 6.9 mg of *6*, 2.0 mg of *7*, 11.7 mg of *1*, 28.5 mg of *2*, 15.2 mg of *3* and 11.2 mg of *4*.

II. A solution of 185 mg of *4* in 12 ml of dioxane − water (3:1) and 0.4 ml of dilute H_2SO_4 (5 %) was kept under nitrogen and at room temperature. Aliquots containing 1 ml of the reaction mixture were taken after the reaction times indicated in Table 2, worked up and examined by HPLC using the conditions described above (*cf.* Table 2).

Treatment of the (1S,2E,4S,6E,8S,11E)- and (1S,-2E,4R,6E,8S,11E)-2,6,11-cembratriene-4,8-diols (1, 2) with acid. A solution of 8.8 mg of *1* in 4 ml of dioxane − water (3:1) and 0.2 ml of aqueous H_2SO_4 (5 %) was kept under nitrogen and at room temperature. Aliquots containing 1 ml of the reaction mixture were taken after 6, 48 and 96 h. They were worked up and analyzed by HPLC using a column packed with Spherisorb/CN and hexane − ethyl acetate (60:40) as an eluent. The results are summarized in Table 2.

A solution of 6.3 mg of *2* in 4 ml of dioxane − water (3:1) and 0.2 ml of aqueous H_2SO_4 (5 %) was kept under nitrogen and at room temperature. Aliquots were taken and analyzed in the same manner as described above (*cf.* Table 2).

Treatment of 10-isopropyl-3,7,13-trimethyl-2,6,11,13-tetradecatetraen-1-al (6) with acid. A solution of 14.2 mg of *6* in 5 ml of dioxane − water (3:1) and 0.2 ml of aqueous H_2SO_4 (5 %) was kept under nitrogen and at room temperature for 5 h. Work-up and examination by TLC and ^1H NMR showed that *6* was recovered unchanged.

Preparation of 10-isopropyl-3,7,13-trimethyl-2,6,11,13-tetradecatetraen-1-al (6). To a solution of 102 mg of *3* in 9 ml of toluene, which was kept at

−45 °C, was added a solution of 0.1 ml of BF_3-etherate in 6 ml of toluene. The reaction mixture was kept at −45 °C for 30 min, then diluted with ice-water and extracted with ether. The organic phase was washed with aqueous $NaHCO_3$ and water and dried. The residue was chromatographed over silica gel using a gradient of hexane – ethyl acetate as an eluent to give 12.3 mg of starting material (*3*) and 12.9 mg of *6*, whose IR, MS and ^1H NMR spectra agreed with published spectral data.[7]

Acknowledgements. This paper is submitted in honour of Professor Holger Erdtman on the occasion of his 80th bithday in appreciation of his contributions to organic chemistry.

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Cleavage of Carbon–Metal Bonds, A Possible Explanation for the Disfavoured Coupling of Carbon and Heteroatoms by Reductive Elimination

B. ÅKERMARK,[a] M. ALMEMARK[b] and A. JUTAND[a,*]

[a] Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden and
[b] Swedish Water and Air Pollution Research Institute (IVL), P.O. Box 21060, S-100 31 Stockholm, Sweden

The dimethyl cobalt complexes $CpCo(CH_3)_2(PPh_3)$ (*1*) $(CH_3)_2$ Co(DO)(DOH)pn (*2*) and $(CH_3)_2$ Co (2,3,9,10-tetramethyl-1,4,8,11-tetraazacyclotetradeca-1,3,8,10-tetraene) (*3*) have been oxidized electrolytically in the presence of nucleophiles. No reaction takes place between the methyl groups and the nucleophiles. With *1*, reductive elimination is the major reaction. Attempts to effect carbon–oxygen coupling by reductive elimination from phenylheterocuprates (*4*) failed, except when excess acetate was added as heteroatom ligand. In the reactions of heterocuprates, reductive elimination with carbon–carbon bond formation is the major reaction. It is suggested that the difference in oxidation potentials of carbon and hetero atom ligands is responsible for the result.

The formations of carbon–oxygen and carbon–nitrogen bonds are common and useful reactions, that are usually accomplished by nucleophilic substitution at carbon. Common leaving groups are stable anions such as halide or tosylate. In principle, metal ions could also act as leaving groups. For instance, a metal alkyl could be transformed into alcohol, the reaction (1), if the attack of hydroxide on carbon were sufficiently rapid to compete with other types of cleavage of the metal–carbon bond and attack on the metal. Even when attack on the metal is preferred, carbon–oxygen bond formation is, in principle, possible by reductive elimination, path (2).

$$HO^- + RCH_2ML_n \rightarrow RCH_2OH + [ML_n]^- \quad (1)$$

$$HO^- + RCH_2ML_n \xrightarrow{-L} [RCH_2M(L_{n-1})OH]^- \xrightarrow{+L} RCH_2OH + [ML_n]^- \quad (2)$$

Since metal alkyls may be intermediates in catalytic activation of hydrocarbons by metals,[2] reactions of the types (1) and (2) offer potential routes to selective oxidation of hydrocarbons. The formation of alkylmetal hydrides, the first step in the anticipated activation process, is probably thermodynamically unfavourable (*cf.* Ref. 3 h). An oxidation process based on the reactions (1) and (2) thus requires that they be very efficient.

Although there are a few examples in the literature of reactions of the type (1), these involve only monoalkylmetals.[3] Furthermore, there are no clear-cut examples of reaction (2) although reactions like the Ullmann biarylether synthesis have been postulated to be of this type.[4] In an exploratory study we have therefore tried to evaluate the feasibility of the reactions (1) and (2) by studying a few stable model system *1*–*4*, which were made to react by means of oxidation.

RESULTS AND DISCUSSION

In order to evaluate the redox potentials of compounds *1*–*4* and also get an idea of their chemical properties after oxidation, the compounds were first studied by cyclic voltammetry. They are all irrevers-

*See Ref. 1.

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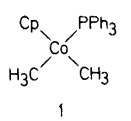

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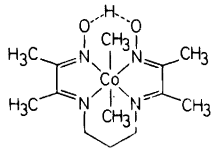

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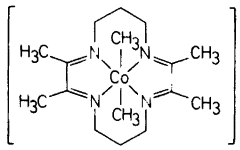

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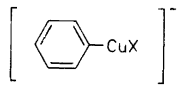


4 a X = AcO^-
b X = PhO^-
c X = $PhCOCH_2^-$

ibly oxidized at room temperature due to rapid decomposition of the oxidized intermediate. At temperatures below 0 °C the oxidation of the compound *1* becomes reversible. The oxidation potentials for the compounds *1* – *3* (Table 1) vary between +0.29 and +0.61 V *vs.* Ag|AgCl|LiCl (aq) (+0.57 – +0.89 V *vs.* standard hydrogen electrode). On oxidative decomposition, *1* – *3* give product cobalt complexes, which are oxidized at a higher potential. For *1*, the decomposition product is reversibly oxidized even at room temperature, while the product from *2* is reversibly oxidized at −25 °C. Compound *4a*, showed only a poorly structured oxidation peak at around +0.5 V *vs.* Ag|AgCl.

Table 1. Cyclic voltammetry of compounds *1* – *4a*.

Compound	Temp. °C	E_p[a] Anodic	E_p[a] Cathodic	E_p, decomposition product Anodic	Cathodic
1	20	0.34[b]	–	0.50	0.40
	−8	0.38[b]	0.28	–	–
	−40	0.41[b]	0.24	–	–
	20	0.29[c]	–		
2	20	0.55[b]	–	1.5	–
	−65	0.61[b]	–	1.54	1.47
	20	0.43[c]	–	1.6	–
	−25	0.48[c]	–	1.56	1.50
3	20	0.40[c]	–	0.80	–
4a	20	~0.5[d]	–		

[a] V relative to Ag|AgCl 0.1 M LiCl (aq). [b] In CH_2Cl_2. [c] In acetonitrile. [d] In THF.

Table 2. Anodic oxidation of the complexes *1* – *3*.[a]

Compound	Solvent	Potential[b] V	Charge passed[c] F/mol	Yield of gaseous products as found above the solution[d] CH_4, %	C_2H_6, %	Estimated total yield of[e] CH_4, %	C_2H_6, %
(*1*)	$CH_2Cl_2 + H_2O$	0.6 – 1	0.9 ± 0.1	0	≅10	0	25
	$CH_2Cl_2 + Bu_4NBr$	0.3 – 0.4	0.9 ± 0.1	0	9	0	36
	$CH_3CN + Bu_4NOH$(aq)	0.1 – 0.4	4 ± 0.5	0	13	0	26
(*2*)	$CH_3CN + H_2O$	0.45 – 1	1 ± 0.1	5	3	6	6
(*3*)	$CH_3CN + H_2O$	0.4 – 0.8	0.5[f]				
		0.8 – 0.9	≅1[g]	4	1	5	2
	CH_3CN	0.3 – 0.7	0.6[f]				
		0.7 – 1	1.2 ± 0.1[g]	4	traces	≅5	traces
	$CH_3CN + Bu_4NBr$	0.3 – 0.5	≅2	1.6	0	≅2	0

[a] On a Pt foil anode, supporting electrolyte 0.1 M Bu_4NBF_4. [b] With ref. to Ag, AgCl|0.1 M LiCl(aq.). As the electrolysis proceeded, the potential rose from the lower to the upper limit. [c] The electrolysis was continued until the anodic peak had disappeared. [d] Determined by GLC analysis of the atmopshere in the anodic chambre. [e] Corrected for solubility of methane and ethane in the solvents. [f] For the first anodic peak. [g] Total for the first and second anodic peak.

The reaction is irreversible even at low temperatures.

On oxidation at room temperature, compound *1* decomposes to give ethane as the only detectable volatile organic product (Table 2). The reaction is probably a concerted reductive elimination since methane was not detected. In the presence of nucleophiles no products other than ethane were detected except when tetrabutylammonium hydroxide was added. In this reaction small amounts of cyclopentadiene and methylcyclopentadiene were detected, but none of the desired alcohol, methanol. The latter reaction is obscure in that 4 equivalents of charge are consumed as compared to 1 equivalent in most of the other oxidations. Compounds *2* and *3* give only low yields of a mixture of methane and ethane, indicating perhaps that with these compounds, decomposition goes *via* methyl radicals. Triphenylmethylphosphonium salts may have been formed from *1* in agreement with the reaction of arylnickel halide species [5a] but in the presence of ionic medium it was difficult to identify. These results indicate that external nucleophilic attack on dialkylmetals is not a favourable reaction, probably both because the high electron density around the metal makes nucleophilic attack unfavourable and because reductive elimination is very efficient upon oxidation (*cf.* Ref. 5).

The preparative oxidation experiments with heterocuprates were done in THF at a potential of about +0.6 V *vs.* Ag|AgCl. The results are summarized in Table 3. The cuprates *4* were prepared *in situ* by addition of the desired nucleophile to copper(I) at −70 °C, followed by oxidation at the same temperature. Only with acetate *4a* could coupling products be detected. The major product was biphenyl, which is formed by oxidation of either polynuclear complexes or diphenylcopper(I). The latter compound would be formed by scrambling of the phenyl and acetate groups, most probably a very rapid reaction. Interestingly, the addition of stabilized carbon nucleophiles like the enolate of acetophenone or the anion of dimethyl malonate failed to give cross coupled products with the phenyl group. The yield of the cross coupled product, phenyl acetate, is low also with the mixed phenylacetate complex but is increased as excess acetate is added. Even with a 5-fold excess, the yield is moderate, 20 %, and the main product is always biphenyl. Both diphenylcopper(I) and the cobalt complex *1* are thus relatively stable compounds, that after oxidation readily undergo reductive elimination. It is possible that symmetry imposed barriers to reductive elimination are important. For the mononuclear copper(I) and copper(II) species, reductive elimination is in principle forbidden, but may become allowed by cluster formation.[6] Especially for the copper(II) species, one could imagine the formation of a mixed copper(III)−copper(I) cluster from which reductive elimination would be permitted when the aryl groups are attached to copper(III). The stability of the cobalt complex *1* is even more surprising since the methyl groups are forced to occupy *cis*-positions. However, the most probable structures for the complex *1* are a pseudotetrahedral one with the cyclopentadienyl ring occupying only one position, or an octahedral one. For a d^6-system like *1*, reductive elimination is symmetry forbidden for the former structure (Table 4) and permitted for the octahedral structure only when the product has a square planar configuration.[6] This is not possible with the cyclopentadienyl ring occupying three positions. On 1-electron oxidation, a d^5-system is formed. Reductive elimination is still symmetry forbidden for the octahedral structure,

Table 3. Oxidation of heterocuprates *4*.

Compound	Oxidant	Potential[c]	Nucleophile (ratio)[d]	Biphenyl (yield)[h]	PhX (yield)[h]
4a	[a]	+0.6 V	NaOAc (1)	80	trace[e]
4a	[a]	+0.6 V	Bu_4NOAc (5)	46	16[e]
4b	[b]		NaOPh (5)	49	0[f]
4a	[b]		Bu_4NOAc (1)	80	5[e]
4a	[b]		Bu_4NOAc (5)	61	24[e]
4a	[b]		NaOAc (2)	61	22[e]
4c	[b]		$LiCH_2COPh$ (1)	62	0[g]

[a] Electrolysis at −70 °C, THF solution, Pt-anode, supporting electrolyte $LiClO_4$. [b] Air stream 2 h at −70 °C. [c] Relative to Ag/AgCl. [d] Ratio nucleophile−copper. [e] X = OAc. [f] X = OPh, none detected by GLC. [g] X = CH_2COPh, none detected. [h] Based on added phenyllitium.

Table 4. Symmetry for reductive elimination from a pseudo-tetrahedral structure.

	Metal *d*-configuration	Number of electrons in orbitals of symmetry[b]			
		a_1	a_2	b_1	b_2
Reactant[a], d^5	$(d_{yz})^1(d_{x^2-y^2})^2(d_{xy})^2$	4	2	2	1
Products d^7	$(d_{yz})^1(d_{xz})^2(d_{x^2-y^2})^2(d_{xy})^2$	4	2	2	1
Reactant[a], d^6	$(d_{xz})^1(d_{yz})^1(d_{x^2-y^2})^2(d_{xy})^2$	4	2	3	1
Products d^8	$(d_{xz})^2(d_{yz})^2(d_{x^2-y^2})^2(d_{xy})^2$	4	2	2	2

[a] Includes the two metal-carbon orbitals. [b] The orbital symmetries are (C_{2v}) d_{z^2}, $d_{x^2-y^2}$: a_1; d_{xy}: a_2; d_{xz}: b_1 and d_{yz}: b_2.

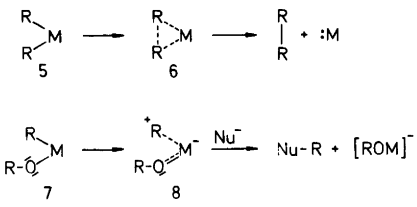
but for the pseudotetrahedral it now becomes allowed (Table 4), in accordance with the experimental result.

In order to investigate the carbon–oxygen coupling reaction in some further detail, we have also done a series of experiments where the arylcopper(I) – nucleophile system was oxidized by air. The results are very similar to those obtained with electrochemical oxidation. Of the nucleophiles used, phenolate, malonate, the enolate of acetophenone and acetate, only acetate couples with the phenyl group, giving phenyl acetate. The yield is low, 5 %, when equimolar amounts of phenylcopper and acetate were mixed and about 20 % when a 5-fold excess of acetate was added. The major product, as in the electrolyses, is biphenyl. The explanation for this result is not clear. Since diphenylcopper(I) would be expected to be oxidized at a lower potential than a mixed arylcopper acetate, continuous phenyl-acetate scrambling and selective oxidation of diphenylcopper would explain the result. An alternative explanation is that phenyl acetate is not formed by reductive elimination but by direct attack on the phenyl ring. Excess nucleophile is then required. The reaction could be regarded either as an attack on a phenyl cation or on a metal bound phenyl cation radical. The observed need for excess acetate ion has also been noted for the electrolytic acetoxylation of arylpalladium species generated *in situ* from arylmercury compounds.[7] The attack on phenyl cation is similar to reactions of alkylmetals according to path (1). In this type of reaction, the carbon attached to metal will probably have considerable carbonium ion character as indicated by phenonium ion formation from (2-phenyl)-ethylpalladium species.[8]

Since diphenyl ether was not formed from phenylcopper and sodium phenolate, it seems unlikely that a phenylcopper phenolate is an intermediate in the Ullmann biaryl ether synthesis. Other mechanisms which must be considered are direct nucleophilic displacement on a coordinated aryl halide and copper catalyzed oxidation of the aryl halide to a cation radical, which undergoes displacement. Both reactions have analogies, the former in the addition of nucleophiles to arenes promoted by chromium carbonyl and similar reagents[9a] and the latter in nucleophilic aromatic substitution catalyzed by electrolysis or strong 1-electron oxidants.[9b] Another possibility is, of course, the classical 4-centre mechanisms.[10] Further studies are clearly desirable.

Although the exact mechanism of the phenyl-acetate coupling is not clear, it is evident that reductive elimination is not facile. This is even more true for the other tested nucleophiles, neither of which gave detectable amounts of coupling products. This reluctance to reductive elimination has been observed earlier, for instance in the cleavage of palladium–carbon bonds by acetate, amines and chloride.[3c,3d] In these reactions the nucleophile is probably coordinated to the metal but the stereochemistry of the reaction shows that the external attack by free nucleophile is favoured. In contrast, alkanide ions, after coordination, preferentially undergo *intra*-molecular reaction. As noted earlier, this preference for intramolecular reaction applies also to the addition of non-stabilized carbanions to π-olefin and π-allyl systems.[11] Again, heteroatom nucleophiles and stabilized carbanions like malonates, prefer external attack.[11]

The reasons for this are not clear, but perhaps the oxidation potentials of the coordinated ligands are important. For instance, reductive elimination from a dialkyl- or diarylmetal, could be pictured as a symmetric electron transfer from the two coordinated carbanions until one electron has been removed from each and coupling takes place. In the process, the carbanions have in principle become



Scheme 1.

oxidized to radicals. When coupling is symmetry allowed, the formation and combination of the radicals is probably concerted (*eg.* 5→6).

For an alkylmetal alkoxide the situation is different and we would like to suggest that the difference in the oxidation potentials for the two ligands is the most important factor. For instance, 2-methylpropanide ion is oxidized at a very much lower potential (−2.2 V) than the corresponding alkoxide, t-butoxide (+1.0 V).[12a,b] Other examples are phenylmethanide ion (−1.58 V)[12a,b] and acetate (+2,4 V).[12a] In fact, one might suspect that even carbon radicals are more readily oxidized than alkoxides since copper(II) (standard potential +0.15 V)[13] will oxidize carbon radicals to carbonium ions.[14] Furthermore, the determined oxidation potential for one member of this group, the triphenylmethyl radical, is considerably lower (+0.48 V)[12a,b] than that of *t*-butoxide. As electron density is transferred from the ligands to the metal, it seems reasonable that electron density is removed from the carbanion while the alkoxy group will be expected to respond by forming a partial double bond to the metal. When electron transfer has proceeded to a point where two electrons have been removed from the ligands, (7→8), the metal bound carbon has acquired carbonium ion character while the alkoxide has mainly become more strongly bound to the metal. As a result, attack by external nucleophiles, if available, will be preferred over internal migration of the coordinated alkoxide to carbon. This is in accordance with earlier experimental evidence,[3] including the carbonium ion character at coordinated carbon.[8] It is also in accordance with our observation that the coupling of phenyl and acetate *via* phenylcopper(I) is promoted by excess acetate.

In conclusion, reductive elimination involving carbon−oxygen bond formation (reaction 2) and nucleophilic substitution at the alkyl groups of dialkyl-metals (reaction 1) appear to be slow relative to other types of reactions, *e.g.* reductive elimination involving carbon−carbon coupling. Other types of reactions must therefore probably be relied upon for selective oxidation of hydrocarbons.

EXPERIMENTAL

Materials. The dimethylcobalt complexes *1*−*3* were prepared by literature procedures.[15-17] Tetrabutylammonium fluoroborate was prepared by mixing solutions of sodium fluoroborate (Riedel − de Haen AG, Seelze-Hannover) and tetrabutylammonium hydrogen sulfate (Bofors Nobel Kemi). The product was dissolved in dichloromethane and the solutions washed with water and dried. After evaporation of the solvent, the product was recrystallized twice from ethyl acetate−pentane and dried *in vacuo* at 80 °C over P_2O_5 for 16 h. Tetrabutyl ammonium bromide (Eastman Kodak Co.) was recrystallized twice from ethyl acetate, then dried over P_2O_5 overnight, first at 80 °C, then at 120 °C. Tetrabutylammonium acetate was prepared according to a literature procedure.[18]

Dichloromethane was purified by refluxing the commercial product over calcium hydride, followed by distillation. Immediately before use, it was distilled over P_2O_5. Acetonitrile was purified by stirring overnight with calcium hydride, followed by distillation over fresh calcium hydride. Also this solvent was distilled over P_2O_5 immediately before use. Dimethyl formamide (DMF), Fluka spectrograde, was dried 48 h over 4Å molecular sieves, then distilled at 0.5 mm Hg from calcium hydride.

The cyclic voltammograms were obtained on platinum button electrodes in a three-compartment cell with an instrument constructed at the Chemistry Center of the University of Lund. It consisted of a potentiostat connected to a sweep generator. The voltammograms were recorded on a Watanabe X-Y recorder.

The ionic medium was 0.1 M Bu_4NBF_4 in acetonitrile or dichloromethane. The reference electrode was an Ag|AgCl electrode from Metrohm, immersed in 0.1 M LiCl(aq). The nominal potential of this electrode is +0.280 V on the normal hydrogen scale at 20 °C and +0.0385 V against the saturated KCl calomel electrode (SCE).[19] In order to check the potential of the electrode in our system, voltammograms of 9,10-diphenylanthracene (DPA), which is known to undergo a reversible one-electron oxidation on platinum at +1.23 V (*vs.* SCE) in CH_3CN (0.1M $Bu_4N\,BF_4$)[20a] and at +1.22 V (*vs.* SCE) in CH_2Cl_2 (0.2 M $Bu_4N\,ClO_4$),[20b] were run. In our hands DPA was reversibly oxidized with an anodic-cathodic peak separation of 90 mV. In CH_3CN, the first anodic peak appeared at +1.20 V

(*vs.* Ag|AgCl), a value which compares well with the cited literature value. However, in CH_2Cl_2 the first anodic peak appeared at +1.28 V (*vs.* Ag|AgCl), 100 mV more anodic than the cited literature value. This difference may be due to the lack of iR compensation in our apparatus and to liquid junction potentials.

The electrolyses were performed in a divided cell where the anode and cathode compartments were separated by a fritted glass disc. A stabilized power supply (Oltronix B504D) was used. The reference half cell (Metrohm, Ag|AgCl) was connected to the cell *via* a bridge, equipped with a fritted glass disc and filled with 0.1 M Bu_4NBF_4 in dichloromethane or acetonitrile. Platinum electrodes were used.

IR-spectra were recorded on a Perkin Elmer 257 spectrometer, NMR spectra on a Varian EM-360 and GC-MS on a Finnègan 4021 instrument.

GC were run on a Varian Aerograph 1400 or a Carlo Erba Fractovap 2350, using stationary phases consisting of 7.5 % SE 30 on Chromosorb for the determination of phenyl acetate and biphenyl and Chromosorb 102 for methane, ethane, bromomethane and methanol.

Preparation of the cuprates 4. Dry, purified copper (I) bromide[21] (0.072g, 0.5 mmol) was suspended in dry THF (1 cm^3) at −70 °C. On addition of phenyl lithium in benzene−ether (0.58 cm^3, 0.5 mmol) a light yellow precipitate of phenyl copper was formed.[22] The appropriate amount of the nucleophile (acetate, sodium phenolate and the anions of dimethyl malonate and acetophenone) in in DMF (2 cm^3) was added and the solution thus formed was used for the oxidation experiments.

Electrochemical oxidation of compounds 1−3. Compounds *1−3* were oxidized as summarized in Table 2. After oxidation, two different work-up procedures were used. In the first, the solvent and the volatile products were distilled into a cooled receiver and analyzed by GLC.

In the second procedure, the ionic medium was precipitated by addition of diethyl ether and the filtrate analyzed by GC.

Oxidation of compounds 4. Compounds *4*, prepared *in situ* as described above, were oxidized as summarized in Table 3, either chemically with air or electrochemically. The yields of the compounds were determined by GLC, using 1,2-diphenylethane as internal standard.

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The Structure of 3-Carene Nitrosate, (3*R*,3′*R*,4*R*,4′*R*)-(*E*)-Di(8-nitrooxy-6-menthen-3-yl)diazene *N*,*N*′-Dioxide. An Unusual Product of a Nitrosation Reaction

JURAJ HARMATHA,[a,b] ZDENEK SAMEK,[b,†] STURE STRÖMBERG[a,c] and TORBJÖRN NORIN[a,c]

[a]Swedish Forest Products Research Laboratory, S-114 86 Stockholm, Sweden, [b]Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia and [c]Royal Institute of Technology, Department of Organic Chemistry, S-100 44 Stockholm, Sweden

The structure of the nitrosate, prepared from (+)-3-carene (*1*) (by nitrosation with isoamyl nitrite and nitric acid in acetic acid at −20 °C) was elucidated by chemical and spectroscopic methods. The structure of the product, known as "*d*-carene nitrosate", is shown to be (3*R*,3′*R*,4*R*,4′*R*)-(*E*)-di(8-nitrooxy-6-methen-3-yl)diazene *N*,*N*′-dioxide (*2*). The nitrosation thus must proceed by the cleavage of one of the cyclopropane bonds leaving the double bond untouched.

(+)-3-Carene (*1*) is one of the main constituents of the turpentine from the kraft pulping of *Pinus silvestris* L. This monoterpene hydrocarbon is of potential interest as a starting material for the syntheses of products of technical and economic interest, such as menthol, carvone, citral, and chrysanthemic acids. In connection with our studies on the utilization of 3-carene we have investigated a product, which is formed upon nitrosation of (+)-3-carene (*1*).

The 3-carene nitrosochloride, which is the product formed by nitrosation with ethyl nitrite and hydrochloric acid in acetic acid, has previously been studied.[1] There is another known procedure of nitrosation, which is reported to give higher yields and simpler isolation procedures from complex turpentine mixtures. This procedure was previously in common use for the preparation of crystalline derivatives of unsaturated monoterpenoids. According to this method isoamyl nitrite and nitric acid in acetic acid at −20 °C are used as reagents. In 1920 Simonsen [2] applied this procedure to 3-carene and isolated a crystalline derivative ($C_{10}H_{16}N_2O_3$) which decomposed at 141.5 °C. In this paper the structure elucidation of the product is presented.

The crystalline compound *2* was prepared from (+)-3-carene (*1*) according to the method of Simonsen.[2] The compound exhibited similar properties to those of the "*d*-carene nitrosate" described by Simonsen. It melted at 142–143 °C with decomposition. The elemental analysis indicated that the composition of our product is $C_{10}H_{16}N_2O_4$ which is different from that of $C_{10}H_{16}N_2O_3$ reported by Simonsen. However, it is evident that our product and that of Simonsen are identical.

The elemental composition and the molecular weight of the compound *2* could not be confirmed by mass spectroscopy because of its thermal decomposition. The ^{1}H NMR spectrum (see Table 1) exhibits signals which demonstrate the presence of two trisubstituted bonds of the type $CH_3-C=CH$ (δ 5.37, 2H; δ 1.69, 6H) and four tertiary methyl groups (δ 1.64, 1.61, 1.34, 1.29). The six methyl signals indicate that the compound *2* is an unsymmetrical dimer of the two C_{10}-units derived from 3-carene.

The characteristic bands in the infra-red (IR) spectrum (KBr disc) of compound *2* at 1615, 1292 and 855 cm^{-1} indicate the presence of nitric ester groups ($-ONO_2$). This fact and the elemental composition suggest the presence of one $-ONO_2$ group for each C_{10} unit in the dimeric C_{20} species. Moreover, the IR spectrum gives no support for the

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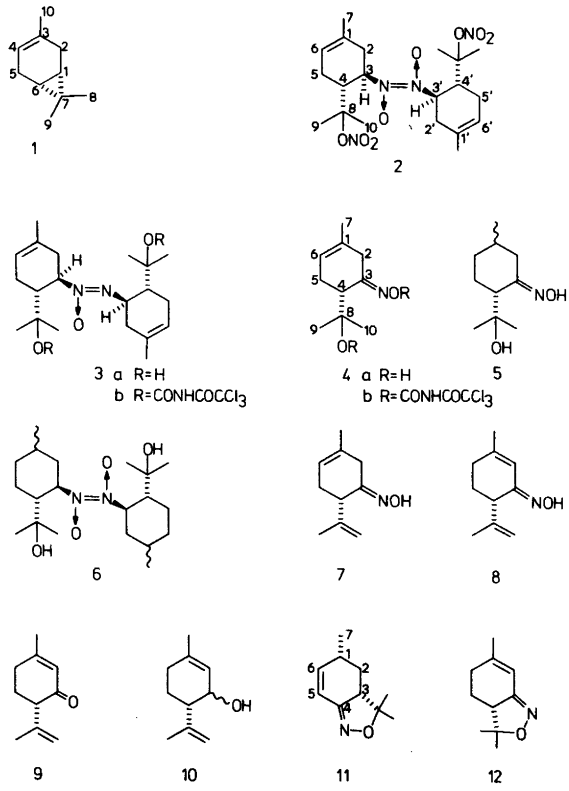

presence of *C*-nitroso or oxime groups. Instead an azodioxy group seems possible.[3]

cis-Azodioxy compounds exhibit characteristic IR bands[4] at 1403, 1392, 1210, 1178, 954 and 721 cm^{-1} and UV absorption[5,6] at 278 nm (ε 7640). The corresponding *trans* azodioxy compounds show IR bands[7-9] at 1227, 1216 and 1193 cm^{-1} and UV absorption[5-7] at 294.5 (ε 8000). In the IR spectrum of compound *2* there are bands at 1232 and 1190 cm^{-1} and the compound exhibits UV absorption at 293 nm (ε 2400). This is clearly in accordance with the presence of a *trans*-azodioxy grouping.

The compound *2* was treated with zinc in acetic acid to yield one main product (*3a*) with m.p. 130 – 132 °C and composition $C_{20}H_{34}N_2O_3$. This compound, unlike the starting material (*2*), was stable and gave rise to a molecular ion (M^+ 350 *m/e*) in its mass spectrum. The IR spectrum of compound *3a* shows hydroxyl bands (3460 cm^{-1}) but no absorption due to nitric ester or azodioxy groups. The spectroscopic data suggest that the azodioxy group has been reduced to a stable azoxy group and that the nitric ester groups have been transformed to the corresponding alcohols. The presence of the azoxy group is shown by IR bands at 1503 cm^{-1} and by UV absorption at 226 nm (ε 9800).[10] The ^1H NMR spectrum of the compound *3a* reveals the presence of two trisubstituted double bonds of the type $CH_3-C=CH$ (δ 5.34, 2H and δ 1.67, 6H) and four tertiary methyl groups (δ 1.25, 1.22, 1.16, 0.97). The presence of the two tertiary hydroxyl groups was confirmed by an *in situ* reaction with trichloroacetylisocyanate (TAI method[11,12]). The ^1H NMR spectrum of compound *3a* also exhibits two signals at δ 4.50 (1 H) and δ 4.28 (1 H) assigned to the protons on the carbons bonded to the azoxy group.

The reduction of the compound *2* with zinc in pyridine[13] yields two main products. One of them is identical with the azoxy derivative *3a*. The second product (*4a*) with m.p. 125 – 127 °C has the elemental composition $C_{10}H_{17}NO_2$. Its molecular weight is confirmed by MS (M^+ 183 *m/z*). The IR spectrum of this compound (*4a*) indicates the presence of a hydroxyl group (3585 cm^{-1}) and an oxime group

Table 1. ^{1}H NMR data of dimeric compounds II and III.

Compound[a]	Assignments $H_{(3)(3')}$	$H_{(4)(4')}$	$H_{(6)(6')}$	$H_{(7)(7')}$	$H_{(9)(9')}$	$H_{(10)(10')}$	Coupling constants[b]
2[c]	5.75 um (2H)	3.52 td (1H) 3.41 td (1H)	5.37 um (2H)	1.69 bs (6H)	1.64 s (3H) 1.61 s (3H)	1.34 s (3H) 1.29 s (3H)	$J_{4,5\alpha}=J_{4,3}=11$ $J_{4,5\beta}=6.5$ $J_{4',5'\alpha}=J_{4',3'}=11$ $J_{4',5'\beta}=6.5$
2[d]	5.78 um (1H) 5.89 um (1H)	3.50 um (2H)	5.24 um (1H) 5.12 um (1H)	1.51 bs (3H) 1.44 bs (3H)	1.16 s (3H) 1.18 s (3H)	1.40 s (3H) 1.42 s (3H)	
3a[c]	4.50 ddd (1H) 4.28 ddd (1H)		5.34 um (2H)	1.67 bs (6H)	0.97 s[e] (3H) 1.25 s[e] (3H)	1.16 s[e] (3H) 1.22 s[e] (3H)	$J_{3,4}=J_{3,2\beta}=10$ $J_{3,2\alpha}=5.5$ $J_{3',4'}=J_{3',2'\beta}=10$ $J_{3',2'\alpha}=5.5$
3b[c,f]	4.65 um (1H) 4.53 um (1H)	3.29 um (2H)	5.35 um (2H)	1.63 bs (6H)	1.41 s[e] (3H) 1.65 s[e] (3H)	1.49 s[e] (3H) 1.53 s[e] (3H)	

[a] The spectra were recorded on a Varian HA-100 instrument. Chemical shifts are given in δ-units (TMS – internal standard). Spin-spin splittings are given in Hz and are obtained from a first order analysis. Multiplicities are presented by the following abbreviations: a singlet, d doublet, t triplet, q quartet, m multiplet, b broad, u unresolved. Assignments of coupling constants were verified by decoupling experiments. [b] Symbols α, β were used for the assignment of protones with reference to the stereoprojection expressed in formula 2. This assignment is evident for methine protons. For methylene protons it depends on the vicinal coupling constant in the particular conformation. We used the transoid assignment $J_{\alpha,\beta}$ for larger couplings tentatively. [c] In $CDCl_3$. [d] In pentadeuteriopyridine, hexamethyldisiloxane (HMDS) – internal standard ($\delta_{HMDS}=0.06$). [e] The pairs of methyl signals due to the gem.-dimethyl groups were assigned on the basis of TAI induced shifts (*cf.* Ref. 12). [f] *In situ* acylation with trichloroacetylisocyanate (TAI-method).[11,12]

(3100 and 3290 cm^{-1}).[8] The structure of the compound *4a* follows from a detailed analysis of its ^{1}H NMR spectrum and also from the ^{1}H NMR spectrum of its diacyl derivative (Table 2) prepared *in situ* by treatment with trichloroacetylisocyanate (TAI method[11,12]). The relative positions of the oxime group and the trisubstituted double bond follow from the presence of an isolated AB system at δ 3.37 and 3.04 with $J_{A,B}=21$ Hz. Obviously such a large coupling constant belongs to the protons of the methylene group between the two sp_2 hybridized C atoms ($\delta\pi$ enhancement of geminal coupling). The signal assigned to the $H_{(4)}$ proton forms a doublet of doublets at δ 2.47 (see Table 2). After acylation of the hydroxyl groups with trichloroacetylisocyanate the signal of this proton is shifted to δ 3.38. Similarly, the signals due to the geminal methyl groups are shifted from δ 1.26 and 1.31 to 1.67 (6 H). This is characteristic of acylation shifts.[12]

The zinc pyridine reduction of the compound *2* yields only one major monomeric species *4a* which indicates that the compound *2* is a dimer composed of two identical units.

The catalytic hydrogenation of the compound *2* yields three crystalline main products, of which one is the compound *4a*. The second product *5*, with the composition $C_{10}H_{19}NO_2$, differs from compound *4* by being saturated. The spectral data (^{1}H NMR, IR and MS, see Experimental) are consistent with the structure *5*. The third product is the major constituent of the reaction mixture. It has the composition $C_{10}H_{19}NO_2$ but is a dimer *6* as indicated by its IR spectrum with characteristic bands at 1192 cm^{-1}, assigned to the *trans*-azodioxy group and at 3610 and 3450 cm^{-1} due to the hydroxyl groups. The UV absorption at 290 nm (ε 15850) also shows the presence of the *trans*-azodioxy group. The transformation of the compound *6* by

Table 2. ^{1}H NMR data of the monomeric compounds.

Compound[a,b]	Assignments $H_{(2)}$	$H_{(4)}$	$H_{(6)}$	$H_{(7)}$	$H_{(9)}$	$H_{(10)}$	Other data[c]
4a	3.26 bd (1H) 2.78 bd (1H)	2.47 dd (1H)	5.49 um (1H)	1.71 bs (3H)	1.26 s (3H)	1.31 s (3H)	$J_{2\alpha,2\beta}=21$ $J_{4\beta,5\alpha}=10.5$; $J_{4\beta,5\beta}=5.5$
4b[d]	3.37 bd (1H) 3.04 bd (1H)	3.38 t (1H)	5.55 um (1H)	1.75 bs (3H)	1.67 bs (6H)		$H_{(5)}$: 2.49 bs (2H) $J_{4\beta,5\alpha}\cong J_{4\beta,5\beta}=6$ $J_{2\alpha,2\beta}=21$
7			5.31 um (1H)	1.92 bs (3H)	1.89 bs (3H)	4.90 bs (1H) 4.80 bs (1H)	
8[e]	5.65 q (1H)	3.05 dd (1H)		1.92 m (3H)	1.82 ddd (3H)	4.90 bd (1H) 4.77 bd (1H)	$J_{4\beta,5\alpha}=7.5$; $J_{4\beta,5\beta}=5.5$ $J_{7,2}=1.5$; $J_{4,9}=0.3$ $J_{10a,9}=1.4$; $J_{10b,9}=0.8$
9[e]	5.90 q (1H)	2.96 dd (1H)		1.94 m (3H)	1.75 dd (3H)	4.94 bd (1H) 4.75 bd (1H)	$J_{4\beta,5\alpha}=9$; $J_{4\beta,5\beta}=6$ $J_{7,2}=1.5$; $J_{4,9}\neq 0$ $J_{10a,9}=1.4$; $J_{10b,9}=0.8$
11	1.90 dtd (1H) 1.28 m (1H)		6.40 dd (1H)	1.17 d (3H)	1.21 s (3H)	1.54 s (3H)	$H_{(1)}$: 2.42 um (1H) $H_{(3)}$: 2.96 dd (1H) $H_{(5)}$: 6.06 bd (1H) $J_{5,6}=10$; $J_{1,6}=3$; $J_{7,1}=6$ $J_{2\alpha,1\beta}=10$; $J_{2\alpha,2\beta}=12.5$ $J_{2\beta,1\beta}=J_{2\beta,\beta}=4.5$ $J_{3\beta,2\alpha}=13.5$; $J_{2\beta,6}=1$
12	6.20 q (1H)	2.85 dd (1H)		1.89 bs (3H)	1.11 s (3H)	1.51 s (3H)	$J_{4\beta,5\alpha}=13.5$; $J_{4\beta,5\beta}=5.5$ $J_{2,7}=1.1$

[a] See footnote *a* of Table 1. [b] In $CDCl_3$. [c] See footnote *b* of Table 1. [d] *In situ* acylation with trichloroacetylisocyanate (TAI-method).[11,12] [e] The olefinic protons on $C_{(10)}$ in the compounds *8* and *9* are named $H_{(10a)}$ and $H_{(10b)}$.

thermolysis at 150 °C or by alkaline hydrolysis to the oxime *5* further confirms the structural assignment.

Alkaline hydrolysis (NaOH/EtOH) of the compound *2* yields a mixture of two main compounds of which one is amorphus and has the composition $C_{10}H_{15}NO$. The second compound is crystalline and has the same composition ($C_{10}H_{15}NO$). The structure *7* and *8* are assigned to these compounds based on analyses of their ^{1}H NMR (Table 2), IR and MS spectra.

The structure of the compound *8* is further confirmed by a direct comparison with an authentic sample of the oxime of isopiperitonone. The isopiperitenone (*9*)[14] is obtained by a Jones oxidation of the mixture of *trans* (80 %) and *cis* (20 %) isopiperitenols (*10*) which in turn is prepared from (+)-3-carene (*1*) *via* an independent series of reactions.[15]

Information about the configuration of the $C_{(3)}$, $C_{(3')}$, $C_{(4)}$ and $C_{(4')}$ positions of compounds *2* and *3* are obtained by an analysis of their ^{1}H NMR

spectra. The protons $H_{(4)}$ and $H_{(4')}$ of the compound *2* display two equal, partially overlapping multiplets at δ 3.52 and 3.41, in both cases with equal splittings (see Table 1). The signals of the protons $H_{(3)}$ and $H_{(3')}$ form an overlapping complex multiplet at δ 5.75. The protons $H_{(3)}$ and $H_{(3')}$ of the compound *3a* display two equal, partially overlapping multiplets δ 4.50 and 4.28, in both cases with practically equal splitting (see Table 1). The multiplets of the protons $H_{(4)}$ and $H_{(4')}$ were not separated in the spectrum of compound *3a*. The pair-wise identical splitting-constants due to the interactions of protons $H_{(3)}$ with $H_{(4)}$ and $H_{(3')}$ with $H_{(4')}$ in the compounds *2* and *3a* demonstrate that these protones are pair-wise similar and have axial configurations.

The fact that the two identical units of the compound *2* give rise to separate NMR signals must be due to the dissymmetry of the molecule. Such an atropisomerism appears as a consequence of hindered rotation about the $C_{(3)}-N$ and $C_{(3')}-N$ bonds.[16] Assuming a preferred conformation (Fig. 1), in which the plane of the azodioxy group is perpendicular to the hypothetical plane of the two six-membered rings, and where the $C_{(3)}-H$ and $C_{(3')}-H'$ bonds have a *syn* orientation to one another and lie in the plane of the *trans*-azodioxy group, the $C_{(3)}-H$ bond has a *cis* periplanar orientation relative to the adjacent $N-O$ bond, whereas the $C_{(3')}-H$ bond and the neighbouring $N-O$ bond have a *trans* periplanar conformation. Such an arrangement is expected to be stable on both steric and electrostatic grounds.

The chemical and spectroscopic data of the "*d*-carene nitrosate" clearly establish its structure *2* and conformation (Fig. 1). It is interesting to note that the double bond did not react in the nitrosation reaction and that the cyclopropane ring of the 3-carene (*1*) preferably underwent cleavage at the $C_{(1)}-C_{(7)}$ bond. Dimers or reaction products which correspond to other cyclopropane ring cleavages, *e.g.* cleavage of the $C_{(1)}-C_{(6)}$ bond, were not isolated.

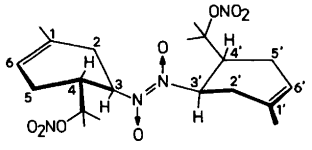

Fig. 1. A possible conformation of (3*R*,3'*R*,4*R*,4'*R*)-(*E*)-di(8-nitrooxy-6-menthen-3-yl)diazene *N*,*N*'-dioxide (*2*).

However, there is evidence for a reaction in which cleavage of the $C_{(6)}-C_{(7)}$ bond occurs. The alkaline degradation of a crude sample of "*d*-carene nitrosate", from which the compound *2* could be obtained, also produced a mixture containing small amounts of the compound *11*.

The structure of the compound *11* follows from its spectral data (see Experimental) and particularly from its ^1H NMR spectrum (Table 2). It is evident that this compound most probably originates from a dimer of the *m*-menthane type. The formation of the oxazole ring in the compound *11* is explained by the addition of a $=N-OH$ group to an isopropenyl group or its equivalent. Analogously, the isomeric compound *12* is formed as a by-product in the preparation of the oxime *8* from isoperitenone *9*.The formation of an oxazole ring in the compound *7* reveals the presence of a *syn* configuration of the $=N-OH$ group and the $C_{(4)}-C_{(8)}$ bond in the reactive oxime *8*. Thus the isolated oximes *4*, *5*, *7* and *8* may tentatively be assigned *anti*-configurations.

The nitrosation product of 3-carene (*1*) described here and the degradation of the nitrosation product reveal interesting routes to a series of 3-oxo-*p*-methane derivatives from a readily available starting material.

EXPERIMENTAL

The melting points were determined on a Kofler micro hot stage and are not corrected. The IR spectra were obtained with a C. Zeiss UR-20 instrument, the UV spectra with an Optica Milano CF 4 spectrophotometer. The ^1H NMR spectra were recorded with a Varian HA-100 instrument using the conditions given in Table 1. The mass spectra were recorded with an AEI MS-902 instrument (IP 70 eV). "Kieselgel G nach Stahl" (Merck) and silica gel (Merck) were used for thin-layer chromatography (TLC) and column chromatography, respectively.

Preparation of "d-*carene nitrosate*", (3R,3'R,4R,4'R)-(E)-*di*(8-*nitrooxy*-6-*menthen*-3-*yl*)*diazene* N,N'-*dioxide*. (+)-3-Carene (*1*, 25 g; $[\alpha]_D$ +17°, *c* 2.5, $CHCl_3$) was mixed with acetic acid (10 ml) and isoamyl nitrite (20 g). Nitric acid (density 1.4, 18 g) was then added dropwise (40 min) under stirring and cooling (−20 °C). A solid slowly deposited and, after one hour, ethanol (100 ml) was added and a solid (6 g; yield approximately 15 %) was collected. The product was purified by column chromatography (light petroleum − dichloromethane, 1:1), and by crystallization from a mixture of dichloromethane

and light petroleum; 142 – 143 °C (vigorous decomposition at the same time). $[\alpha]_D^{20}$ −31.15° (*c* 0.183, $CHCl_3$). IR ($CHCl_3$): 1198 (*trans*-azodioxy), 1298, 1630 (−ONO_2), 1377, 1393 (*gem* dimethyl group), 1688 (C=C) cm^{-1}. IR (KBr): 1190, 1232 (*trans*-azodioxy), 1615, 1292, 855 (−ONO_2) cm^{-1}. UV (abs. EtOH): 293 nm (ε 2400). ^1H NMR see Table 1. Found: C 52.47; H 7.10; N 12.40. Calc. for $C_{20}H_{32}N_4O_8$: C 52.62; H 7.07; N 12.27.

Reduction of compound 2 *with Zn in acetic acid.* A mixture of compound *2* (0.3 g) and zinc-dust (0.4 g) in acetic acid (15 ml) and tetrahydrofuran (30 ml) was stirred at room temperature overnight. Water was added to the mixture and the products were extracted with diethyl ether. According to TLC the product mixture contained 5 compounds. No starting material was detected. The main compound *3a* m.p. 130 – 132 °C was isolated by column chromatography on silica gel using light petroleum – methanol (20:1). IR ($CHCl_3$): 3460 (hydroxy), 1503 (azoxy), 1688 (C=C), 1379, 1393 (*gem* dimethyl) cm^{-1}. UV (abs. EtOH): 226 nm (ε 9800). ^1H NMR see Table 1. MS (*m/e*): 350 (M^+). Found: C 68.27; H 9.85; N 8.25; Calc. for $C_{20}H_{34}N_2O_3$: C 68.53; H 9.78; N 7.99.

Reduction of compound 2 *with Zn in pyridine.* To the mixture of compound *2* (0.3 g) in pyridine (7 ml) was added zinc dust (0.6 g) and acetic acid (15 drops).[13] The mixture was shortly heated up to the boiling point. Compound *2* dissolved and the mixture was allowed to stand until it cooled to room temperature (15 min). The mixture was filtered to remove excess Zn and the residue was washed (3 ×) with benzene. The combined pyridine and benzene solutions were evaporated at reduced pressure to a small volume and aqueous HCl (1 N, 20 ml) was added dropwise. The solution was heated on a water bath (60 °C) for a short time and allowed to cool to room temperature. Water was added and the mixture was extracted with diethyl ether. The extract contained 6 products according to TLC. The two major products were isolated by chromatography on silica gel using increasing amounts of diethyl ether in benzene (0→5%). One of the products was identical with the compound *3a*. The second product, *4a*, had m.p. 125 – 127 °C. IR ($CHCl_3$): 3585 (hydroxy), 3290, 3100 (oxime), 1650 (C=C), 1382, 1395 (*gem* dimethyl) cm^{-1}. ^1H NMR see Table 2. MS (*m/e*): 183 (M^+). Found: C 64.73; H 9.33; N 7.52. Calc. for $C_{10}H_{17}NO_2$: C 65.54; H 9.35; N 7.64.

Catalytical hydrogenation of compound 2. Compound *2* (0.5 g) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate (30 ml) and tetrahydrofurane (30 ml) using 5% Pd/$SrCO_3$ (0.25 g) as catalyst. When the hydrogen uptake ceased (after 20 h), the catalyst was removed by filtration and the product was separated by chromatography on silica gel using dichloromethane – diethyl ether (20:1). The main constituent, compound *6*, was isolated by crystallization from the first fractions. M.p. 140 – 144 °C. $[\alpha]_D^{20}$ −16.04° (*c* 0.349, $CHCl_3$). IR ($CHCl_3$): 3610, 3450 (hydroxy), 1192 (azodioxy), 1374, 1389 (*gem* dimethyl) cm^{-1}. UV (abs. EtOH): 291 nm (ε 1530). Found: C 65.06; H 10.42; N 7.62. Calc. for $C_{20}H_{38}N_2O_4$: C 64.83; H 10.34; N 7.56.

A minor product (*5*) was isolated by crystallization from the later fractions. M.p. 94 – 103 °C. High resolution MS (*m/e*): 185 (M, $C_{10}H_{19}NO_2$); 170 ($C_9H_{16}NO_2$, M−CH_3); 167 ($C_{10}H_{17}NO$, M−H_2O); 127 ($C_7H_{13}NO$, M−C_3H_6O). The ^1H NMR spectrum is in accordance with the structure *5* showing the presence of both $C_{(1)}-CH_3$ epimers in the mixture.

A minute amount of the compound *9* was obtained from the last fractions.

When the hydrogenation was carried out using palladium on charcoal (30%) in a mixture of ethanol and tetrahydrofurane (1:1) only the compounds *4a* and *5* could be isolated from the reaction mixture.

Thermolysis of the compound 6. The compound *6* (0.02 g) was heated in a tube inserted into a thermoregulated heating block at 150 – 160 °C for 15 min and at 200 °C for another 15 min. The major part distilled and solidified in the non-heated part of the tube. According to TLC the reaction mixture consisted of three components, one of which was unchanged starting material. The major product was isolated by chromatography on silica gel using dichloromethane – diethyl ether (50:1). It was shown to be identical to *5* (TLC, mixed m.p., IR, MS and NMR). The second product was not further investigated.

Alkaline degradation of compound 2. Compound *2* (0.5 g) was added to a solution of sodium hydroxide (2 g) in 96% ethanol (25 ml). The mixture was stirred under reflux. After 25 min the starting material had dissolved. Refluxing was continued for another 90 min. After cooling to room temperature the mixture was neutralized with aqueous H_2SO_4 (2 N) and extracted with diethyl ether. Two products were detected in the reaction mixture by TLC. The products were separated by chromatography on silica gel using benzene – diethyl ether (20:1). A non-crystalline compound (*7*) was isolated from the first fractions. High resolution MS (*m/e*): 165 (M^+, $C_{10}H_{15}NO$). ^1H NMR see Table 2. The major component, compound *8*, was isolated by crystallization from the following fractions. M.p. 135 – 137 °C. $[\alpha]_D^{20}$ −125.7° (*c* 0.212, $CHCl_3$). IR ($CHCl_3$): 3590, 3270 (−OH), 1643 (C=N) cm^{-1}. High resolution MS (*m/e*): 165 (M^+, $C_{10}H_{15}NO$). ^1H NMR see Table 2.

When a crude sample of "*d*-carene nitrosate" was hydrolyzed in the same way, a small amount of an additional non-crystalline product (*11*) was formed.

High resolution MS (*m/e*): 165 (M^+, $C_{10}H_{15}NO$). ^1H NMR see Table 2.

Alkaline degradation of the compound (6). The compound (*6*) (0.2 mg) was added to a solution of sodium hydroxide (1 g) in 96% ethanol (13 ml). The mixture was heated and stirred. The starting material dissolved after 10 min. The reaction mixture was refluxed for 60 min. After cooling the mixture was neutralized with aqueous H_2SO_4 (2 N) and extracted with diethyl ether. The products present in the diethyl ether phase were separated by chromatography on silica gel using dichloromethane–diethyl ether (20:1). The major component, isolated from the first fractions, was identified as compound (*5*) by TLC, mixed m.p., IR, MS and NMR. Two constituents obtained in very small quantities were not further investigated.

Oxidation of isopiperitenol (10). A mixture (80:20) of *trans* and *cis* isopiperitenol (*10*) (0.6 g) was dissolved in acetone (30 ml). Jones reagent was added dropwise under stirring until the color of the reaction mixture was persistently brown. The course of the reaction was followed by TLC. After 3 h, water was added and the acetone and water were partly removed by evaporation under reduced pressure. The remaining mixture was extracted with diethyl ether. After evaporation of the solvent the reaction mixture was chromatographed on silica gel using benzene as eluent. Pure isopiperitenone (*9*) was thus obtained. For ^1H NMR of IX see Table 2.

Oxime 8 from the isopiperitenone 9. A mixture of isopiperitenone (*9*, 0.15 g) in 96% ethanol (5 ml), water (1 ml) and hydroxylamine hydrochloride (0.25 g) was treated with powdered sodium hydroxide (0.5 g) added in portions. The mixture was refluxed for 5 min and then allowed to stand overnight at room temperature. After acidiciation with aqueous HCl (1 N) the reaction mixture was extracted with diethyl ether. According to TLC two products were formed. The mixture was separated by chromatography on silica gel with benzene–ethyl acetate (20:1). The first fractions yielded a minor non-crystalline constituent, which according to its spectral data was assigned to be the compound *12*. For ^1H NMR see Table 2.

The major product was obtained by crystallization from the later fractions. It was shown to be identical with the compound *8* by TLC, mixed m.p., IR, MS and NMR. $[\alpha]_D^{20}$ −122.3° (*c* 0.038, $CHCl_3$).

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Chlorination of Carboxylic Acid Derivatives. VIII. Liquid Phase Chlorination of the Aliphatic C_5-Carboxylic Acids and Their Chlorides, Methyl Esters and Chloromethyl Esters with Chlorine

ILPO O. O. KORHONEN

Department of Chemistry, University of Jyväskylä, Kyllikinkatu 1–3, SF-40100 Jyväskylä 10, Finland

The chlorinations of pentanoic, 3-methylbutanoic, 2-methylbutanoic and 2,2-dimethylpropanoic acids and their derivatives with chlorine in the liquid phase have been investigated. The monochloro products formed were determined by gas-liquid chromatography (GLC) and gas-liquid chromatography–mass spectrometry (GLC–MS) as their esters through comparison with authentic samples. The deactivation of position 2 decreases in the order $COCl > CO_2H > CO_2CH_2Cl > CO_2CH_3$, the effect of the COCl-group in pentanoic acid derivatives being 4.3 times stronger than that of the CO_2CH_3-group. The deactivation is smallest in 2-methylbutanoic acid derivatives owing to the electron-donating methyl group. The EI mass spectra of the methyl and chloromethyl esters have been studied in detail.

In earlier studies we have reported the chlorination of methyl esters of aliphatic C_3-C_{18} n-carboxylic acids,[1-3] propanoyl chloride,[4] butanoyl chloride[5] and its monochloro derivatives,[6] and chloromethyl esters of aliphatic C_3-C_{12} n-carboxylic acids.[7]

Some papers have appeared on the chlorination of pentanoic,[8-14] 3-methylbutanoic[15-18] and 2,2-dimethylpropanoic[18-23] acids and their derivatives, but none on the chlorination of 2-methylbutanoic acid or its derivatives.

This work has been undertaken to study the effect of the COR-group on the isomer distributions of monochloro products formed in the chlorination of aliphatic C_5-carboxylic acids and their derivatives. The EI mass spectra of chlorinated methyl and chloromethyl esters were studied in detail, with deuterium labelling and metastable ion analysis used to elucidate the mechanism of fragmentations.

RESULTS AND DISCUSSION

The structures and notation of compounds studied are given in connection with Table 1. The chlorinations of carboxylic acids and their derivatives were carried out at room temperature by passing dry chlorine through a sample of the neat compound. To minimize the formation of higher chlorinated products, less than an equimolar quantity of chlorine was used. For gas-liquid chromatographic analysis the mixtures of monochloro acids and acid chlorides were converted to methyl esters.

Identification. Products were identified by GLC and GLC-MS, through comparison with separately prepared reference esters, and estimated by GLC without weight response factors.[2] Monochloro chloromethyl esters were identified by GLC as described earlier:[7] acid chlorides were chlorinated, whereafter one part of the monochloro products was esterified with methanol and the other part was converted to chloromethyl esters.[24] The combined mixtures of the esters were analyzed by GLC, the separation of compounds indicating similar isomer distributions. A chromatogram of the mixture of methyl and chloromethyl 2-methylbutanoates is illustrated in Fig. 1.

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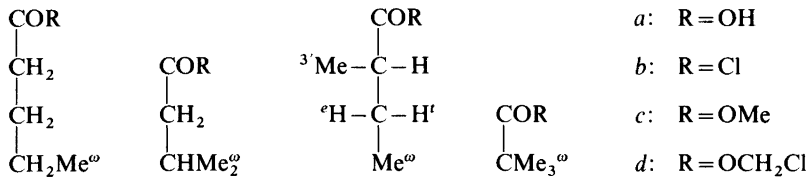
Table 1. Relative reactivity (r_x) of each hydrogen atom ($r_\omega = 100$) in the chlorination of the title compounds.



Cpd.	x	a	b	c	d
1	R	–	–	9	–
	2	14	4.3	21	18
	3	151	101	162	175
	4	282	278	279	304
2	R	–	–	13	–
	2	20	7	29	23
	3	339	357	387	341
3	R	–	–	16	–
	2	76	57	132	110
	3e	200	239	249	275
	3t	183	204	229	235
	3'	29	15	42	29
4	R	–	–	36	–

3-Chloro-2-methylbutanoic acid and its derivatives exist as pairs of diastereomers, the *erythro* and the *threo* forms. The addition of hydrogen chloride to methyl tiglate (methyl *trans*-2-methyl-2-butenoate) leads almost quantitatively to the *erythro* form. GLC analysis of the reaction mixture established the amount of the *threo* isomer to be only 1%. The free-radical substitution in the saturated compound, however, gives nearly equivalent amounts of the isomers (*3c3e* and *3c3t*), which were identified by comparing their behaviours with those of methyl *erythro*- and *threo*-2,3-dichlorobutanoates.[2,5] The following similarities are observed: the *erythro* form has a shorter retention time (GLC) than the *threo* isomer (Fig. 1); most chemical shifts (^{1}H NMR) for the *threo* form are slightly downfield as compared with the *erythro* isomer (see the Experimental section); the *threo* form gives a more abundant peak (MS) at *m/z* 115, M – Cl$^+$, than the *erythro* form, the mass spectra of the compounds being otherwise nearly identical (Table 3).

Isomer distribution. The results of the quantitative analyses of monochloro products formed in the chlorinations are presented in Table 1. The relative reactivity (r_x) of each hydrogen atom of the compounds is given, relative to ω-chloro isomers ($r_\omega = 100$). Values are the averages of two experiments, agreeing within $\pm 3\%$. It can be seen that the deactivating effect of the carbonyl group at the adjacent 2-position decreases in the order $COCl > CO_2H > CO_2CH_2Cl > CO_2CH_3$. The respective amounts of 2-chloro isomer formed in the pentanoic acid series were in the ratios 1.0:3.4:4.2:5.0. In the 2-methylbutanoic acid series, however, the electron-donating 2-methyl substituent strongly decreases this deactivation as well as the disparities between the four compounds, the ratios being 1.0:1.3:2.0:2.3. An opposite influence of the electronegative chlorine substituent at the 2-position has been reported earlier.[4-6] As can be seen from Table 1, the effect of the methyl substituent further away (3-methylbutanoic acid derivatives) is smaller.

Earlier studies have always reported [1,2] the (ω-1)-chloro isomers to be the main chlorination products of methyl esters of aliphatic short- and medium-chain n-acids such as the 4-chloro isomers of

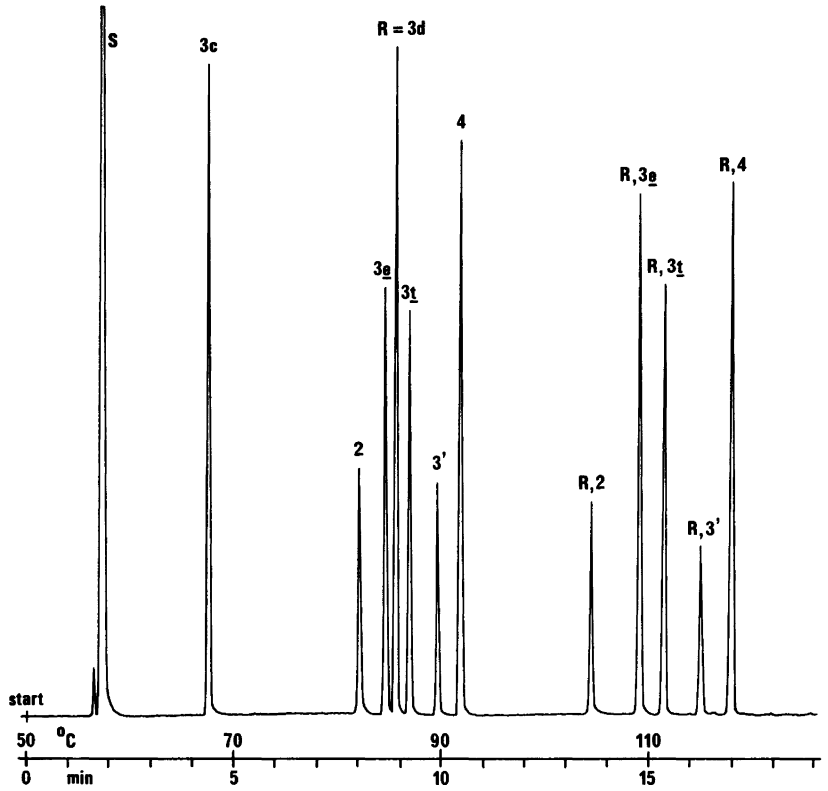


Fig. 1. Mixture of *3c* and its chloro derivatives analyzed by GLC on SE-30. S = solvent; the peak numbers indicate the chlorinated positions.

pentanoic acid derivatives in this work. However, in the case of 3-methylbutanoic acid derivatives, which contain six ω-hydrogen and only one (ω-1)-hydrogen, the main products were the ω-chloro isomers. It has been reported[18] that the chlorinations of 3-methylbutanoic acid and its derivatives give the following ratios of 2-, 3- and 4-chloro isomers: acid 26:69:5, acid chloride 32:60:8 and methyl ester 49:49:2. Although the chlorinations of acid chloride and methyl ester were carried out in the gas phase, the isomer distributions reported must be incorrect, since chlorination temperature has a relatively small effect on the product proportions.[1]

Table 1 shows the chlorinations of 2-methylbutanoic acid (*3a*) and its derivatives (*3b* – *3d*) favour the 3-position, the total amount of stereoisomers being between 44 and 52%. By contrast, substitution at a second 3-position giving 2-chloromethylbutanoic acid derivatives seems to be difficult and in most cases even more difficult than substitution at the 2-position.

The chlorinations of methyl esters lead to a reaction in the methoxyl group, the substitution decreasing with increase in chain length.[1,2] With the branched-chain esters studied in this work the greatest amount of chloromethyl ester was obtained for methyl 2,2-dimethylpropanoate (11%), the quantity being smaller than earlier reported[1] for methyl propanoate (18%).

Mass spectra. The mass spectra of methyl esters and their monochloro derivatives are given in Tables 2 and 3, all peaks greater or equal to 5% of the base peak (100%) being tabulated. Ions containing ^{37}Cl are not shown. Deuterium labelling (trideuteriomethyl esters) and metastable ion analysis were used to elucidate the mechanism of fragmentations.

The molecular ion peaks of chlorinated methyl esters are small and can be seen only at lower

Table 2. 70 eV EI-MS data for Cl-free methyl esters.

Ion	*m/z* >38	Rel. int. ≧5			
		1c	*2c*	*3c*	*4c*
M − Me·	101		8	15	
$M-C_2H_4$	88			74	
M − Et·	87	24		5	
M − MeO·	85	27	18	23	9
$M-C_3H_6$	74	100	79		
M − Me· − CO	73				10
$C_5H_9^+$, $C_4H_5O^+$	69		9	9	
$MeOCO^+$	59	28	34	25	8
$C_4H_9^+$	57	44	25	100	100
$C_4H_8^{\cdot+}$, $C_3H_4O^{\cdot+}$	56	8	6	22	25
$C_4H_7^+$	55	26		16	
$C_2H_5O^+$	45	7		7	
$C_3H_7^+$	43	74	100		15
$C_3H_6^{\cdot+}$, $CH_2CO^{\cdot+}$	42	19	17		
$C_3H_5^+$	41	52	43	78	73
$C_3H_3^+$	39	19	20	24	31

molecular weights.[1] The loss of a methyl radical from the molecular ion is more important in the parent esters than in chloro esters. Judging from the mass spectra of trideuteriomethyl esters, the loss of a methyl radical occurs from the acyl group and never from the methoxyl group. This fragmentation is the most prominent in *3c*; in chloro esters it occurs only in 3-chloro isomers (*1c3*, *2c3*, *3c3* and *4c3*) the peaks being small (< 5 %).

α-Cleavages give in the case of parent esters M − MeO· and M − MeOCO· ions at *m/z* 85 and 57, respectively, the latter peak always being more intense (base peak in *3c* and *4c*). In chloro esters the corresponding fragment ions appear at *m/z* 119 and 91. In all monochloro isomers of methyl 2-methylbutanoate (*3c*) and in methyl 3-chloro-2,2-dimethylpropanoate (*4c3*), and always in 2-chloro esters,[1] the M − MeOCO· ions are more abundant than the M − MeO· ions. In *1c3*, *1c4*, *2c3* and *2c4*, however, the opposite relation is observed.

The McLafferty rearrangement gives, in general, intense peaks (base peak in *1c*, *1c4*, *2c2*, *2c4* and *3c4*) at *m/z* 74 or 88 in the spectra of parent esters and at *m/z* 74, 88, 108 or 122 in those of chloro esters. The isomers substituted at the 2- or 3'-positions can easily by identified and distinguished from the other isomers on the basis of this fragment ion. In the case of 3- and 5-chloro esters, however, the β-cleavage with hydrogen rearrangement gives only small peaks.[26]

The β-cleavage produces $C^{\cdot}\ R^1R^2-CO_2CH_3$ ions in appreciable abundance only in the case of 3-chloro isomers, the fragmentation in *1c3* and *2c3* (base peak in *2c3* at *m/z* 73) being even more important than the McLafferty rearrangement.

γ-Cleavage in the parent esters gives the peak at *m/z* 87 or 101. In chloro esters, except *1c5* (34 %), it is weak and in 2- and 3-chloro isomers the corresponding chlorine-containing fragment ion is missing.

The loss of a chlorine atom from the molecular ion is appreciable only in the 3- and 5-chloro isomers, being intense in stereoisomers (*3c3e* and *3c3t*), 51 and 66 %, respectively. The intensity of the M − Cl· peak shows the greatest variation in the spectra of these different forms. On the basis of metastable ion analysis, there would seem to be losses of small neutral fragments such as CO, CH_2CO and CO_2 from the M − Cl· ion after migration of the methoxyl or methyl group, giving peaks at *m/z* 87, 73 and 71, respectively. Proton transfer from the adjacent carbon atom to the ether oxygen allows elimination of CH_3OH from the M − Cl· ion,[27] giving a peak at *m/z* 83.

The loss of hydrogen chloride from the molecular ion produces the unsaturated methyl ester, the fragmentation in *2c3* being even more important than the loss of Cl·. The loss of CH_3OH after HCl-elimination gives a peak at *m/z* 82, which in *1c3* and *2c3* is more intense than the M − Cl· − CH_3OH

Table 3. 70 eV EI-MS data (rel. int. ≧5) for methyl esters chlorinated at position *x*.

Ion	*m/z* >38	*1c*, *x* =					*2c*, *x* =				*3c*, *x* =						*4c*, *x* =	
		R (=*1d*)	2	3	4	5	R (=*2d*)	2	3	4	R (=*3d*)	2	3′	3*e*	3*t*	4	R (=*4d*)	3
$M-C_2H_4$	122										10	22	22					
$M-C_2H_5^{\cdot}$	121												12					
$M-MeO^{\cdot}$	119			9	13	14			25	12			9	8	11	10		6
$M-Cl^{\cdot}$	115		9	23		18			12				11	51	66			35
$M-HCl$	114			12		8			20					9				5
$M-C_3H_6$	108	12	79	5			11	100										
$M-HCl-Me^{\cdot}$	99													14	14			
$C_4H_8Cl^+$	91		9	6		19		8	18	6		42	18	20	25	14		33
$C_4H_7Cl^{\cdot+}$	90											20						14
$M-C_2H_3Cl$	88													9	15	100		
$M-C_2H_4Cl^{\cdot}$	87					34												
$M-Cl^{\cdot}-CO$	87												67	6				
$C_4H_6O_2^{\cdot+}$	86										15							
$M-ClCH_2O^{\cdot}$	85	55					30				43						15	
$C_4H_7CO^+$	83		11	32	22	22		9	28			7		16	9			10
$C_4H_6CO^{\cdot+}$	82			38	10	20			43	8				9	6			7
$ClCHCO^{\cdot+}$	76		10					20			9							
$M-C_3H_5Cl$	74		6	13	100	19			15	100								
$C_3H_5O_2^+$	73	6		18	10	16			100									16
$C_3H_4O_2^{\cdot+}$	72	13		16		8	14	7										
$C_5H_9^+, C_4H_5O^+$	69						7	8	5		6		6					
$MeCHCl^+$	63		7	6	8			6	5			8	8	9	15			8
$MeOCO^+$	59		57	51	29	51		34	37	34		39	38	75	78	37		25
$C_4H_9^+$	57	34	8		6	13	28				98			26	21	23	100	
$C_4H_8^{\cdot+}, C_3H_4O^{\cdot+}$	56	15	8		11		7		9		24		26	34	39	25	10	53
$C_4H_7^+$	55	27	100	100	72	100	5	39	60	31	15	100	100	100	100	69	5	100
$C_4H_6^{\cdot+}$	54			16	7	11			6				6	7	6			
$C_4H_5^+$	53		11	15	8	10		10	7		5	9	8	14	14	9		10
CH_2Cl^+	49	8	5	6	5	6	8			8	10		5			5	7	7
$C_2H_5O^+$	45	6		8	6								8	6	7	6		
$C_3H_7^+$	43	50	63	28	41	64	56	56	26	42	21	67		25	23	9	7	46
$C_3H_6^{\cdot+}, CH_2CO^{\cdot+}$	42	32	26	13	15	32	27	24	7	26	14		6	7	6	21	33	
$C_3H_5^+$	41	100	45	38	20	51	100	44	68	36	100	24	24	32	35	39	66	34
$C_3H_3^+$	39	29	37	34	22	43	33	52	34	20	37	27	25	42	36	33	32	59

Table 4. 70 eV EI-MS data (rel. int. ≧5) for chloromethyl esters chlorinated at position *x*.

Ion	*m/z* >38	*1d*, *x* =				*2d*, *x* =			*3d*, *x* =					*4d*
		2	3	4	5	2	3	4	2	3′	3*e*	3*t*	4	*x* = 3
$M-C_2H_4$	156								7					
$M-Cl^{\cdot}$	149										25	19		5
$M-C_3H_6$	142	38				72								
$M-C_2H_3Cl$	122										5		54	
$M-CO-Cl^{\cdot}$	121									16				
$M-ClCH_2O^{\cdot}$	119	17	39	41	30	22	66	52	6	16	46	46	44	15
$M-ClCH_2OH$	118		18		15		23	8			12	9		
$M-C_3H_5Cl$	108			70	5		5	100						
$M-C_3H_6Cl^{\cdot}$	107					6	21							
$C_3H_3ClO_2^{\cdot+}$	106	41	9			100								
$M-HCl-ClCH_2^{\cdot}$	99		12									6		
$C_4H_8Cl^+$	91	25	10		26	38	32	18	98	17	46	47	31	85
$C_4H_7Cl^{\cdot+}$	90		17	5	11		41	13	20		13	12		10
$C_4H_6O_2^{\cdot+}$	86										13	14	48	
$C_4H_7CO^+$	83	22	42	35	19	15	32	6	12	11	40	45		21
$C_4H_6CO^{\cdot+}$	82		17		7		15				12	13		7
Me_2CCl^+	77			6			33	8						9
$ClCHCO^{\cdot+}$	76	21				64								
$C_3H_4O_2^{\cdot+}$	72		6	38	8		6	59						
$C_5H_9^+$	69		6			17	8	13	7	7				5
$MeCHCl^+$	63	9	9	19	6	9	6	8	15	8	25	33	7	15
$C_2H_3Cl^{\cdot+}$	62	8	15						5					
$C_4H_9^+$	57		11	7	12		6				6	9		
$C_4H_8^{\cdot+}$	56	11	18	19	10	7	21		7	11	55	78	45	17
$C_4H_7^+$	55	100	100	100	100	63	68	70	100	100	100	100	100	100
$C_4H_6^{\cdot+}$	54		13	6	11		6			6				
$C_4H_5^+$	53	7	9	12	10	9	12	10	10	8	11	14	7	11
CH_2Cl^+	49	33	36	32	32	34	42	45	28	37	29	34	32	34
$C_3H_7^+$	43	38	39	35	33	70	57	36	20	17	67	34		13
$C_3H_6^{\cdot+}$, $CH_2CO^{\cdot+}$	42	9	15	41	30	13	21	58		5	6	13		12
$C_3H_5^+$	41	21	38	59	34	56	100	58	30	30	22	56	62	32
$C_3H_3^+$	39	18	34	30	41	43	53	35	30	28	25	39	19	36

peak at *m/z* 83.

The mass spectra of the chloromethyl esters differ somewhat from the spectra of methyl esters. EI–MS data for parent chloromethyl esters and their chlorinated derivatives are given in Tables 3 and 4. One α-cleavage gives the $M-ClCH_2O^{\cdot}$ ion, which in *1d* (=*1cR*) and *2d* (=*2cR*) at *m/z* 85 is more abundant than the other α-cleavage ion $M-ClCH_2OCO^{\cdot}$ at *m/z* 57. In chlorinated chloromethyl esters, in general, the former is more abundant, whereas in *3d* (=*3cR*) and *4d* (=*4cR*) the latter α-cleavage gives the more intense peak, as in the corresponding methyl esters and in all 2-chloro isomers (very intense, 98%, in *3d2*).

The β-cleavage with hydrogen rearrangement gives peaks of lower intensity in chloromethyl esters than in the corresponding methyl esters (base peak only in *2d4*). The McLafferty rearrangement and subsequent loss of HCl give a very characteristic peak of chloromethyl esters at *m/z* 72, $C_3H_4O_2^{\cdot+}$, or 86, $C_4H_6O_2^{\cdot+}$, the latter in *3d* derivatives. The corresponding chlorine-containing fragment at *m/z* 106 or 120 appears in the spectra of 2-chloro isomers (base peak in *2d2*), but in all 3-chloro esters it is weak owing to the small peak of the McLafferty rearrangement.

The elimination of CH_2ClOH from the $M-Cl^{\cdot}$ ion produces in the spectra of chlorinated chloromethyl esters a peak at *m/z* 83, and the loss of CO from this fragment gives in many cases the base peak at *m/z* 55. The α-cleavage and subsequent (or simultaneous) loss of HCl may also give the fragment ions at *m/z* 55 and 83.

EXPERIMENTAL

Materials and methods. Pentanoic (*1a*) and 3-methylbutanoic acid (*2a*) were commercial products from Fluka, AG. 2-Methylbutanoic (*3a*)[28] and 2,2-dimethylpropanoic acid (*4a*)[29] were obtained by a general method involving the carboxylation of the corresponding Grignard reagent; pentanoyl (*1b*), 3-methylbutanoyl (*2b*), 2-methylbutanoyl (*3b*) and 2,2-dimethylpropanoyl chloride (*4b*) by the reaction of benzoyl chloride with the corresponding acid;[30] methyl pentanoate (*1c*), 3-methylbutanoate (*2c*), 2-methylbutanoate (*3c*) and 2,2-dimethylpropanoate (*4c*) from the corresponding acid chlorides with methanol; chloromethyl pentanoate (*1d*), 3-methylbutanoate (*2d*), 2-methylbutanoate (*3d*) and 2,2-dimethylpropanoate (*4d*) from the corresponding acid chlorides and paraformaldehyde in the presence of a trace amount of zinc chloride.[24]

Authentic methyl monochloro esters were obtained as follows: 2-chloropentanoate (*1c2*), 2-chloro-3-methylbutanoate (*2c2*) and 2-chloro-2-methylbutanoate (*3c2*) from the corresponding 2-chloro acid chlorides[31] with methanol; 3-chloropentanoate (*1c3*), 3-chloro-3-methylbutanoate (*2c3*) and *erythro*-3-chloro-2-methylbutanoate (*3c3e*) from α,β-unsaturated methyl esters[32] (methyl *trans*-2-methyl-2-butenoate and methyl 3-methyl-2-butenoate were prepared from Merck commercial acids) with hydrogen chloride;[33] *threo*-3-chloro-2-methylbutanoate (*3c3t*), 2-chloromethylbutanoate (*3c3'*), 3-chloro-2,2-dimethylpropanoate (*4c3*), 4-chloropentanoate (*1c4*), 4-chloro-3-methylbutanoate (*2c4*) and 4-chloro-2-methylbutanoate (*3c4*) by isolation from the reaction mixtures of monochloro esters obtained by chlorinating the parent esters with chlorine; 5-chloropentanoate (*1c5*) from commercial acid (Merck) by esterification.

The mixtures of monochloro acid chlorides obtained by chlorination of the parent acid chlorides with chlorine were converted to trideuteriomethyl esters with CD_3OD and to authentic chloromethyl monochloro esters with paraformaldehyde.[24] The products were identified by GLC-MS.

The purity of separately prepared esters were checked by GLC and when required the products were purified by preparative GLC and structures confirmed by ^1H NMR and MS.

The chlorinations were carried out at room temperature as described earlier.[1] Monochloro acids were converted *via* thionyl chloride and methanol treatment and acid chlorides with methanol to the corresponding methyl esters. The conversion of isomeric monochloro derivatives was supposed to be nearly similar. Esters were analyzed by GLC, along with the chlorination products of methyl and chloromethyl esters. The amounts of higher chlorinated products were at greatest about 5%.

GLC analyses were run with a Perkin-Elmer Model Sigma 3 gas-liquid chromatograph with a flame ionization detector. A 25 m × 0.22 mm (I.D.) vitreous silica SE-30 WCOT column was used with a nitrogen flow-rate of 1 ml/min. The column temperature was programmed from 50 °C at 4 °C/min, the splitting ratio was 1:20 and the temperatures of injector and detector were 230 and 250 °C, respectively. The chromatographic data were analyzed with a Hewlett-Packard Model 3390A Reporting Integrator using standard programs.

The products were purified by a Perkin-Elmer Model 800 instrument, adapted for preparative work, on a 6 m × 9.5 mm (O.D.) aluminium tube packed with 10% Carbowax 20 M on Chromosorb W (60–80 mesh). Appropriate temperatures were

used, with a nitrogen flow-rate of 120 ml/min.

^1H NMR spectra were obtained on a Perkin-Elmer Model R 12 B 60 MHz spectrometer in carbon tetrachloride solutions using TMS as an internal standard. The spectra will be published later (only the chemical shifts for *3c3e* and *3c3t* are given: s = singlet; d = doublet; c = complex absorption).

Methyl erythro-*3-chloro-2-methylbutanoate (3c3e).* ^{1}H NMR (60 MHz, CCl_4): δ 1.27 (3 H, d), 1.48 (3 H, d), 2.56 (1 H, c), 3.63 (3 H, s), 4.22 (1 H, c).

Methyl threo-*3-chloro-2-methylbutanoate (3c3t).* ^{1}H NMR (60 MHz, CCl_4): δ 1.22 (3 H, d), 1.48 (3 H, d), 2.75 (1 H, c), 3.66 (3 H, s), 4.27 (1 H, c).

Mass spectra. MS and GLC-MS data were recorded with a Varian MAT-212 mass spectrometer connected with a Varian Model 3700 gas-liquid chromatograph. It was equipped with a 25 m × 0.30 mm (I.D.) 5 % SE-54 glass capillary column with a helium flow-rate of 1 ml/min. Electron ionizing energy was 70 eV and ion source temperature 250 °C. Data were acquired and processed on a Spectro System MAT-188. All peaks ($m/z > 38$) greater or equal to 5 % of the base peak (100 %) are tabulated. Metastable transitions were obtained by linked scans using a Varian Metascan unit.

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Structural Studies of Curcuminoids. I. The Crystal Structure of Curcumin

HANNE HJORTH TØNNESEN,[a] JAN KARLSEN[a] and ARVID MOSTAD[b]

[a] Department of Galenical Pharmacy, University of Oslo, Oslo 3, Norway and [b] Department of Chemistry, University of Oslo, Oslo 3, Norway

The crystal and molecular structure of the food-additive 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione (curcumin) has been determined at 121 K by X-ray crystallographic methods using 3368 reflections observed by counter methods. The crystals are monoclinic, space group $P2/n$ with unit cell dimensions $a=20.028(3)$ Å, $b=7.073(1)$ Å, $c=12.609(2)$ Å, $\beta=94.94(1)°$. The structure was refined to a conventional R-factor of 0.055. Estimated standard deviations are 3×10^{-3} Å and 0.2° in interatomic distances and angles when hydrogen atoms are not involved.

The rhizomes of *Curcuma longa* L. (Zingiberaceae) and extracts of the rhizomes are commercial products produced in large quantities.[1] They are used as ingredients in "Indian curries" and as a natural colouring matter in food processing around the world.[2,3] The yellow compounds of *Curcuma longa* belong to the group of diarylheptanoids. Since many countries have issued a ban on the use of synthetic dyes in food and drugs, the interest in natural colouring matter is increasing. Much to our surprise not too much is known about the stability, analysis and structure of the curcuminoids. As a natural colouring agent it is known to be unstable and has been replaced by stable synthetic dyes when possible.

In addition curcumin is known to possess important pharmacological properties.[4,5] It is known to relieve gastro-intestinal disorders.

During our investigations on the analysis of curcumin and extracts of *Curcuma longa* L. the response of the coloured compounds during HPLC analysis differed from what was expected.[7] The compounds gave considerable negative peaks, not stable signals *etc.* A reason for this may be the fluorescence of the compounds. Curcumin is also known for its ability to make complexes with other molecules and this may also count for the anomalous behaviour during HPLC analysis.

Curcumin is a symmetrical molecule, but – surprisingly – biosynthetic experiments indicate that two different pathways, *e.g.* the phenylpropane and the acetate pathways, contribute to the formation of the molecule.[6] Furthermore, there seems to be some dispute as to which structure can be given to curcumin.[11,13] We therefore found it of interest and a necessity to investigate this before further analysis on the stability could be undertaken.

EXPERIMENTAL

Deep red plate-formed crystals of curcumin were prepared by recrystallization of a sample of Koch-Light quality 1324 h. The compound was dissolved in ethanol at 70 °C in the dark. A small amount of water was added to the solution and after a few days in the refrigerator the crystals separated. A diamond shaped crystal of dimensions $0.6\times0.4\times0.1$ mm. was used for the experimental procedure which is described in 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 $\sigma(I)=[C_T+(0.02C_N)^2]^{\frac{1}{2}}$, 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. The variations in the intensities of the test reflections were between 1 to 2 % and no corrections were made on this basis. Scattering factors used were those of Doyle and Turner[8] for O and C, and of Stewart, Davidson and Simpson[9] for H.

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CRYSTAL DATA

Curcumin, $C_{21}O_6H_{20}$, monoclinic, $a=20.028(3)$ Å, $b=7.073(1)$ Å, $c=12.609(2)$ Å, $\beta=94.94(1)°$, $V=1779.6$ Å^3, $M=368.37$, $Z=4$, $F_{(000)}=776$, space group $P2/n$.

EXPERIMENTAL CONDITIONS

Instrument	SYNTEX $P\bar{1}$
Radiation	Graphite Crystal Monochromated MoK_α $\lambda=0.71069$ Å
Crystal dimensions/mm	$0.6\times0.4\times0.1$
Scanning mode	$\theta/2\theta$
Scan speed/° min^{-1}	3–6 depending on intensity
Scan range/°	$2\theta_{\alpha_1}-1.1$ to $2\theta_{\alpha_2}+1.3$
Background counts	For 0.35 of scan time at scan limits
Temperature/K	121
2θ range/°	$2<2\theta<60$
Number of reflections meas.	3774
Number of reflections $I>2.5\sigma(I)$	3331
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,[10] and a successive Fourier synthesis indicated the positions of all the non-hydrogen atoms. The positions of 17 of the 20 hydrogen atoms were readily found from a difference synthesis. The positions of the hydrogen atoms at O1 and O6 were introduced from con-

Table 1. Fractional atomic coordinates and thermal parameters multiplied by 10.[4] The anisotropic temperature factor is given by $\exp -2\pi^2(U_{11}a^{*2}h^2+\cdots+2U_{12}a^*b^*hk+\cdots)$. Estimated standard deviations in parameters are given in parentheses.

Atom	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O1	5993(1)	6670(2)	0517(1)	198(7)	464(11)	187(7)	39(7)	53(6)	−8(7)
O2	4776(1)	6297(2)	1202(1)	164(7)	435(10)	215(8)	−11(7)	30(6)	−45(7)
O3	5823(1)	8366(2)	7055(1)	128(7)	468(11)	197(7)	9(7)	32(5)	−16(7)
O4	5307(1)	8721(2)	8734(1)	165(7)	478(11)	204(8)	9(7)	32(6)	−31(7)
O5	2272(1)	11231(2)	11201(1)	156(7)	413(10)	345(9)	−53(7)	90(6)	−102(8)
O6	1352(1)	8440(2)	11107(1)	159(7)	392(10)	352(9)	−33(7)	135(6)	−20(8)
C1	5278(1)	6701(3)	1970(2)	168(9)	143(12)	209(10)	16(9)	25(8)	−11(9)
C1	5278(1)	6701(3)	1970(2)	168(9)	143(12)	209(10)	16(9)	25(8)	−11(9)
C2	5211(1)	6928(3)	3040(2)	151(9)	267(12)	211(10)	19(9)	48(8)	−8(9)
C3	5778(1)	7307(3)	3750(2)	164(9)	238(12)	202(10)	36(9)	43(8)	2(9)
C4	6406(1)	7452(3)	3347(2)	139(9)	315(13)	219(10)	−38(9)	12(8)	−7(10)
C5	6473(1)	7247(3)	2267(2)	146(9)	320(13)	236(11)	43(9)	62(8)	−1(10)
C6	5917(1)	6866(3)	1577(2)	207(10)	251(12)	170(10)	35(9)	49(8)	−1(9)
C7	5738(1)	7569(3)	4889(2)	164(9)	271(12)	194(10)	29(9)	12(8)	−20(9)
C8	5186(1)	7651(3)	5429(2)	169(9)	296(13)	193(10)	19(9)	28(8)	−10(9)
C9	5231(1)	8014(3)	6565(2)	175(9)	240(12)	201(10)	35(9)	39(8)	18(9)
C10	4662(1)	8014(3)	7139(2)	138(9)	275(13)	219(10)	2(9)	53(8)	−11(9)
C11	4717(1)	8399(3)	8226(2)	181(10)	232(12)	238(11)	32(9)	61(8)	13(9)
C12	4154(1)	8537(3)	8881(2)	192(10)	292(13)	193(10)	27(9)	55(8)	−9(10)
C13	3540(1)	7835(3)	8601(2)	187(10)	297(13)	135(11)	22(9)	68(8)	−16(10)
C14	2962(1)	8052(3)	9235(2)	157(9)	286(13)	226(10)	18(9)	41(8)	16(9)
C15	2926(1)	9566(3)	9940(2)	124(9)	314(13)	250(11)	−8(9)	46(8)	8(10)
C16	2382(1)	9745(3)	10550(2)	142(9)	296(13)	216(10)	−10(9)	33(8)	−26(10)
C17	1883(1)	8345(3)	10490(2)	126(9)	340(13)	233(10)	−3(9)	54(8)	50(10)
C18	1911(1)	6859(3)	9793(2)	144(9)	316(14)	311(12)	−36(9)	40(8)	11(10)
C19	2448(1)	6717(3)	9154(2)	201(10)	293(13)	289(12)	1(10)	51(9)	−25(10)
C20	4103(1)	6329(4)	1522(2)	159(10)	425(15)	263(11)	−12(10)	19(8)	−19(11)
C21	2729(1)	12788(4)	11209(2)	252(11)	259(15)	353(13)	−36(11)	71(10)	−67(11)

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

	X	*Y*	*Z*	*B*
H2	0.478(1)	0.685(3)	0.330(2)	2.4
H4	0.679(1)	0.776(3)	0.386(2)	2.3
H5	0.689(1)	0.734(3)	0.197(2)	1.8
H7	0.615(1)	0.777(3)	0.352(2)	1.7
H8	0.473(1)	0.746(3)	0.506(2)	1.6
H10	0.426(1)	0.780(3)	0.678(2)	2.6
H12	0.427(1)	0.924(3)	0.953(2)	1.6
H13	0.347(1)	0.707(3)	0.796(2)	1.3
H15	0.325(1)	1.056(4)	1.001(2)	2.3
H18	0.157(1)	0.592(4)	0.973(2)	2.8
H19	0.245(1)	0.566(3)	0.862(2)	2.0
H201	0.383(1)	0.610(3)	0.086(2)	1.8
H202	0.397(1)	0.764(4)	0.181(2)	3.1
H203	0.405(1)	0.536(3)	0.205(2)	2.3
H211	0.255(1)	1.385(4)	1.159(2)	3.0
H212	0.277(1)	1.320(3)	1.044(2)	2.9
H213	0.315(1)	1.236(3)	1.166(2)	4.0
HO1	0.569(2)	0.693(5)	0.018(3)	7.2
½HO3	0.577(2)	0.848(6)	0.764(3)	1.6
½HO4	0.560(2)	0.869(6)	0.822(4)	2.5
HO6	0.134(1)	0.947(3)	1.141(2)	3.0

siderations of the hydrogen bond system. A final difference map suggested two possible positions for the last hydrogen atom supposed to be attached to the O3 or O4 atom. The best result was obtained by placing a half hydrogen atom in each of the two positions indicating a statistical distribution of the hydrogen atom at the two positions. 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 an *R*-factor of 0.055 and a goodness of fit $S=(\Sigma w\Delta^2/m-n)^{\frac{1}{2}}=2.52$.

The final parameters are given in 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 indicated in Fig. 1, the bond lengths and angles are given in Table 3 and some torsion angles in Table 4. Fig. 1 illustrates the molecule as it appears in the crystal as well as the molecular packing and the hydrogen bond

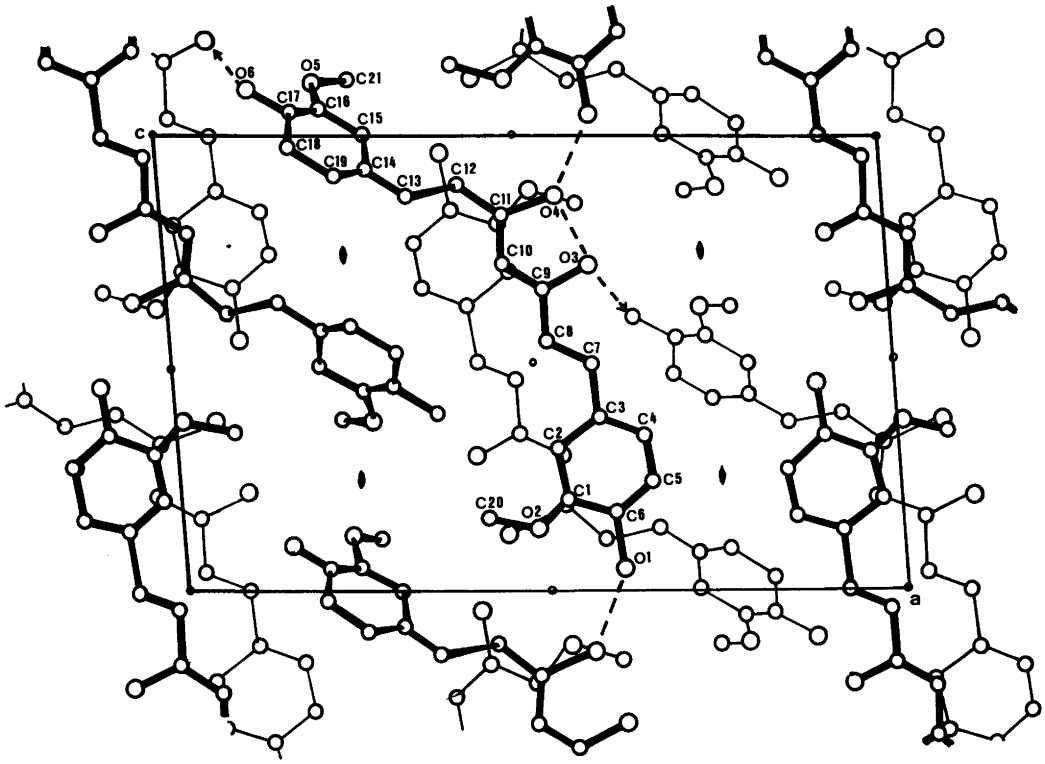

Fig. 1. Numbering of the atoms and the molecular packing in crystals of curcumin.

Table 3. Bond lengths and angles in curcumin. Estimated standard deviations are given in parentheses.

Bond lengths (Å)		Bond angles (°)	
O1 – C6	1.364(2)	C20 – O2 – C1	116.7(2)
O2 – C1	1.366(2)	O2 – C1 – C2	126.2(2)
O2 – C20	1.440(2)	O2 – C1 – C6	113.7(2)
C1 – C2	1.377(3)	C1 – C2 – C3	120.2(2)
C2 – C3	1.410(3)	C2 – C3 – C7	122.7(2)
C3 – C4	1.401(3)	C2 – C3 – C4	118.9(2)
C4 – C5	1.388(3)	C3 – C4 – C5	120.9(2)
C5 – C6	1.379(3)	C4 – C3 – C7	118.4(2)
C6 – C1	1.417(3)	C4 – C5 – C6	120.0(2)
C3 – C7	1.457(3)	C5 – C6 – O1	119.1(2)
C7 – C8	1.348(3)	O1 – C6 – C1	120.9(2)
C8 – C9	1.450(3)	C3 – C7 – C8	128.3(2)
C9 – O3	1.312(2)	C7 – C8 – C9	121.5(2)
C9 – C10	1.403(3)	C8 – C9 – O3	118.4(2)
C10 – C11	1.392(3)	C8 – C9 – C10	121.7(2)
O4 – C11	1.316(2)	O3 – C9 – C10	119.9(2)
C11 – C12	1.457(3)	C9 – C10 – C11	120.7(2)
C12 – C13	1.344(3)	C10 – C11 – O4	120.3(2)
C13 – C14	1.471(3)	C10 – C11 – C12	124.9(2)
C14 – C15	1.397(3)	O4 – C11 – C12	114.8(2)
C15 – C16	1.392(3)	C12 – C13 – C14	124.3(2)
O5 – C16	1.364(2)	C13 – C14 – C15	120.8(2)
O5 – C21	1.432(3)	C13 – C14 – C19	120.0(2)
C16 – C17	1.404(3)	C14 – C15 – C16	120.5(2)
O6 – C17	1.372(2)	C15 – C16 – C17	119.6(2)
C17 – C18	1.375(3)	C15 – C16 – O5	125.3(2)
C18 – C19	1.402(3)	C16 – O5 – C21	117.5(2)
C19 – C14	1.394(3)	C16 – C17 – C18	120.3(2)
		C16 – C17 – O6	121.2(2)
The mean value of		O6 – C17 – C18	118.5(2)
the X – H distances		C17 – C18 – C19	119.9(2)
C – H	0.98(3)	C18 – C19 – C14	120.4(2)
O – H	0.81(7)	C19 – C14 – C15	119.2(2)

Table 4. Torsion angles in curcumin.

Angle	(°)
C20 – O2 – C1 – C2	7.7
C2 – C3 – C7 – C8	– 5.7
C3 – C7 – C8 – C9	– 176.9
C7 – C8 – C9 – C10	– 177.0
C7 – C8 – C9 – O3	3.7
C8 – C9 – C10 – C11	178.8
O3 – C9 – C10 – C11	0.5
C9 – C10 – C11 – O4	– 1.7
C9 – C10 – C11 – C12	176.4
C10 – C11 – C12 – C13	18.4
O4 – C11 – C12 – C13	– 163.3
C11 – C12 – C13 – C14	– 176.9
C12 – C13 – C14 – C15	25.2
C15 – C16 – O5 – C21	4.1

system. The molecule may be described as consisting of three substituted planar groups interconnected through the two double bonds C-7 – C8 and C12 – C13. The two terminal groups are identical but the inherent symmetry of the molecule is distorted in the crystal by a rotation of – 162° about the C11 – C12 bond.

Electron delocalization and intramolecular hydrogen bonding in the fragment – CO – HC = COH – has been studied in a number of molecules.[13] Of the possible tautomeric forms it appears that in the crystal phase, the β-diketones prefer the *cis*-enol configuration stabilized by a strong intramolecular H-bond. This hydrogen bond appears moreover invariably to be asymmetrical, the hydrogen atom always found to be bonded to one unique oxygen atom. A possible exception to this may exist

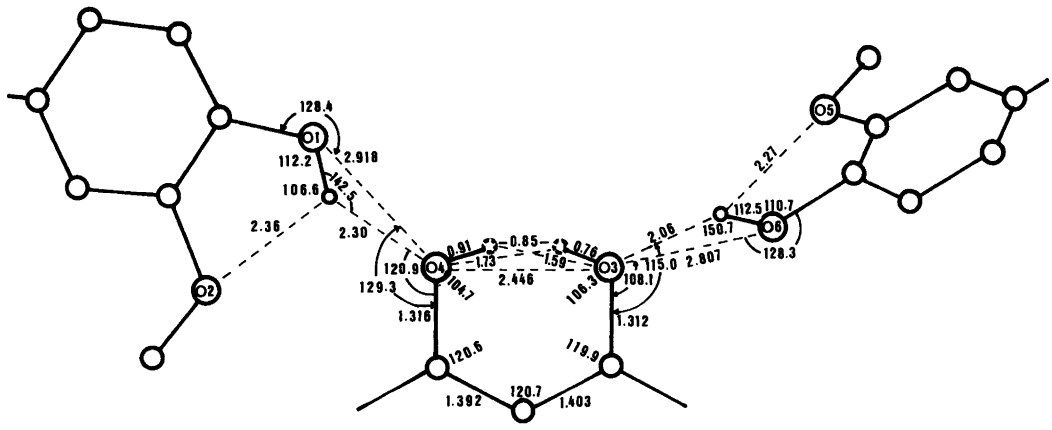

Fig. 2. Geometry in the enol ring and the hydrogen bond system.

in dibenzoylmethane[13] even if the asymmetry in the two C–O bonds seems to be persistent.

In the present structure, the oxygen atoms of the enol-ring are engaged in intermolecular as well as in intramolecular hydrogen bond and the geometry of this particular group and the hydrogen bond system is illustrated in Fig. 2. It may be noticed that both the phenolic hydrogen atoms are oriented towards the neighbouring oxygens, the torsion angles C1–C6–O1–HO1 and C16–C17–O6 –HO6 being 27 and 9°, respectively. The asymmetry of the external angles at C1 and C6 are as expected for such conformations. It may also be seen from Fig. 2 that there are no significant differences in the C–C or the C–O bonds in the enol ring. Furthermore, the best model to fit the data is the one with the hydrogen atom statistically distributed between the two oxygen atoms.

A certain conjugation between the aromatic ring I and the pseudo aromatic ring II seems to be indicated by the distances between the atoms connecting the two ring systems which also are essentially coplanar, the angle between the two ring planes being only about 3°. The interaction between the π-electron systems in ring II and III is probably somewhat less as the angle between these two ring planes is about 45°. The significant difference in the two bond lengths C3–C7 and C13–C14 may be a result of the rotation of 25° about the C13–C14 bond. The total twist of the molecule is indicated by the angle of 47° between ring planes I and III. It is interesting to notice that in the structure of dibenzoylmethane[12] there is a similar difference in the ring twists relative to the enol ring plane; namely −3.8 and 16.9°.

The pseudo aromatic character of the enol ring is finally reflected in the molecular packing in the crystal (Fig. 1). Molecules related by a center of symmetry are stacked along the direction of the *b*-axis, the distance between molecular planes being about 3.45 Å and the respective atomic distances being 3.475 *Å* (C1---C11), 3.517 *Å* (C1---C10), 3.513 *Å* (C2–C10) and 3.650 *Å* (C2–C9). The stacks are finally bonded together by the hydrogen bonds.

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Addition of Maleic Anhydride to Esters of Mono-unsaturated Fatty Acids

KRISTER HOLMBERG and JAN-ALLAN JOHANSSON

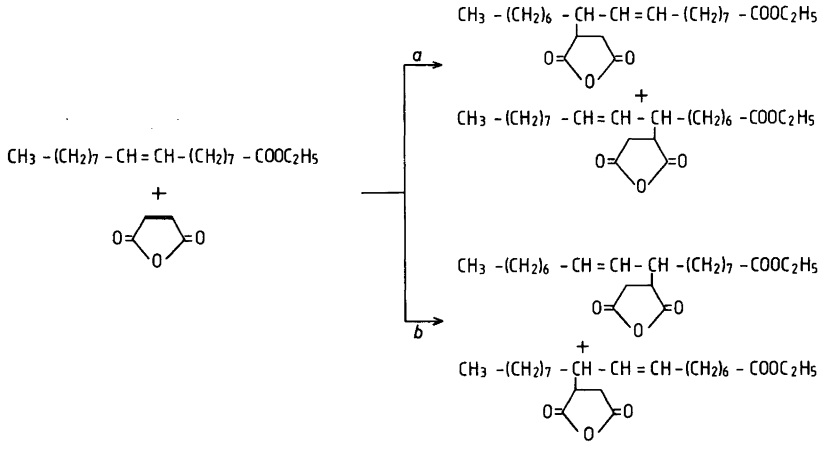
EKA AB, S-445 01 Surte, Sweden

Maleic anhydride adds to ethyl oleate and ethyl elaidate at elevated temperatures to form 1:1 adducts. The product compositions of these reactions are clarified by oxidative cleavage of the remaining olefinic bond of the adducts and subsequent analysis of the products. In both cases, an addition reaction with retention of the double bond of the fatty acid competes with the ene reaction which is the expected mode of addition.

The reaction between maleic anhydride and unsaturated systems is well known and the process, when applied to fatty acids, is referred to by the term "maleinization".[1] The reactions which occur during maleinization fall into two major categories. With conjugated systems, capable of attaining a *cisoid* configuration of the double bonds, a Diels-Alder reaction takes place at 60–80 °C. With systems containing isolated double bonds, on the other hand, temperatures around 200 °C are required and a complex mixture of products is usually obtained.

Early investigations showed that methyl oleate reacts with 1 mol, methyl linoleate with 2 mol and methyl linolenate with 2.5 mol of maleic anhydride at 200–210 °C.[2] Analysis indicated that the degree of unsaturation of the fatty acids was not affected by the reaction; the composition of the products, however, proved to be more difficult to elucidate.

Although the simplest maleinization, that of oleic acid, has been widely studied, neither the reaction mechanism, nor the product composition is clarified today. Two different routes leading to different reaction products have been proposed. In the first of these, the reaction occurs at the allylic positions in the fatty acid, leading to reaction products with the unsaturation retained at the 9–10-position (Scheme 1, reaction *a*).[2,3] By the alternative route, an ene reaction takes place, giving rise to products



Scheme 1. Maleinization of ethyl 9-octadecenoate proceeding by two different routes.

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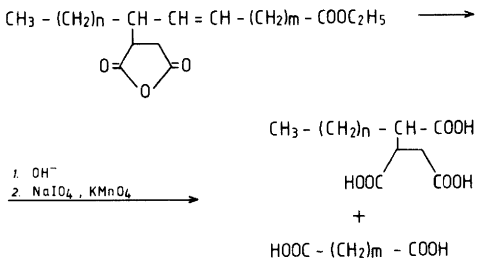


Scheme 2. Oxidative cleavage of the addition product of maleic anhydride and ethyl 9-octadecenoate.

where the double bond of the fatty acid has undergone an allylic shift (Scheme 1, reaction *b*).[4,5] It has also been claimed that both types of reactions take place simultaneously in the maleinization.[6,7] The present work aims at providing a contribution to the understanding of this process.

RESULTS AND DISCUSSION

Ethyl oleate (ethyl *cis*-9-octadecenoate) was treated with maleic anhydride at 210 °C. The anhydride ring was opened with alkali and the reaction product was oxidized in an aqueous solution of periodate containing catalytic amounts of permanganate (Scheme 2).[8] Under these conditions, the olefins are readily cleaved to carboxylic acids.

If the addition of maleic anhydride proceeds according to reaction *a* of Scheme 1, the reaction sequence of Scheme 2 would give nonanoic acid and nonanedioic acid, as well as succinic acid derivatives from the substituted part of the ethyl oleate. If, on the other hand, the addition follows path *b* of Scheme 1, a mixture of octanoic acid and octanedioic acid would be obtained along with succinic acid derivatives.

Analysis by GLC shows an approximate 1:2 ratio of cleavage products according to reactions *a* and *b* (Table 1). Obviously, the reaction is more complex than many related addition reactions.[9]

There seems to be two possible explanations of the product composition:

(1) The addition of maleic anhydride is purely a free radical chain reaction. Assuming a restricted rotation about the carbon–carbon bonds in the allyl radical residue,[15] two pairs of *cis-trans* isomers will be formed. After oxidative cleavage, a mixture of C-8 and C-9 carboxylic acids will be obtained (Scheme 3).

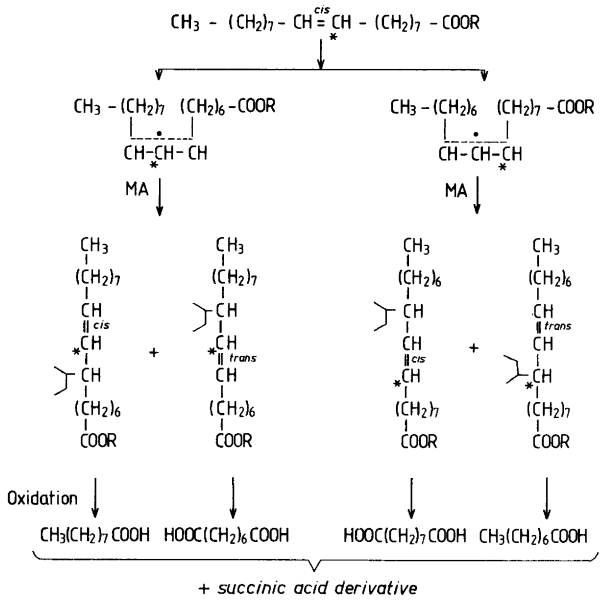
(2) An ene reaction takes place parallel to the free radical chain reaction of Scheme 3. The ene reaction gives only the cleavage products corresponding to a movement of the double bond, *i.e.* octanoic acid and octanedioic acid along with the succinic acid derivatives.

At first sight, the latter hypothesis, *i.e.* that of two simultaneous reactions, seems attractive since this would explain the predominance of the products resulting from migration of the olefinic bond in the fatty acid. However, since the double bond shifts of Scheme 3 involve changes from the original *cis* to the more stable *trans* configuration, the free radical chain route would also be expected to favour the formation of C-8 acid derivatives.

In order to obtain more information about the reaction mechanism, the maleinization was carried out with the ethyl ester of elaidic acid, the *trans* isomer of oleic acid. In a free radical chain reaction, there would, in this case, be no special driving force for a double bond shift since the double bond of the allylic starting material already possesses the *trans* configuration. Hence, if the reaction proceeds according to alternative (1) above, cleavage

Table 1. Relative amounts of C-8 and C-9 acids after maleinization of ethyl 9-octadecenoates and subsequent oxidative cleavage.

Starting products	Fission products (%)			
	Octanoic acid	Octanedioic acid	Nonanoic acid	Nonanedioic acid
Ethyl oleate	28	36	12	24
Ethyl elaidate	46	41	5	8
Ethyl oleate with radical inhibitor	41	42	6	11



Scheme 3. Addition of maleic anhydride (MA) to ethyl 9-octadecenoate by a free radical mechanism followed by oxidative cleavage of the adducts.

products derived from adducts with retention of the double bond (C-9 acids) would be expected to be at least equal in amount to those derived from adducts with an olefinic shift (C-8 acids).

In an ene reaction elaidic acid derivatives would be expected to react faster than their *cis* isomers with maleic anhydride.[10] Whereas the *trans* olefin can easily attain the favoured *exo* transition state, the *cis* isomer must react either *via* a sterically hindered *exo* approach or *via* an *endo* approach. This is reflected in a lower entropy of activation of the *trans* isomer.[10] Consequently, if the ene reaction contributes to the product composition, a higher ratio of C−8 to C−9 fission products, as compared with the reaction with ethyl oleate, would be expected.

The fission products obtained with ethyl elaidate after treatment with maleic anhydride and subsequent oxidation according to Scheme 2 appears from Table 1. In this case, the ratio of C−8 to C−9 acids is around 6:1, indicating a strong preference for the ene reaction. Although direct experimental proofs are lacking, it now seems reasonable to assume that the ene reaction contributes to the product composition also in the addition to the oleate ester. The relative amounts of ene reaction products *vs.* products from a free radical chain mechanism, however, cannot be determined from these results.

The addition of maleic anhydride to ethyl oleate was also performed in the presence of 2,6-di-*tert*-butyl-4-methylphenol, which is known to be an efficient radical chain inhibitor. The ratio of C−8 to C−9 acids was then considerably increased compared to the reaction without inhibitor, as can be seen from Table 1. This result supports the view that the product composition is the result of two competing types of reaction. It is interesting that also in the presence of the phenolic inhibitor the free radical chain reaction takes place to a considerable extent.

Consequently, this work implies that the maleinization of the ethyl esters of 9-octadecenoic acids proceeds by two competing modes of addition: a stepwise reaction, having a free radical chain mechanism, and an ene reaction. This work does not differentiate between a concerted or a diradical mechanism of the latter reaction.

The fact that addition *via* a free radical chain mechanism competes so favourably with the ene reaction in the addition of maleic anhydride to ethyl oleate may seem surprising. Alder and coworkers, who were the first to recognize the two modes of addition, coined the terms "direkte" and "indirekte substituirende Additionen" to describe the addition

involving double bond retention and double bond shift, respectively.[11] They claim that "die indirekte substituirende Addition offenbar den Normalfall aller Anlagerungen von Maleinsäure-anhydrid an Monoolefin-kohlenwasserstoffe vorstellt". Even more categorical statements in favour of the ene reaction have been made by other authors.[12] However, the reactions described in Alder's works, as well as in later studies of the addition of maleic anhydride to monoolefins, where the product compositions have been clarified,[9,13] almost invariably involve olefins with terminal double bonds or with double bonds adjacent to a terminal methyl group. In both cases the ene reaction is particularly favoured: in the former structure because of reduced steric hindrance in the *suprafacial* approach of the reactants and in the latter structure because of the particular facility with which a primary hydrogen atom is abstracted.[9] On the other hand, the ene reaction is known to be promoted by electron-rich ene components with a high HOMO energy level, a fact which should favour disubstituted olefins.

The energy factor, however, has earlier been found to be over-shadowed by steric requirements in the formation of the transition state.[10] It may be that with relatively big ene components, having a *cis* double bond in a central position, the most favoured geometry of approach of the reactants is difficult to attain, thus enabling other modes of addition to take place.

The order of reactivity in the ene reaction of the *cis*- and *trans*-olefins found in this work is opposite to that suggested by Hoffmann for additions of maleic anhydride to olefins.[9] It is, however, in accordance with later kinetic studies of the ene reaction.[10]

In order to ensure that ethyl oleate does not undergo a *cis, trans* isomerization prior to reacting with maleic anhydride, a model experiment was carried out in which the ester was treated with phthalic anhydride under the same conditions as were used for maleic anhydride. After completed treatment, the starting materials were intact; GLC analysis showed no traces of ethyl elaidate.

EXPERIMENTAL

Addition of maleic anhydride to ethyl oleate and ethyl elaidate. The ethyl ester of 9-octadecenoic acid (*cis* or *trans*, 6.21 g, 0.020 mol) was heated at 210 – 220 °C with maleic anhydride (2.94 g, 0.030 mol) for 2 h in a nitrogen atmosphere under reflux.

Oxidative cleavage of the adducts.[8] The reaction product from the above addition was treated with 100 ml KOH in ethanol for 16 h at 23 °C. The solvent was evaporated, 1000 ml water was added, the pH was adjusted to 7.5 – 8.0 with HCl and a mixture of $NaIO_4$ (34.2 g, 0.16 mol) and $KMnO_4$ (1.69 g, 0.011 mol) was added. After 20 h at 23 °C, the solution was made acidic with 10% sulfuric acid and extracted with diethyl ether. The ether extract yielded 8.67 g of an oil.

Analysis of the fission products. The residue from the oxidative cleavage above was treated with ethanol (5.52 g, 0.12 mol) and dicyclohexylcarbodiimide (24.7 g, 0.12 mol) in pyridine (80 mol) in the presence of a catalytic amount of p-toluenesulfonic acid (0.80 g). After 20 h at 23 °C, acetic acid (2 g) was added and the mixture was kept at 4 °C for 16 h. After the work-up procedure given in Ref. 14, an oily residue was obtained. GLC was performed using 3% SP-2310 on 100/120 mesh Supelcoport, a temperature profile of 8 °C/min and an interval of 80 – 200 °C. The pure ethyl esters of octanoic, nonanoic, octanedioic and nonanedioic acid were used as references.

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Short Communications

The Importance of Hydrophobic Interactions in the Antagonist Binding to the Muscarinic Acetylcholine Receptor

JAAK JÄRV[a,*] and TAMAS BARTFAI[b]

[a] Department of Organic Chemistry, Tartu State University, 202 400 Tartu, Estonian SSR, USSR and [b] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The list of the muscarinic antagonists has been largely extended since Dale defined muscarinic receptors on the basis of stimulation by muscarine and blockade by atropine.[1] Similarly to acetylcholine or atropine all of these compounds possess quaternary ammonium group, hydrophobic substituents and often also polar fragments like ether, ester or alcohol groups.[2,3] Despite extensive studies it is not known which physico-chemical and structural factors govern the binding effectiveness of the muscarinic antagonists to the receptor site and are responsible for the more than millionfold variation in affinity of these ligands.[2,3] In the present communication the role of hydrophobic interaction in the ligand – receptor complex formation is characterized and two principally different mechanisms of hydrophobic binding are identified.

The hydrophobic properties of antagonists were characterized proceeding from the octanol – water partition system and the effective hydrophobicity constants π' for the whole ligand molecule were calculated from the fragmental constants, making use of the tabulated f-parameters,[4] as described by Rekker *et al.*[4,5] As all compounds used in the following analysis involve a quaternary nitrogen atom, its contribution was not taken into account and thus all calculated π-constants are equally shifted relative to the actual log P values for antagonists.

It is well established that binding of the muscarinic antagonists to the receptor follows the mass-action law for simple equilibrium (eqn. 1),[2,3]

$$R + A \overset{K_d}{\rightleftharpoons} RA \qquad (1)$$

and thus the constants pK_d refer to the free energy of the binding reaction and can be used in LFE relationships. The experimental K_d values for muscarinic receptor from rat brain were selected from literature[2,3,6,7] to compile systematic series of antagonists in which hydrophobicity can be considered as a single variable structural factor. Altogether, the following series were constructed: *a*, alkyl- or arylsubstituted ammonium ions; *b*, carboxylic esters containing ammonium group in the alcohol portion; *c*, esters of hydroxyacids, containing ammonium group in their alcohol portion.

The compounds included in these three series are shown in Table 1.

Within each of these series the binding affinity (K_d) of antagonists is governed by hydrophobicity of the drug molecule and the linear relationships

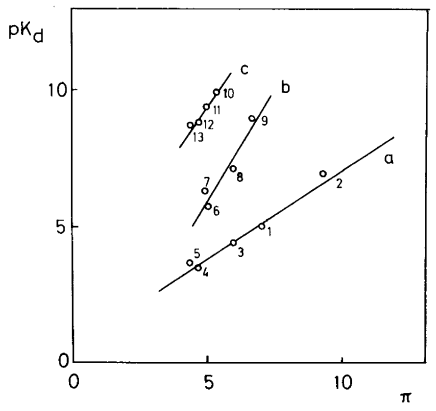

Fig. 1. Linear relationship between pK_d values, derived from ligand binding experiments and the hydrophobicity constants π'. Three series, *a*, *b*, and *c*, were constructed.

* Part of this work was carried out in Stockholm during Dr. Järv's sabbatical visit within the frame of the scientific exchange program between the Royal Swedish Academy of Sciences and Soviet Academy of Sciences.

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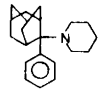
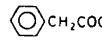
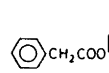
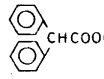
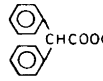
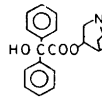
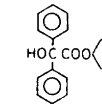
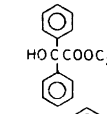
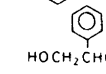
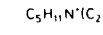
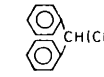
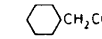
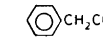
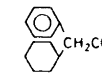
Table 1. The structural formulae of the compounds included in the study and the available pK_d and pK'_d values derived from binding and bioassay experiments, respectively. Compounds *1* – *5* constitute series *a*, *6* – *9* series *b*, *1* – *5* and *14* – *17* series *d*, *6* – *9* and *18* – *20* series *e*.

	Formula	pK_d	pK'_d	π'
1		5 04	4.64	7 2
2		7 03	7 02	9 2
3		4 45	4 77	5 9
4		3 53	2 70	4 6
5		3 77	3 49	4 3
6		6 34	6 20	4 9
7		5 80	5 50	5.0
8		7 20	7 16	5 9
9		9 00	9 06	6 6
10		9 85	9 8	5 25
11		9 46		4 9
12		8 85		4 6
13		8 80		4 35
14			4 59	6 4
15			5 18	6 5
16			5 39	7 6
17			7 02	8 2
18			5 07	5 3
19			4 53	4 25
20			8 44	7 0

between pK_d and π'-values can be described by a single-parameter, eqn. (2),[8] where ϕ is the intensity

$$pK_d = C + \phi_{\pi'} \qquad (2)$$

factor of the hydrophobic effect (Fig. 1). Thus it is assumed that all parts of the antagonist molecules are involved in hydrophobic interaction with the receptor site which must be large enough to accommodate the bulky substituents. (The present data do not allow more detailed analysis of the binding site "topography".) The following ϕ-values were obtained for series $\phi_a = 0.7 \pm 0.2$; $\phi_b = 1.4 \pm 0.3$; $\phi_c = 1.4 \pm 0.4$, respectively.

Proceeding from these values of the intensity factor of the hydrophobic effect the muscarinic antagonists can be clearly divided into two groups; with $\phi > 1$ and $\phi < 1$.

The number of ligands involved in the analysis can be increased if not only binding data are used but also affinity constants determined by measurement of the contractile response of the guinea pig ileum are used. Appropriate data can be found in the work of Abramson *et al.*[9] on a large number of antagonists. It can be shown that there is a linear interrelationship with slope of approximately one between the pK_d and pK'_d values, obtained from direct binding experiments

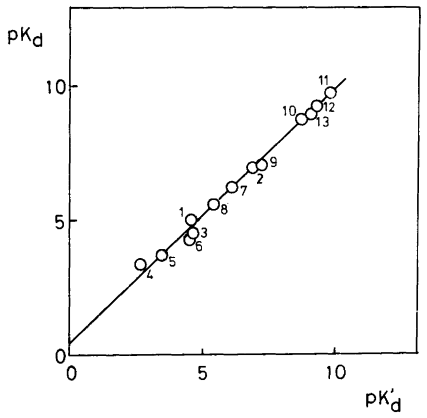


Fig. 2. The linear relationship between pK_d and pK'_d values derived from binding experiments or from bioassay on guinea-pig ileum, respectively.

to the receptor from rat brain and by measurements at the guinea pig ileum, respectively (Fig. 2). Thus the latter constants (pK'_d) also characterize the free energy of the ligand binding to the muscarinic receptor and may be used for structure-activity analysis.

From the pK'_d values and the π' values two series of compounds *d* and *e* were compiled. Fig. 3 shows that the same distinction of the intensity of the hydrophobic effect can be observed as in Fig. 1. Different ϕ-values are obtained for the two series *d* and *e* also in the case of the ileum receptor: $\phi_d = 0.8 \pm 0.3$ and $\phi_e = 1.5 \pm 0.2$. These differences seem to

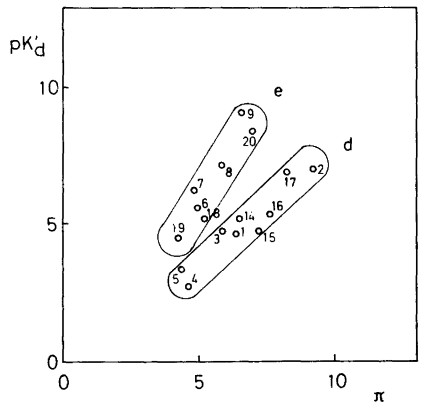


Fig. 3. The linear relationship between the pK'_d values and the hydrophobicity constants for the two series *d* and *e* of the compounds: Series *e* and *d* include series *a* and *b'*, respectively. Formulae of the compounds are given in Table 1.

be connected with the presence of the ester group in the antagonist molecule as this seems to be the main difference between the reaction series *a* and *d*, and series *b*, *c*, and *e*, respectively.

The intensity factor of the LFER's characterizes the mechanism of the appropriate interaction.[10,11] Therefore, proceeding from the ϕ-values obtained above, two different binding mechanisms for the muscarinic antagonists can be postulated.

The first binding mechanism, characterized by $\phi < 1$, corresponds to the simple extraction model of the hydrophobic interaction and is characteristic for many ligand binding processes to proteins with hydrophobic sites.[10]

The extra high affinity, characterized by $\phi > 1$, cannot be explained using this simple model, although such ϕ-values have been observed previously in the case of some enzyme-catalyzed reactions.[10,11] In these instances, multi-step processes were assumed and thus the observed ϕ-value for the overall process is an apparent constant consisting of several ϕ-increments; $\phi_{obs} = \sum_i \phi_i$. For all elementary steps $\phi_i < 1$ and thus the simple extraction model is valid for the individual steps.

In the light of these data the high intensity factor ϕ for some ester bond containing antagonists can be explained by a two-step binding mechanism, eqn. (3), where R, RA and RA* stand for the

$$R + A \overset{K_A}{\rightleftharpoons} RA \overset{K_i}{\rightleftharpoons} RA^* \qquad (3)$$

receptor, receptor–antagonist complex and its isomerized form. Indeed for some of the ester-type antagonists such as 3-quinuclidinyl benzilate and 4-*N*-methylpiperidinyl benzilate such a two-step binding mechanism has been established from kinetic studies.[12] One may hypothesize that both constants K_A and K_i could depend on hydrophobicity of the ligand to give $\phi_{obs} > 1$ when the observed dissociation constant is $K_d \approx K_A K_i$. Therefore further discussion of this binding mechanism calls first of all for thorough kinetic analysis of the antagonist binding reaction to obtain separately the constants K_A and K_i for the whole reaction series. It is possible that for the less active antagonists of the *a*-series of Fig. 1 the binding process involves only one step or there are other reasons why the isomerization constant K_i may be independent of the hydrophobicity of the ligand.

It should be noted furthermore that there is precisely twofold difference between the ϕ-values for the series *a* and *b*. A possible mechanism for such "double effects" of hydrophobic interaction in

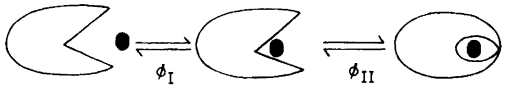


Fig. 4. The schematic picture of a ligand binding model involving isomerization of the receptor-ligand complex. The two reaction steps: binding and isomerization are characterized by intensity factors ϕ_I and ϕ_{II}, respectively.

enzyme catalyzed reactions has been offered by Aaviksaar *et al.*[11] and further discussed by Kljosov and Berezin.[10] This mechanism explains the "two-fold binding" of a ligand molecule in a hydrophobic site by a conformational change consisting of the "shutting" of the hydrophobic slit of the binding site on the protein (according to Fig. 4).

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Biosynthesis of Peroxisomal Proteins *

EEVA-LIISA APPELKVIST and
ÅKE ELHAMMER

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm and Department of Pathology at Huddinge Hospital, Karolinska Institutet, S-141 86 Huddinge, Sweden

The proteins of the different intracellular membranes are synthesized both on bound and free ribosomes.[1] In most cases, however, it is not known to what extent the individual ribosomal compartments contribute for peroxisomes. It is known that catalase is synthesized on free ribosomes and transferred directly to peroxisomes without hydrolytic processing of the polypeptide chain.[2] Morphological investigations suggested that peroxisomes originate from the endoplasmic membrane system as an outgrowth but the peptide patterns on slab gel differ from those of the microsomes.[3] Naturally the transport of proteins could be selective and involve only a few proteins; consequently the peptide pattern is not necessarily identical in the two membranes. The purpose of this investigation was to find out whether peroxisomal precursor peptides were present in the microsomal fraction or in the supernatant after *in vivo* labeling.

In order to identify peroxisomal peptides in the various fractions, peroxisomes were prepared and soluble proteins (content) were removed by treatment of the fraction with a low concentration of deoxycholate. The membranes were solubilized with a high concentration of detergent and the particulate urate oxidase core was removed by centrifugation. The supernatant containing exclusively peroxisomal membrane proteins was used to prepare antibodies against all proteins of the peroxisomal membranes.

The antibodies prepared against proteins of the peroxisomal membranes could be used to follow the biosynthesis and transport of these proteins. Rats were injected *via* the portal vein with an amino acid mixture of high specific radioactivity. Microsomes, peroxisomes and supernatant from liver were prepared at various times after injection and the peroxisomes were treated to isolate

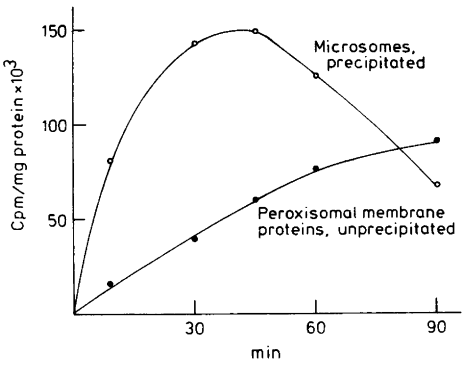

Fig. 1. Labeling of peroxisomal precursor proteins in microsomes and of peroxisomal membrane proteins. Rats were injected into the portal vein with 0.5 mCi [^{3}H]amino acid mixture (Amersham) and after various times the liver was removed followed by preparation of microsomes and peroxisomes. The microsomes were precipitated by antibodies against peroxisomal membranes and adsorbed on a protein A-Sepharose column. The eluate and the peroxisomal membranes freed from the content were used to determine protein content and radioactivity.

membrane proteins free from the luminal content. There was no precipitation of any of the proteins in the supernatant. On the other hand, a part of the microsomal proteins reacted with antibodies and the radioactivity could be measured in the precipitate (Fig. 1). Highest specific activity was obtained after 45 min and after this time a decay occurred. Incorporation into total peroxisomal membrane proteins was considerably lower in the initial period and increased continuously in the first 90 min.

For closer identification of the proteins in the microsomal fraction which serve as precursors for peroxisomal membranes, *in vivo* labeling with injection of [^{35}S]methionine and [^{35}S]cysteine was performed. The microsomal membranes were precipitated with the antibody against peroxisomal membranes and after adsorption to protein A-Sepharose separation on slab gel was performed (Fig. 2). After 45 min 8 bands were visible on the autoradiographs ranging in MW between 30 000 and 90 000. Five of these bands possess high labeling while the remaining three gave low intensity band on the autoradiograph. After 90 min the radioactivity decreased considerably in all bands.

To follow the transport of phospholipids, rats were labeled *in vivo* with [^{3}H]glycerol and

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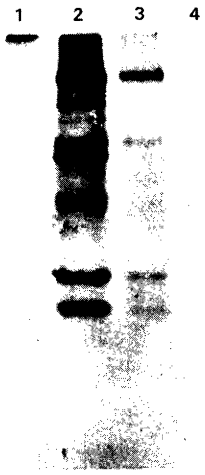

Fig. 2. Slab gel of peroxisomal precursor proteins isolated from microsomes. Rats were injected in the portal vein with 0.5 mCi [^{35}S]methionine and 0.5 mCi [^{35}S]cysteine and the liver was removed after 5, 45, 90, or 180 min (lanes 1, 2, 3 and 4, respectively). Microsomes were precipitated with antibodies against peroxisomal membrane protein. The figure shows the result after autoradiography.

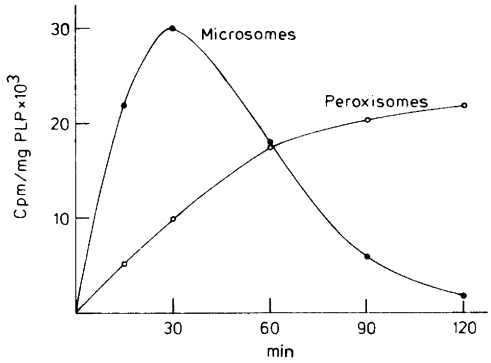

Fig. 3. Labeling of microsomal and peroxisomal phospholipids. Rats were injected in the portal vein with 0.5 mCi [^{3}H]glycerol, the livers were removed after various times, microsomes and peroxisomes were prepared. The lipids were extracted with chloroform: methanol (2:1) and phospholipids were separated by chromatography on silicic acid column. The eluate was used to measure radioactivity and phospholipid.

incorporation of this label into total phospholipid was followed (Fig. 3). Peak incorporation into microsomes occurred at 30 min followed by a rapid decay. As expected, the specific radioactivity in total peroxisomal phospholipid was lower at this time but increased continuously during the first two hour period. Since peroxisomes are considered to lack an enzyme system for synthesis of phospholipids, the experiments indicate relatively rapid transfer of lipids from endoplasmic membranes to peroxisomes.

Peroxisomal membranes were subjected to gel electrophoresis and few bands exhibited staining with Schiff's reagent, indicating the presence of carbohydrate residues. There are two possibilities for explaining the presence of protein-bound carbohydrates in peroxisomes: they are either synthesized at their final location or they are transported from the endoplasmic reticulum. The data presented in Table 1 demonstrate that both dolichol and dolichol-associated glycosylation reactions are present in peroxisomes.

The dolichol concentration is higher in peroxisomes than in microsomes and both dolichol mono- and pyrophosphatase activities are present in this fraction. In spite of the low activity of

Table 1. Dolichol and dolichol-associated reactions in peroxisomes. Dolichol and the various enzyme activities were measured as described earlier.[4,5]

	Peroxisomes Amount or activity	% of microsomal amount or activity
Dolichol[a]	0.85	163
Dolichol monophosphatase[b]	32	59.3
Dolichol pyrophosphatase[b]	69	32.4
NADPH-cytochrome *c* reductase[c]	0.002	1.9
UDP-*N*-acetylglucosaminyl transferase[d]	0.023	12.8
UDP-galactosyltransferase[d]	0.043	11.9

[a] μg/mg protein. [b] pmol Pi/[min(mg protein)]. [c] μmol/[min(mg protein)]. [d] pmol/[min(mg protein)].

NADPH-cytochrome *c* reductase activity, which indicates a low microsomal contamination, both UDP-*N*-acetylglycosaminyl and UDP-galactosyl transferase activities are found at a level which cannot be explained by contamination with microsomal vesicles.

The experiments described in this paper indicate that some of the proteins of the peroxisomal membranes are synthesized in the endoplasmic reticulum and transferred through the cytoplasm to the peroxisomes. This transfer probably does not involve membrane flow and may involve only some individual proteins of the peroxisomal membranes. The cytoplasmic pool in this case is very minute and its precipitation with antibodies is not possible with currently available methods. Glycoproteins appear to be constitutive components of these membranes and the oligosaccharide chain is either synthesized here or completed by the dolichol mediated glycosyltransferase system.

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The Effect of Chemical Carcinogens on the Dolichol Mediated Glycosylation of Rat Liver Microsomes *

CONNY EDLUND and GUSTAV DALLNER

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm and Department of Pathology at Huddinge Hospital, S-141 86 Huddinge, Sweden

Membranes of the malignant cell, particularly the plasma membrane, exhibit characteristic properties different from the normal cell. Chemical analysis of membranes prepared from experimental and human tumors has established the presence of glycoproteins which possess oligosaccharide chains with specific properties concerning structure and composition.[1] Glycosyl transferases are present in various cell membranes but the synthesis of the complete oligosaccharide chain requires the participation of enzymes in the endoplasmic reticulum. Dolichol phosphate is an obligatory intermediate in several steps of oligosaccharide synthesis and consequently its amount, composition and functional capacity is of great importance, as its role in rate limiting the biosynthetic process.[2] For this reason we performed a study of the initial effect of some chemical carcinogens on dolichol and dolichol mediated glycosylation in rat liver.

The amount and composition of dolichol in lipid extracts can be measured by high pressure liquid chromatography using a C18 reversed phase column.[3] Both liver homogenate and isolated microsomes prepared as described previously,[4] were analyzed for the effect of three chemical carcinogens and also phenobarbital, which is a known inducer of microsomal membranes and cytochrome P-450 (Table 1). Dolichol content in microsomes is changed significantly only after treatment of the rat with N-nitroso-diethylamine, which more than doubled the amount of lipid. On the other hand, treatment with the other substances decreased the amount of dolichol in the homogenate. The explanation for these differences between homogenate and microsomes is that the dolichol content of some intracellular membranes is considerably higher than in microsomes and, consequently, carcinogens exert obviously a differentiated effect on dolichol content at various locations. By comparison, dolichol content was also estimated in conditions where rapid synthesis takes place. In regenerating liver, the polyprene content in homogenate is significantly higher, while in microsomes it is somewhat lower than in the control. The first 3 days after birth, the amount of dolichol both in homogenate and microsomes is only 1/3 of that found in adult liver.

Microsomes were isolated from rat liver after various treatments and incubated with the 3 nucleotide sugars known to interact with dolichol monophosphate. This interaction is clearly influenced by the type of carcinogen administered (Table 2). Nitroso-diethylamine and methylcholanthrene decreased GlcNAc incorporation into the lipid intermediate, acetyl-aminofluorene and methylcholanthrene increased mannosylation of the lipid, while glucose transfer was increased by acetyl-aminofluorene and nitroso-diethylamine, and decreased by methylcholanthrene and phenobarbital. These experiments indicate that one of the factors regulating the biosynthesis of the oligosaccharide chain is the amount of the glycosylated lipid intermediate, which is the direct substrate of the glycosyl transferase acting for completion of the chain.

In addition to the glycosylated intermediate, the various glycosyl transferases also influence the structure of the carbohydrate chain on the protein (Table 3). The various treatments change only slightly the transfer of GlcNAc to the protein, but the transfer of mannose is decreased by nitroso-diethylamine and phenobarbital. Glycosylation of the protein on the other hand displays a uniform pattern since all the treatment employed lower the level of glucose transfer.

Rat liver homogenate contains a characteristic composition of dolichols dominated by C90 and C95 species. C85 and C100 forms are present in smaller amounts, while the C105 content is only around 4% (Table 4). This composition appears to be stable since none of the treatments cause any significant change in the distribution pattern of dolichols with various numbers of isoprenol residues.

The experiments described above demonstrate that the amount of dolichol, its active phosphorylated form and its capacity to function as a sugar acceptor are among the factors which determine the type of oligosaccharide chain which is synthesized and transferred to the protein, and participate in an *N*-glycosydic binding. Treatment of rats with chemical carcinogens changes the amount of dolichol in the liver but has no effect on the qualitative composition. Dolichol phosphate glycosylation is also modified as early as in the initial phase of the action of carcinogens the effect of which is a modified glycosylation of the endogenous protein acceptor.

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Table 1. Dolichol contwnt in homogenate and microsomes from rat liver. Dolichol was isolated and quantitated by high pressure liquid chromatography. In exp . 1 rats were given intraperitoneally 2-acetyl-aminofluorene, 10 mg/100 g body weight; *N*-nitrosodiethylamine, 2 mg/100 g; 3-methylcholanthrene, 2 mg/100 g; and phenobarbital, 8 mg/100 g once a day during 5 days. Homogenate and microsomes were prepared on day 6. In exp. 2 partial hepatectomy was performed and the outgrown liver was removed 8 days later. In exp. 3 livers of newborn 6, 18 and 72 hours after birth were investigated. The values are the means ±S.E.M. of 6 experiments.

Exp.	Homogenate	Homogenate Dolichol μg/mg protein	Microsomes
1	Control	0.181±0.020	0.260±0.029
	2-Acetyl-aminofluorene	0.126±0.013	0.230±0.022
	N-Nitrosodiethylamine	0.379±0.028	0.605±0.052
	3-Methylcholanthrene	0.112±0.010	0.282±0.016
	Phenobarbital	0.078±0.005	0.230±0.019
2	Control	0.174±0.012	0.255±0.025
	Regenerating liver	0.271±0.027	0.219±0.029
3	6-Hour old	0.056±0.003	0.080±0.007
	18-Hour old	0.051±0.004	0.082±0.007
	72-Hour old	0.062±0.007	0.076±0.005

Table 2. Glycosylation of endogenous dolichol-P in liver microsomes of rats treated with chemical carcinogens. Microsomes were incubated with nucleotide-activated sugars as described earlier.[5] After incubation at 30 °C for 15 min the lipids were extracted by chloroform – methanol, 2:1 and after partition the radioactivity was determined in the chloroform fraction. The radioactive product was identified as dolichol-P by thin layer chromatography. The values represent the means of 4 experiments.

Treatment	GlcNAc	Mannose cpm per mg protein	Glucose
None	419	6 217	392
2-Acetyl-aminofluorene	427	12 306	512
N-Nitrosodiethylamine	340	3 873	461
3-Methylcholanthrene	349	17 850	305
Phenobarbital	447	5 262	336

Table 3. Glycosylation of endogenous protein in liver microsomes of rats treated with chemical carcinogens. Microsomes were incubated as described in Table 2 and after incubation the microsomes were extracted with chloroform – methanol, 2:1, and chloroform – methanol – H_2O, 1:1:0.3. The protein pellet was dissolved in 1 ml 2% sodium dodecyl sulfate and radioactivity was measured by scintillation counting. The values are the means of 4 experiments.

Treatment	GlcNAc cpm per mg protein	Mannose	Glucose
None	169	239	422
2-Acetyl-aminofluorene	165	280	289
N-Nitrosodiethylamine	139	168	276
3-Methylcholanthrene	161	209	313
Phenobarbital	190	123	173

Table 4. Distribution of different types of dolichols in liver homogenates of rats treated with chemical carcinogens. The experimental conditions are described in Table 1. The individual dolichols were measured by high pressure liquid chromatography. The values represent the means of 3 experiments.

Treatment	Type of dolichol (% of total)				
	C 85	C 90	C 95	C 100	C 105
None	11.7	37.9	34.0	12.6	3.9
2-Acetyl-aminofluorene	11.7	41.5	32.7	10.6	3.5
N-Nitrosodiethylamine	12.1	35.7	33.0	14.3	4.9
3-Methylcholanthrene	8.3	39.3	34.3	13.2	4.9
Phenobarbital	9.2	38.1	32.7	13.5	6.5

Acknowledgement. This work was supported by the Swedish Medical Research Council and the Swedish Society of Medical Sciences.

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Return of Drug-metabolizing Systems to Control Levels after Induction with 3-Methylcholanthrene

JOSEPH W. DePIERRE, GERD LUNDQVIST and LARS ERNSTER

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The induction of drug-metabolizing systems by treatment of animals with xenobiotics such as phenobarbital and 3-methylcholanthrene has been extensively investigated for almost 20 years now.[1] The time course for the induction of cytochrome P-450, epoxide hydrolase, glutathione *S*-transferase(s) and other drug-metabolizing systems has been characterized. However, the time course with which these activities return to control levels after cessation of treatment with the inducer has received considerably less attention. The return of drug-metabolizing systems to control levels after induction must necessarily involve protein degradation and, in some cases, membrane degradation as well. Consequently, this return may provide a useful experimental system for answering questions about the mechanisms and control of protein and membrane degradation.

Treatment of rats with phenobarbital causes an approximately 4-fold increase in the specific activity of the cytochrome P-450 system in the hepatic endoplasmic reticulum, as well as an approximately 2–2.5-fold increase in the content of endoplasmic reticulum membranes per gram liver.[2] We have shown that after the final injection of phenobarbital the induced protein and phospholipid components of the endoplasmic reticulum return in parallel to control levels within five days.[3] In conjunction with results from other laboratories[4] this finding has led us to conclude that the endoplasmic reticulum induced by phenobarbital is degraded in large pieces by autophagic vacuoles after cessation of the treatment.

On the other hand, treatment of rats with 3-methylcholanthrene results in an approximately 4-fold increase in the content of cytochrome P-450 in the hepatic endoplasmic reticulum without causing proliferation of this organelle.[1,5] Thus, in this case it would seem to be highly wasteful to degrade whole segments of the endoplasmic reticulum during the process of return to control levels. In an initial attempt to characterize the return to control levels after induction with 3-methylcholanthrene, we have examined here the time course of this return for a number of different drug-metabolizing enzymes.

Male Sprague-Dawley rats weighing 180–200 g were injected intraperitoneally once daily for 1–5 days with 20 mg 3-methylcholanthrene/kg body weight in corn oil or with a corresponding volume of the vehicle alone. The animals were starved overnight before sacrifice by decapitation and the total microsomal and high-speed supernatant fractions were prepared in the usual manner.[6] Cytochrome P-450,[7] cytocgrome b_5,[7] glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene,[8] and DT-diaphorase activity[9] were all assayed using published procedures.

In the experiments designed to characterize the excretion of 3-methylcholanthrene and its metabolites, 3 rats were injected intraperitoneally with 20 mg 3-methylcholanthrene/kg body weight in corn oil containing 185 MBq [^{14}C]-3-methylcholanthrene (The Radiochemical Centre, Amerham-Searle, England) once daily for 5 days. These animals were maintained in metabolism cages and their urine and feces collected in 24-h periods during the period of treatment and for 14 days after the final injection. The feces were dried and weighed, aliquots of both the urine and feces were bleached using H_2O_2, and the radioactivity present was determined by scintillation counting in Lumagel.

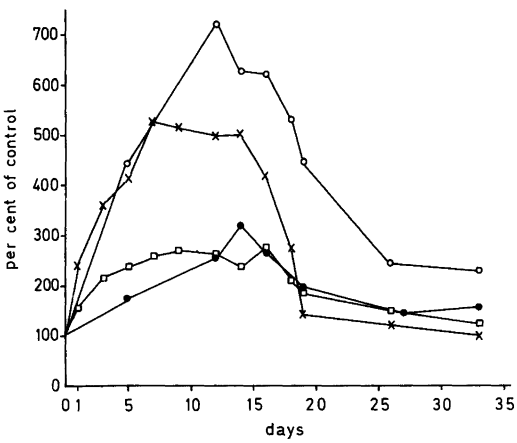

Fig. 1. Induction of drug-metabolizing systems by 3-methylcholanthrene and subsequent return to control levels. The rats were injected on days 0, 1, 2, 3 and 4. Each point represents an average value for 3–9 different animals. The symbols used are as follows: × = cytochrome P-450; □ = cytochrome b_5; ● = glutathione *S*-transferse(s); ○ = DT-diaphorase. For further details see the text.

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Fig. 1 shows the time course of induction of cytochromes P-450 and b_5, glutathione *S*-transferase(s) and DT-diaphorase by 3-methylcholanthrene and the return of these enzymes to control levels after cessation of the treatment. It can be seen that the amounts and/or activities of these enzymes present in the liver continue to rise for several days after the cessation of 3-methylcholanthrene treatment. Cytochrome P-450, cytochrome b_5, glutathione *S*-transferase(s) and DT-diaphorase do not return to control levels until 2–3 weeks after the final injection.

As mentioned above, the return of drug-metabolizing enzymes to control levels after induction with phenobarbital requires about 5 days.[3] This is also the case after induction with *trans*-stilbene oxide[10] or with 2-actylaminofluorene.[11] One possible explanation as to why the return to control levels requires considerably longer after induction with 3-methylcholanthrene may be that this xenobiotic is excreted only slowly and consequently remains in the rat's body for several weeks.

In order to investigate this possibility, the experiment illustrated in Fig. 2 was performed. It can be seen that about 75 % of the total amount of 3-methylcholanthrene injected into the animals has been excreted by the fifth day after the final injection. Thus, it does not seem likely that retention of 3-methylcholanthrene in the body can explain the relatively long time period required for return to control conditions. It can also be seen from Fig. 2

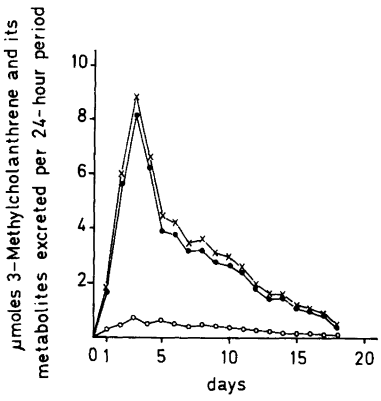

Fig. 2. Excretion of 3-methylcholanthrene and its metabolites by the rat. The animals were injected with [^{14}C]-3-methylcholanthrene on days 0, 1, 2, 3 and 4. Each point represents an average value for 3 different animals. The symbols used are as follows: ● = excretion in the feces; ○ = excretion in the urine; × = total excretion. For further details see the text.

that almost all the 3-methylcholanthrene is excreted in the feces. This may be due to the uptake of 3-methylcholanthrene from the peritoneal cavity directly into the intestine, followed by excretion, and/or to the excretion of conjugated metabolites of 3-methylcholanthrene into the bile. We are in the process of identifying the form in which the radioactivity is excreted.

Thus, the return of drug-metabolizing systems to control levels requires considerably more time after induction with 3-methylcholanthrene than after induction with other xenobiotics. This may indicate that a different mechanism of protein degradation is involved in the case of 3-methylcholanthrene. In addition, the mechanism for degrading membrane proteins such as cytochromes P-450 and b_5 may differ from the degradation of cytosolic enzymes such as glutathione *S*-transferase(s) and DT-diaphorase – *e.g.*, degradation of membrane proteins may require their selective removal from the membrane.

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Determination of Enantiomeric Composition of Partly Racemized Carotenols

KÅRE AARESKJOLD and SYNNØVE LIAAEN-JENSEN

Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

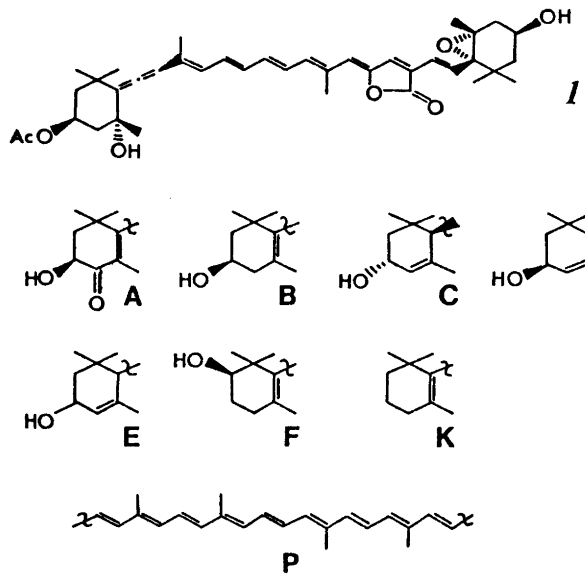
Methods for determining the enantiomeric composition of various racemized carotenols by converting them into diastereomeric esters with subsequent analysis have been studied.

Diastereomeric esters of (−)-camphanic acid with carotenols other than α-ketols could not be separated by HPLC. No separation was achieved for diastereomeric esters of methoxytrifluoromethylphenylacetic acid (MTPA).

^{1}H NMR analysis in the presence of Eu(fod)$_3$ of diastereomeric MTPA esters allowed quantitative determination of the enantiomeric composition of carotenols with 2-hydroxy-β- and 3-hydroxy-β-type end groups.

Naturally occurring carotenoids contain up to six chiral centres, as examplified by peridinin (*1*, Scheme 1).[1] For a long time it was assumed that chiral carotenoids occurred in nature as a single chiral isomer. More recently it was demonstrated that astaxanthin occurred in the (3*S*,3′*S*)- and (3*R*,3′*R*)-configuration (*2a* and *2b*) in different sources.[2,3] Furthermore, lutein (*3a*) and calthaxanthin (*3b*) are shown to be 3′-epimers[4-7] and six different configurations of ε,ε-carotene-3,3′-diol (*4*) have now been established.[8-11]

However, until very recently[10,12-14] only one configuration has been encountered for a carotenoid of given constitution within the same biological



2a A-P-A
2b ent A-P-ent A
2c A-P-ent A
3a B-P-C
3b B-P-D
4 E-P-E
5 F-P-K

Scheme 1.

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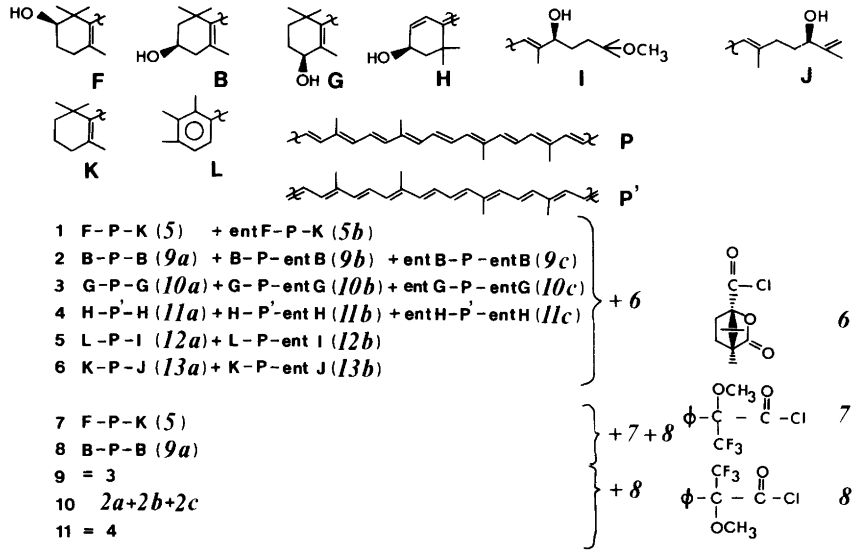

Scheme 2.

source. Lack of suitable methods for demonstrating enantiomeric composition may partly be responsible for this situation.

In our collaboration with Kayser on insect carotenoids[15,16] it was observed that β,β-caroten-2-ol from insects had variable and smaller Cotton effect than earlier found for (2*R*)-β,β-caroten-2-ol (*5*) in our laboratory.[17] This prompted us to search for a method for quantitative analysis of partially racemized carotenols. A common principle for separation of enantiomers involves conversion into diastereomers and separation on the basis of different physical properties, including chromatography. Alternatively, the quantitative composition of a diastereomeric mixture may be estimated by NMR spectroscopy.

RESULTS AND DISCUSSION

From the work of Gerlach[18,19] and Müller *et al.*[20] the commercially available (−)-camphanic acid chloride (*6*, Scheme 2) appeared to be a favourable acylating agent for the preparation of diastereomeric carotenols for subsequent attempts of HPLC separation.[21] Meanwhile Müller and Vecchi[22] succeeded in separating (3*S*,3′*S*), (3*R*,3′*R*) and (3*R*, 3′*S*) astaxanthin (*2a,b,c*) by this principle.

A micro scale procedure for preparation of (−)-camphanic esters of selected carotenols (Scheme 2) and subsequent HPLC analysis was adapted. When testing our procedure with synthetic Roche standards (*2b,2c*)[23,24] and natural astaxanthin *ex Hommarus gammarus*, presumed to be (3*S*,3′*S*) (*2a*),[3] the latter unexpectedly turned out to be a mixture of all three optical isomers (*2a,b,c*). These results have been published.[12]

HPLC separation was attempted for esters of (−)-camphanic acid prepared from carotenol mixtures containing 2 – 3 optical isomers. The carotenol samples esterified with (−)-camphanoyl chloride were as specified in Scheme 2:

(1) Racemic natural β,β-caroten-2-ol (*5,5b*) *ex Cerura vinula;* racemic nature proved by the ^{1}H NMR method below.[16]

(2) Synthetic mixture of racemic (*9a,c*) and meso (*9b*) zeaxanthin.[25]

(3) Racemic (*10a,c*) and meso (*10b*) isozeaxanthin,[26] prepared by $LiAlH_4$ reduction of synthetic[27] canthaxanthin.

(4) Racemic (*11a,c*) and meso (*11b*) eschscholtzxanthin,[28] prepared by $LiAlH_4$ reduction of synthetic[29] rhodoxanthin.

(5) Racemic okenol (*12a,b*)[30] prepared by $LiAlH_4$ reduction of synthetic[30] okenone.

(6) Synthetic, racemic aleuriaxanthin (*13a,b*)[31].

In no case was reproducible separation achieved by HPLC at conditions where the camphanates of

the α-ketols *2a*+*2b*+*2c* were separated.

We now turned to the resolving agent methoxy-trifluoromethylphenylacetic acid chloride (MTPA chloride)[32] which offered two advantages. Both enantiomers of the acid chloride (*7* and *8*) could be synthesized,[30] and this enabled testing the separation of diastereomeric pairs even when only one enantiomer of the carotenol was available. Secondly this ester moiety offered the possibility of performing NMR-analysis[32-35] in addition to HPLC.

R(+)-MTPA chloride (*7*) and *S*(−)-MTPA chloride (*8*) were prepared in six steps from trifluoroacetic acid by published methods.[32,36]

Diastereomeric esters were prepared as follows (see Scheme 2):

(7) (2*R*)-β,β-Caroten-2-ol (*5*) *ex Trentepohlia iolithus*[17] was esterified with *R*(+)- and *S*(−)-MTPA chloride, respectively, and mixed, *cf.* Sample 1.

(8) Natural optically active (3*R*,3′*R*)-zeaxanthin (*9a*) *ex Flavobacterium* sp.[37] was esterified with *R*(+)- and *S*(−)-MTPA chloride and mixed, *cf.* Sample 2.

(9) Sample 3 = racemic and meso isozeaxanthin (*10a,c,b*)[26] was esterified with *S*(−)-MTPA chloride.

(10) Pure synthetic (*R,R*)-, (*S,S*)- and (*R,S*)-astaxanthin (*2a,c,b*)[23,24] were esterified with *S*(−)-MTPA chloride and mixed.

(11) Sample 4 = racemic and meso eschsholtzxanthin (*11a,c,b*)[28] was esterified with *S*(−)-MTPA chloride.

None of the diastereomeric mixtures (7−11) could be separated by HPLC at the conditions employed.

The possibility of analyzing the enantiomeric composition of carotenols by ^{1}H NMR was now investigated. Sample 7 above gave only one methoxy signal. However, upon careful stepwise addition of the shift reagent Eu(fod)$_3$ the methoxy signal shifted downfield and was split into two signals. By using a 2:1 ratio of the two diastereomeric esters it could be demonstrated that the (*R,R*) isomer contained the methoxy group resonating at lowest field. This is in accordance with previous experience. Thus it has been concluded[38] that if the absolute configuration is defined according to the bulkiness of the substituents the "*R,R*" (or "*S,S*") isomer should give a larger shift for the methoxy signal than the "*R,S*" (or "*S,R*") isomer. In our case the chiral C-2 center of (2*R*)-β,β-caroten-2-ol (*5*, Scheme 2) has ("*R*")-configuration, also by using bulky group priority in the sequence rule.

The same experiment was carried out with (3*R*, 3′*R*)-zeaxanthin (*9a*, Sample 8, Scheme 2). In this case the methoxy group of the *R,S* configurated *S*(−)-MTPA ester showed the largest shift. The C-2 and C-4 substituents of the carotenoid moiety are less different in bulkiness.

CONCLUSION

In conclusion, the demonstration of enantiomeric composition by HPLC of esters of (−)-camphanic acid is not generally applicable to carotenols. Positive results are so far obtained for α-ketols such as astaxanthin (*2a,b,c*)[22] and adonirubin.[39]

Separation of diastereomeric MTPA esters of selected carotenols was not achieved in the present study.

^{1}H NMR analysis of MTPA esters in the presence of Eu(fod)$_3$ shift reagent of 2-hydroxy-β- and 3-hydroxy-β-type carotenols was successful and allows a quantitative determination of the relative percentage of *R*- and *S*-configurated carotenol. Application of this method for the demonstration of the racemic nature of β,β-caroten-2-ol (*5,5b*) from insects will be published elsewhere.[16] For dichiral carotenoids such as zeaxanthin (*9a,b,c*) the ^{1}H NMR method does not allow determination of the amount of meso compound present.

EXPERIMENTAL

General methods and instruments. All operations were carried out in inert atmosphere in subdued light and at temperatures not exceeding room temperature. HPLC was carried out on a Dupont 830 Liquid Chromatograph with a Spherisorb S5W column (250 × 4.6 mm) with acetone − hexane (0:100 − 40:60 v/v) containing 0.1 % methanol as mobile phase at a flow rate of 2 ml/min with gradient 1 %/min[21] at conditions where the camphanates of astaxanthin (*2a*+*2b*+*2c*) were separated into three peaks with near-to-base-line separation. Peak components were detected by a Varian 634 UV/vis. spectrometer. Otherwise vis. spectra were recorded on a Coleman-Hitachi 124 spectrometer and ^{1}H NMR spectra on a Jeol JNM-FX 100 Fourier-Transform NMR instrument.

Synthesis of resolving agents

1-Phenyl-2,2,2-trifluoroethan-1-one was prepared by the literature procedure[36] from phenylmagnesium bromide (9 mol) and trifluoroacetic acid (3 mol)

in 64 % yield (334 g); b.p. 150–153 °C, lit.[36] 150–152 °C.

3,3,3-Trifluoro-2-methoxy-2-phenylpropanenitrile was prepared from the above ketone (1 mol), sodium cyanide and dimethyl sulfate as described elsewhere[32] in 90 % yield (194 g); b.p. 68–72 °C/5–6 mm Hg, lit.[32] 85–89 °C/20 mmHg.

(±)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoic acid (MTPA) was prepared by hydrolysis of the above nitrile (0.90 mol) as described[32] in 61 % yield (128 g); b.p. 193–200 °C/40–55 mm Hg, lit.[32] 105–110 °C/1 mmHg.

(−)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoic acid [(−)-MTPA]. Racemic MTPA (45 g, 0.19 mol) was resolved by fractional crystallization of the salt with L(−)-1-phenylethanamine (23.25 g, 0.19 mol) and subsequent treatment with HCl as described in the literature[32] to give the (−)-acid (12.0 g, 0.05 mol); $[\alpha]_D^{20}$ −67.7° ± 1.1 (c = 1.13, ethanol), lit.[32] $[\alpha]_D^{24}$ −71.8° ± 0.6 (c = 3.28, methanol).

(+)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoic acid [(+)-*MTPA*]. The remaining MTPA salts in the mother liquor above was converted to the acid by HCl treatment, and the regenerated MTPA treated with D(+)-1-phenylethanamine (9.3 g, 0.076 mol). The resulting salt was fractionally crystallized as described[32] to give the (+) acid (4.7 g, 0.02 mol); $[\alpha]_D^{20}$ +69.7° ± 1.8 (c = 0.95, ethanol), lit.[32] $[\alpha]_D^{25}$ +68.5° ± 1.3 (c = 1.49, methanol).

(−)-MTPA chloride. (−)-MTPA (12.0 g, 0.051 mol) was treated with thionyl chloride[32] to give the acid chloride (10.5 g, 0.039 mol); b.p. 97.5–99 °C/12 mmHg; $[\alpha]_D^{20}$ −125.7° ± 1.3 (c = 0.9, CCl_4).

(+)-MTPA chloride. (+)-MTPA (4.7 g, 0.02 mol) was reacted as above to give the (+) acid chloride (3.8 g, 0.016 mol); b.p. 96.5–99 °C/11–12 mmHg, lit.[32] 54–56 °C/1 mmHg; $[\alpha]_D^{24}$ +129° ± 2 (c = 5.17, CCl_4).

(±)-MTPA chloride. (±)-MTPA (20.5 g, 0.087 mol) treated as above gave (±)-MTPA chloride (20 g, 0.074 mol); b.p. 96–100 °C/13 mmHg.

HPLC analysis of diastereomeric esters

Standard procedure for the reduction of oxocarotenoids. To the appropriate oxocarotenoid (0.5–5 mg) in anhydrous ether (5–25 ml) at 0 °C was added an excess of a filtered solution of $LiAlH_4$ in dry ether. After 5 min at 0 °C the reaction mixture was poured into a half saturated solution of Rochelle salt, the organic layer was separated, washed with water and the solvent evaporated. Water was removed by azeotropic distillation with benzene and the carotenoids isolated by TLC (SiO_2) and characterized by R_F-values and vis.spectra.

Standard procedure for preparation of carotenol esters. A solution of the appropriate acid chloride (25–100 mg) in anhydrous pyridine (1–3 ml) was added to a solution of the appropriate carotenols (0.1–10 mg) in pyridine (1 ml) at 0 °C. After 45 min at this temperature hexane (10 ml) and water were added. The organic phase was washed 5–7 times with water. Solvent and water were removed by azeotropic distillation with benzene and the carotenoids isolated by TLC (SiO_2) and characterized by R_F-values and vis. spectra.

Camphanic esters, *cf.* Scheme 2, were prepared by esterification with (−)-camphanoyl chloride.

1. β,β-Caroten-2-ol (*5,5b*, 0.1 mg) *ex Cerura vinula*[40] was esterified by the standard procedure. HPLC analysis gave one peak.

2. Synthetic zeaxanthin[25] (*9a,b,c*, 0.5 mg) was esterified. HPLC gave one major peak.

3. Synthetic canthaxanthin[27] (2 mg) was reduced by the standard procedure and of the resulting isozeaxanthin (*10a,b,c*) 0.7 mg was esterified. HPLC gave one peak.

4. Synthetic rhodoxanthin[29] (5 mg) was reduced. Of the resulting eschscholtzxanthin (*11a,b,c*)[28] 3 mg was esterified. HPLC showed one major peak.

5. Okenone[30] (0.7 mg) was reduced. Of the resulting racemic okenol (*12a,b*)[30] 0.25 mg was esterified. HPLC gave one major peak.

6. Synthetic racemic aleuriaxanthin[31] (*13a,b*, 2 mg) was esterified. HPLC analysis resulted in one main peak. MTPA esters, *cf.* Scheme 2, were prepared by esterification with the appropriate acid chlorides.

7. (2*R*)-β,β-Caroten-2-ol (*5*, 3 mg) *ex Trentepohlia iolithus*[17] was esterified with *S*(−)-MTPA chloride, providing the *R,S*-ester.

Similarly (2*R*)-β,β-caroten-2-ol (*5*, 2 mg) was esterified with *R*(+)-MTPA chloride to give the *R,R*-ester. The two diastereomeric esters were mixed 1:1 (7a). HPLC of the mixture showed one peak. A 63:37 mixture (7b) was used for ^{1}H NMR.

8. (3*R*,3′*R*)-β,β-Carotene-diol (zeaxanthin, *9a*, 10 mg) *ex Flavobacterium* sp.[37] was esterified with *S*(−)-MTPA chloride. The same diol (5 mg) was esterified with *R*(+)-MTPA chloride. The crude esters were purified separately providing 11.2 mg (14.8 μmol) and 5.6 mg (7.4 μmol), respectively, of the two diastereomeric esters. In 1:1 mixture (7a) HPLC showed one main peak. A 2:1 mixture (8b) was used for ^{1}H NMR.

9. Racemic and meso isozeaxanthin (*10a,c,b*, 0.7 mg) prepared as for Sample 3, was esterified with *S*(−)-MTPA chloride. HPLC of the esters showed one peak.

10. (*R,R*),(*S,S*)- and (*R,S*,meso)-astaxanthin[23,24] (*2a,c,b*, 0.5 mg of each) were esterified separately with *S*(−)-MTPA chloride. The resulting esters were

Table 1. LIS of the $-OCH_3$ signal in $CDCl_3$ of the (*R,S*)-MTPA esters of sample 7b: (2*R*)-β,β-caroten-2-ol (63 % *R,S* and 37 % *R,R* monoesters) and of sample 8b: (3*R*)-β-β-carotene-3,3'-diol (67 % *R,S* and 33 % *R,R* diesters).

Sample	$Eu(fod)_3$ μmol/300 μl	δ-OCH_3 *R,R*	δ-OCH_3 *R,S*
7b	0	3.53	3.53
	1.00	3.53	3.53
	1.20	3.70	3.70
	1.35	3.89	3.80
	1.50	4.06 (*ca.* 62 %)[a]	3.92 (*ca.* 38 %)[a]
8b	0	3.53	3.53
	0.55	3.70	3.70
	0.70	4.45	4.20
	0.95	4.67	4.53
	1.30	5.02 (*ca.* 67 %)[a]	4.82 (*ca.* 33 %)[a]

[a] Relative integrals.

mixed in 1:1:1 ratio. HPLC analysis showed one major peak.

11. Racemic and meso eschscholtzxanthin (*11a, c,b*, 2 mg) prepared as for sample 4 above was esterified with *S*(−)-MTPA chloride. HPLC showed one main peak.

^{1}H NMR analysis of diastereomeric esters

General procedure. The diastereomeric mixture of the carotenol esters (3−8 mg) was dissolved in $CDCl_3$ (300 μl), a few drops of 1 % TMS in $CDCl_3$ was added and the ^{1}H NMR spectrum recorded.

A freshly prepared 0.005 N solution of $Eu(fod)_3d_{30}$ in $CDCl_3$ was then added in 10,50 or 100 μl portions. Each time the volume was adjusted to 300 μl and the shifts recorded.

β,β-Caroten-2-ol MTPA ester (Sample 7b), δ($CDCl_3$), 0.97 s (3H, 16-CH_3), 1.03 s (6H, 16',17'-CH_3), 1.27 s (3H, 17-CH_3), 1.50 s (CH_2), 1.70 s (6H, 18,18'-CH_3), 1.97 s (12H, in-chain-CH_3), 3.53 s (3H, -OCH_3), 4.85−5.15 (1H, H-2), 5.9−7 m (14H, olefinic), 7.3−7.5 m (5H, aromatic).

Addition of $Eu(fod)_3$ caused no significant shifts except for the $-OCH_3$ signal, see Table 1.

When (2*R*)-β,β-caroten-2-ol esterified with (*S*)-MTPA alone was tested, this pure diastereomer gave no splitting of the $-OCH_3$ signal upon stepwise addition of 0−1.5 μmol $Eu(fod)_3$/300 μl.

β,β-Carotene-3,3'-diol (zeaxanthin) di-MTPA ester (Sample 8b). δ($CDCl_3$) 1.03 s and 1.13 s (6H, 16,17,16',17'-CH_3), 1.70 s (6H, 18.18'-CH_3), 1.95 s (12H, in-chain-CH_3), 3.53 s (3H, $-OCH_3$), 6.0−6.8 m (14H, olefinic), 7.3−7.5 m (5 H, aromatic).

The LIS of the $-OCH_3$ signal are given in Table 1.

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Enamine Chemistry. XXVII.* Reduction of Enaminones, Enaminothiones and Thioamides by $LiAlH_4$ and $NaBH_4$

A.-B.A.G. GHATTAS,** K. A. JØRGENSEN and S.-O. LAWESSON

Department of Organic Chemistry, Chemical Institute, University of Aarhus, DK-8000 Aarhus C, Denmark

Enamines, *1*, prepared from cycloalkanones, (cyclopentanone and cyclohexanone), and secondary amines (pyrrolidine, piperidine and morpholine), are reacted with phenylisocyanate and phenylisothiocyanate to give inseparable mixtures of enaminones (enaminothiones) *2* and enamino carboxanilides (thiocarboxanilides), *2'*. Reduction of compounds *2a* and *b* with LAH affords 1-(*N*-phenyl)-aminomethylcyclopentene, *4*, whereas reduction of compound *2c* yields three compounds, *4*–*6*. Reaction of compounds *2a*–*d* with $NaBH_4$ in refluxing acetonitrile affords compounds *7a*–*d* and further reduction of compound *7b* with $NaBH_4$ gives 1-(*N*-phenyl)aminomethyl-2-(1-piperidino)cyclopentane, *8b*. The thiocompound *2e* is reduced by LAH, to give compound *4*, whereas $NaBH_4$ produces a mixture of *N*-phenylcyclopentanethiocarboxamide, *9*, and (*N*-phenyl)aminomethylcyclopentane, *10*. Reduction of *N,N*-disubstituted thioamides, *11* with $NaBH_4$ produces disulfides, mercaptans and amines. Mechanistic considerations are presented.

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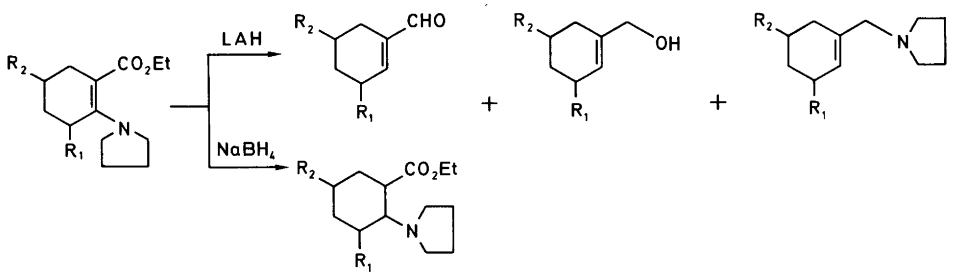
**On leave from the Faculty of Science, Assiut University, Sohag, Egypt.

Recently,[1] the reduction of some enaminones[2] by lithium aluminium hydride (LAH) and sodium borohydride has given some unexpected results (Scheme 1) and especially the formation of unsaturated aldehydes is noteworthy. In continuation of our earlier work, this paper will report the reduction of another type of enaminones (vinylogous ureas and thioureas) and also $NaBH_4$-reduction of thioamides.

RESULTS AND DISCUSSIONS

The starting enamines, *1*, are prepared according to the literature.[3–5] By reacting *1* with phenylisocyanate and phenylisothiocyanate in acetone or benzene at room temperature,[6,7] the products, *2,2'*, are smoothly isolated in good yields. Pyrrolidinocyclopentene, *1a*, and piperidinocyclopentene, *1b*, produce only the enaminones *2a* and *2b*, respectively, (no vinylic proton observed, *cf.* Table 1). However, the morpholino derivatives, *1c–d*, give a mixture of two isomers, *2c–d* and *2'c–d* and from their ^1H NMR spectra (Table 1 and Scheme 2)



Scheme 1.

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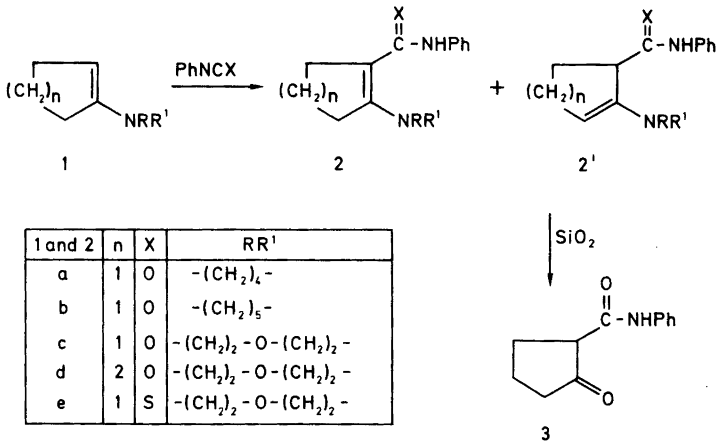


1 and 2	n	X	RR'
a	1	O	$-(CH_2)_4-$
b	1	O	$-(CH_2)_5-$
c	1	O	$-(CH_2)_2-O-(CH_2)_2-$
d	2	O	$-(CH_2)_2-O-(CH_2)_2-$
e	1	S	$-(CH_2)_2-O-(CH_2)_2-$

Scheme 2.

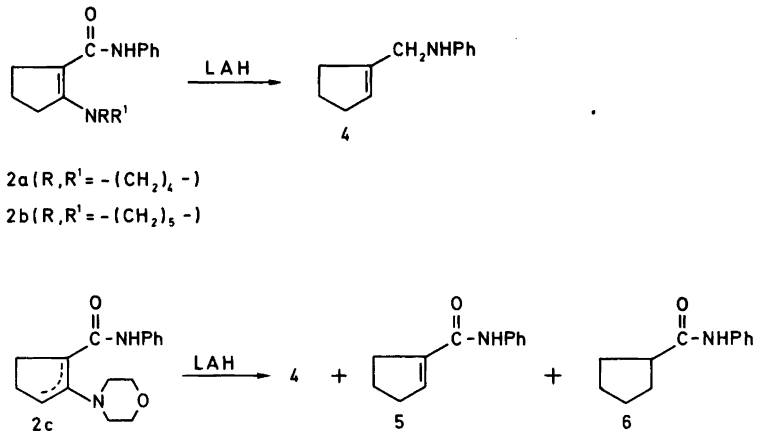
the ratios *2c/2'c*, *2d/2'd* and *2e/2'e* are found to be 38/62, 47/53 and 1/1, respectively.

Attempts to separate the two isomers *2c* and *2'c* (Scheme 2) using silica gel-column chromatography give only the hydrolysis product, *N*-phenyl-2-oxo-cyclopentanecarboxamide,[8] *3*.

Reduction of 2-(1-pyrrolidino)cyclopentene-1-(*N*-phenyl)carboxamide, *2a*, and 2-(1-piperidino)cyclopentene-1-(*N*-phenyl)carboxamide, *2b*, with LAH in refluxing tetrahydrofuran affords 1-(*N*-phenyl)aminomethylcyclopentene, *4*, in 16 and 24 % yields, respectively, whose spectroscopical and analytical data (see Experimental) are clearly consistent with the assigned structure. Thus, in IR absorption at 3550 cm^{-1} (NH) is observed, in ^1H NMR there is one vinylic hydrogen (δ 5.52) and in the ^{13}C NMR spectra the vinylic carbons are observed at δ 125.61 and 142.18. Besides precise measurements MS also shows the expected fragmentation pattern, *e.g.* an allylic scission to give *m/e* 81 ($M^+ - 92$). Reduction of 2-(1-morpholino)cyclopentene-1-(*N*-phenyl) carboxamide, *2c*, with LAH in refluxing tetrahydrofuran affords compound *4* as main product (33 %; Scheme 3). Thus, a number of by-products are

Table 1. ^1H NMR spectra of the compounds *2a–e* and *2'a–e*.

2 and *2'*	δ ($CDCl_3$)
a	8.71 (s, 1H) NH, 7.70–7.00 (m, 5H) Ph, 3.48 (t, *J* 6.5 Hz, 4H) CH_2-N-CH_2, 2.95–2.63 (m, 2H), 2.60–2.30 (m, 2H), 2.08–1.68 (m, 6H)
b	9.63 (s, 1H) NH, 7.68–6.75 (m, 5H) Ph, 2.95–2.50 (m, 4H), 2.50–2.05 (m, 4H), 1.92–1.28 (m, 8H)
c	9.90 (s, 0.62 H) NH, 9.00 (s, 0.38H) NH, 7.68–6.90 (m, 5H) Ph, 4.79 (t, *J* 1 Hz, 0.38H)C=CH, 3.90–3.30 (m, 4H) CH_2OCH_2, 3.11–1.52 (m, 10H)
d	12.22, 9.80 (s, 1H) NH, 7.78–6.98 (m, 5H) Ph, 5.10 (t, *J* 4 Hz, 0.47H)C=CH, 3.93–3.53 (m, 4H) CH_2OCH_2, 3.30–1.32 (m, 12H)
e	11.58 (s, 1H) NH, 7.85–7.00 (m, 5H) Ph, 3.80–3.42 (t, *J* 4 Hz, 4H), 4.80 (t, *J* 2 Hz, 0.5H), 3.00–2.20 (m, 8H), 2.00–1.50 (m, 2H)

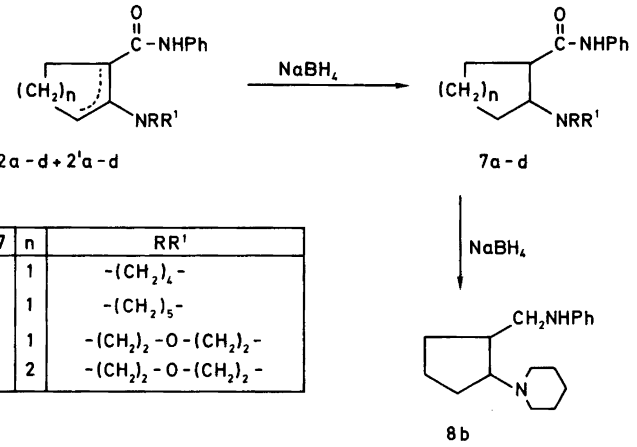


Scheme 3.

observed, two of them are isolated which are *N*-phenylcyclopentene-1-carboxamide,[9] *5*, (6%) and *N*-phenylcyclopentanecarboxamide,[10] *6*, (1%). The structures of the known compounds *5*[9] and *6*[10] are also proved by spectroscopy and precise mass measurements (*cf.* Experimental). As to the mechanism for the formation of *4*–*6* it is suggested that compound *5* is formed from *2c* by 1,4–H^- addition followed by elimination of the amine. Compound *5* by another H^- 1,2-addition. Similarly, *5* gives *6*.

The compounds *2a–d* are also reduced by $NaBH_4$ in refluxing acetonitrile to give cyclopentane and cyclohexane carboxanilides, *7a–d*, respectively, in good yields. These results are in accordance with a similar and exclusive 1,4-reduction of enaminones.[1] Further reduction of 2-(1-piperidino)cyclopentane-1-(*N*-phenyl)carboxamide, *2d*, with $NaBH_4$, *7b*, produces 1-(*N*-phenyl)aminoethyl-2-(1-piperidino)cyclopentane, *8b*, in 72 % yield. The structures of the reduced products are confirmed by precise mass measurements, ^1H NMR and IR spectra (Scheme 4, Table 2). The *7b*→*8b* reduction is unexpected since amides to our knowledge are not reduced by $NaBH_4$ alone (for other methods of amide reductions: $NaBH_4 + CH_3SO_3H$,[11] or $NaBH_4$ + transition metal salts[12]).

Also *2e*+*2'e* (Scheme 4) are reduced by LAH in refluxing THF to give a mixture of products, of which only compound *4* is isolated in 25 % yield. Reduction of *2e*+*2'e* by $NaBH_4$ in refluxing acetonitrile produces *N*-phenylcyclopentanethio-



2 and 7	n	RR¹
a	1	-(CH₂)₄-
b	1	-(CH₂)₅-
c	1	-(CH₂)₂-O-(CH₂)₂-
d	2	-(CH₂)₂-O-(CH₂)₂-

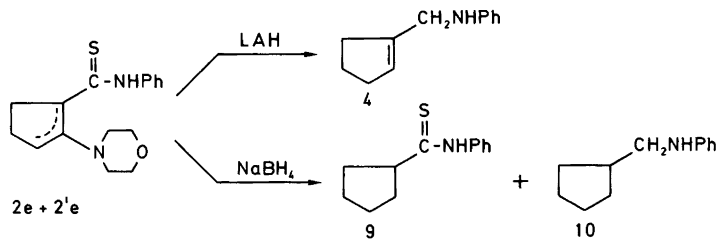
Scheme 4.

Table 2. Experimental data for compounds *7a–d* and *8*.

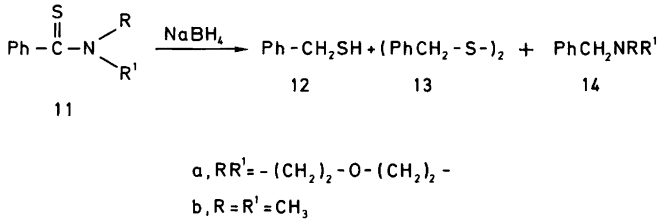
Compound	Yield (%)	M.p. (°C)	MS: *m/e* (rel.int. %)	^{1}H NMR ($CDCl_3$) δ	IR (cm^{-1})
7a	84	80	258 (M^+, 100), 201(36), 187(16), 156(38), 138 (45). Precise measurement on *m/e* 258 (calc. for $C_{16}H_{22}N_2O$: 258.1733, found: 258.1733)	12.30 (s, 1H) NH, 7.70–6.93 (m, 5H) Ph, 3.00–2.33 (m, 6H), 2.12–1.68 (m, 10H)	NH, 3400 CO, 1680
7b	100	162	272 (M^+, 52), 180(10), 152(86), 125(100). Precise measurement on *m/e* 272 (calc. for $C_{17}H_{24}N_2O$: 272.18889, found: 272.1888)	12.40 (s, 1H) NH, 7.70–6.80 (m, 5H) Ph, 3.00–2.35 (m, 6H), 2.19–1.00 (m, 12H)	NH, 3250 CO, 1700
7c	70	100	274 (M^+, 100), 257(12), 232(17), 182(19), 126 (60). Precise measurement on *m/e* 274 (calc. for $C_{16}H_{22}N_2O_2$: 274.1682. found: 274.1680)	11.82 (s, 1H) NH, 6.80–7.68 (m, 5H) Ph, 4.05–3.38 (t, *J* 15 Hz, 4H) 3.09–2.20 (m, 6H), 1.32–1.82 (m, 6H)	NH, 3300 CO, 1700
7d	70	145	288 (M^+, 65), 245(29), 194(27), 166(32), 126 (100). Precise measurement on *m/e* 288 (calc. for $C_{17}H_{24}N_2O_2$: 288.1838, found: 288.1837)	11.80 (s, 1H) NH, 7.70–6.81 (m, 5H) Ph, 3.92–3.52 (t, *J* 4 Hz, 4H) CH_2OCH_2, 3.03–2.13 (m, 6H), 2.13–0.90 (m, 8H)	NH, 3480 CO, 1705
8b	72		258 (M^+, 100), 173(49), 166(98), 124(98). Precise measurement on *m/e* 258 (calc. for $C_{17}H_{26}N_2O$: 258.2091, found: 258.2096)	7.20–6.30 (m, 5H) Ph 3.20–2.78 (m, 2H), 2.68–2.10 (m, 4H), 1.92–0.70 (m, 14H)	NH, 3250

carboxamide,[13] *9*, and (*N*-phenyl)aminomethylcyclopentane,[14] *10*, in 33% and 2% yields, respectively, (Scheme 5), the structures of which are confirmed by spectral and analytical data. Compound *9* is suggested to be formed from *2e* by 1,4-hydride addition, elimination of morpholine and subsequently another 1,4-reduction. Compound *10* is produced from *9* by a simple reduction (C=S→ >CH_2).

As to the reduction of *2e*+*2'e* with $NaBH_4$ no disulfides or mercaptans are isolated, which is contrary to a recent note[15] on $NaBH_4$-reduction of



Scheme 5.



Scheme 6.

certain simple thioamides (Scheme 5). Only the solid products have been isolated, according to the above cited note, without mentioning the reaction time and temperature. So we are strongly prompted to investigate the same reaction on a few simple thioamides which are of special interest.

It has now been found that the reduction of thioamides (*11*, $RR' = -(CH_2)_2-O-(CH_2)_2-$ or $-(CH_3)_2$) with $NaBH_4$ in refluxing isopropanol gives a mixture of three compounds: The corresponding mercaptans, *12*, disulfides, *13* and amines, *14*, in reasonable yields. Higher yields of all products are found with acetonitrile as solvent. Running the experiments in an inert atmosphere (N_2) does not give any change of product composition. (*cf.* Experimental, Scheme 6).

To our knowledge *N,N*-disubstituted thioamides have not been reduced by $NaBH_4$ to amines or to mercaptans. It is also observed (Experimental) that the yield of mercaptan is increased and that of disulfide is decreased at longer reaction time due to the known $NaBH_4$ reduction of disulphides.[16-19] As to the mechanism it is suggested that the first step is a 1,2-hydride addition to the thiocarbonyl group to give a salt of a semithioaminal, which subsequently produces the amine and mercaptan. *N*-Monosubstituted thioamides (*e.g.* thiobenzanilide[20]) give a complex product composition when reacted with $NaBH_4$ (for reduction of such thio-amides as *S*-salts, see Refs. 21 and 22).

EXPERIMENTAL

^1H NMR spectra were recorded at 60 MHz on a Varian A-60 spectrometer ($CDCl_3$) and the ^{13}C NMR spectra at 20 MHz on a CFT-20 Varian instrument ($CDCl_3$). TMS was used as internal standard. Chemical shifts are expressed in δ-values. IR spectra were recorded on a Beckman IR 18A spectrometer. Mass spectra and precise measurements were recorded on a Micromass 7070 mass spectrometer operating at 70 eV using direct inlet. M.p.s are uncorrected.

Starting materials. The enamines *1* are prepared by known methods.[3-5] Enamine carboxamides, enaminone carboxamides and thioanalogues, *2*, are obtained by the reaction of enamines *1* with phenylisocyanate and phenylisothiocyanate (0.01 mol: 0.01 mol) in an anhydrous solvent. Compounds *2c–d* are known.[6,7]

Compound 2a. 2-(1-Pyrrolidino)cyclopentene-1-(*N*-phenyl)-carboxamide. Benzene (30 ml), 20 min. Yield: 82 %. M.p. 138 °C (C_6H_6). Precise measurement of M^+ (calc. for $C_{16}H_{20}N_2O$, 256.1576, found: 256.1576). MS: *m/e* (rel.int. %): 2.56 (M^+, 100), 164 (49), 136 (29), 93 (71). ^1H NMR ($CDCl_3$): δ 8.71 (s, 1H) NH, 7.70–7.00 (m, 5H) Ph, 3.48 (t, *J* 6.5 Hz, 4H), CH_2NCH_2, 2.95–2.63 (m, 2H), 2.60–2.30 (m, 2H), 2.08–1.68 (m, 6H). IR (cm^{-1}) KBr: 1700 (C=O), 3300 (NH).

Compound 2b. 2-(1-Piperidino)cyclopentene-1-(*N*-phenyl)-carboxamide. Acetone (3 ml), 30 min. Yield: 98 %. M.p. 102 °C (ether). Precise measurements of M^+ (calc. for $C_{17}H_{22}N_2O$, 270.1733, found: 270.1731. MS: *m/e* (rel.int. %): 270 (M^+, 73), 203 (41), 178 (100), 150 (47), 93 (69), 74 (62). ^1H NMR ($CDCl_3$): δ 9.63 (s, 1H) NH, 7.68–6.75 (m, 5H) Ph, 2.95–2.50 (m, 4H), 2.50–2.05 (m, 4H), 1.92–1.28 (m, 7H). IR (cm^{-1}) KBr: 1670 (C=O), 3500 (NH).

General procedure of reduction with LAH. Enamine-derivatives (2+2'; 0.01 mol) are added dropwise on stirring to the ice-cooled mixture of LAH (0.03 mol) in anhydrous THF (100 ml) under N_2 atmosphere. The reaction mixture is refluxed for 1 h after the addition.

Work-up procedure. At 0 °C 1.14 g H_2O is added dropwise followed by 0.85 g of 30 % NaOH + 8.5 g H_2O. The ice-bath is removed and stirring is continued for 20 min. The mixture is then filtered, extracted with ether and dried ($MgSO_4$), the solvent is evaporated under reduced pressure and the product is purified on silica gel plates using 50 % ether–light petroleum as eluent.

Compound 4. 1-(*N*-Phenyl)aminomethylcyclopentene. Precise measurement of M^+/e: 173 (calc. for $C_{12}H_{15}N$, 173.1204, found: 173.1205). MS: *m/e*

(rel.int. %): 173 (M^+, 100), 107 (41), 93 (59), 81 (29), 77 (29). ^1H NMR ($CDCl_3$): δ 7.30–6.39 (m, 5H) Ph, 5.52 (t, J 3 Hz, 1H) C=CH, 3.68 (s, 2H), 2.50–1.42 (m, 6H). IR(cm^{-1}): 3550 (NH).

Compound 5. Cyclopentene-1-(*N*-phenyl)carboxamide,[9] m.p. 125 °C (lit.[9] 126 °C), yield: 6 %, precise measurement of M^+ (calc. for $C_{12}H_{13}NO$, 187.0997, found: 187.0997). IR (cm^{-1}) KBr: 1725 (C=O), 3400 (NH).

Compound 6. *N*-Phenylcyclopentanecarboxamide,[10] m.p. 160 °C (lit.[10] 160.1–161.2 °C), yield: 1%, precise measurement of M^+ (calc. for $C_{12}H_{15}NO$, 189.1153, found: 189.1155). IR (cm^{-1}) KBr: 1725 (C=O), 3300 (NH).

General procedure for reduction with $NaBH_4$. Sodium borohydride (0.03 mol) and enamine-derivatives (2+2′; 0.01 mol in acetonitrile (30 ml) are refluxed for 1 h.

Work-up procedure. To the ice-cooled reaction mixture, 1.14 g H_2O is added dropwise followed by 0.85 g of 30 % NaOH + 8.5 g H_2O. The ice-bath is removed and the mixture dried ($MgSO_4$); then the solvent is evaporated under reduced pressure and the product purified by crystallization from ether–light petroleum.

Compounds 7a–d *and* 8: (*cf.* Table 2).

Compound 9. *N*-Phenylcyclopentanethiocarboxamide,[11] m.p. 75 °C (lit.[11] 80 °C). Precise measurement of M^+ (calc. for $C_{12}H_{15}NS$, 205.0925, found: 205.0925). MS: *m/e* (rel. int. %): 205 (M^+, 92), 172(100), 164(87), 130(27). ^1H NMR ($CDCl_3$): δ 9.01 (s, 1H) NH, 7.70–6.90 (m, 5H) Ph, 3.28–2.63 (m, 1H), 2.20–1.18 (m, 8H). IR (cm^{-1}) KBr: 3400 (NH).

Compound 10. (*N*-Phenyl)aminomethylcyclopentane,[12] yield: 2 %. Precise measurements on M^+ (calc. for $C_{12}H_{17}N$, 175.1361, found: 175.1361). MS: *m/e* (rel.int. %): 175 (M^+, 20), 106 (100). IR (cm^{-1}): 3400 (NH).

Reduction of thioamide 11a *with $NaBH_4$.* Sodium borohydride (1.14 g, 0.03 mol) is added to a solution of thioamide, *11a*, (2.07 g, 0.01 mol) in isopropanol (20 ml) and the mixture is refluxed for 3 h. The solvent is evaporated under reduced pressure and water (25 ml) is added to the reaction mixture. The products are extracted several times with CH_2Cl_2, the organic layer is extracted with dil. NaOH to separate benzyl mercaptane,[23] *12a*, as sodium salt (water phase) and on acidification, it is isolated in 31 % yield. The CH_2Cl_2 layer is then extracted again with dil. H_2SO_4 to separate benzyl morpholine,[24] *14a*, as sulfate (27 %). The CH_2Cl_2 layer is finally washed with water and dried ($MgSO_4$), the solvent is evaporated to yield dibenzyl disulfide,[15–19] *13a*, in 28 % yield.

The products are in all respects identical with authentic samples.

Reduction of the same thioamide, *11a*, in refluxing acetonitrile (2 h) (TLC) as described above produces benzyl mercaptan, benzyl morpholine and dibenzyl disulfide in 55, 22 and 14 % yields, respectively.

Reduction of the thioamide 11b *with $NaBH_4$ in acetonitrile. 11b* is reduced as above for 26 h under reflux (TLC) to yield benzyl mercaptan, *12a*, (68 %). *N,N*-Dimethylbenzylamine,[25] (23 %) *14b* and dibenzyl disulfide, *13a*, (6 %). The three products are identical in all respects with authentic samples.

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Silver Imidazolate-assisted Glycosidations. Part 7.* Synthesis of 1,2-*trans*-Linked Aryl Glycosides

PER J. GAREGG, HANS HULTBERG, CARMEN ORTEGA and BERTIL SAMUELSSON

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Efficient preparations of 1,2-*trans*-linked aryl glycosides starting from fully acetylated glycopyranosyl bromides are described. The promoting system is silver imidazolate and zinc chloride.

p-Nitrophenyl glycosides are useful intermediates for linking carbohydrates to proteins in the preparation of artificial antigens.[1,2] An efficient way of achieving this is to first reduce the nitro group to an amino group and then transform the latter into an isothiocyanato group by reaction with thiophosgene. On reaction of the resulting *p*-isothiocyanatophenyl glycoside with a free amino group in a protein, the carbohydrate moiety becomes linked to the protein *via* a phenyl thiourea residue.[3]

We now present an efficient synthesis of 1,2-*trans*-linked *p*-nitrophenyl glycosides, starting from fully acetylated glycopyranosyl bromides using silver imidazolates and zinc chloride as promotors.[4] The molar proportions of glycosyl bromide, *p*-nitrophenol, zinc chloride, and silver imidazolate

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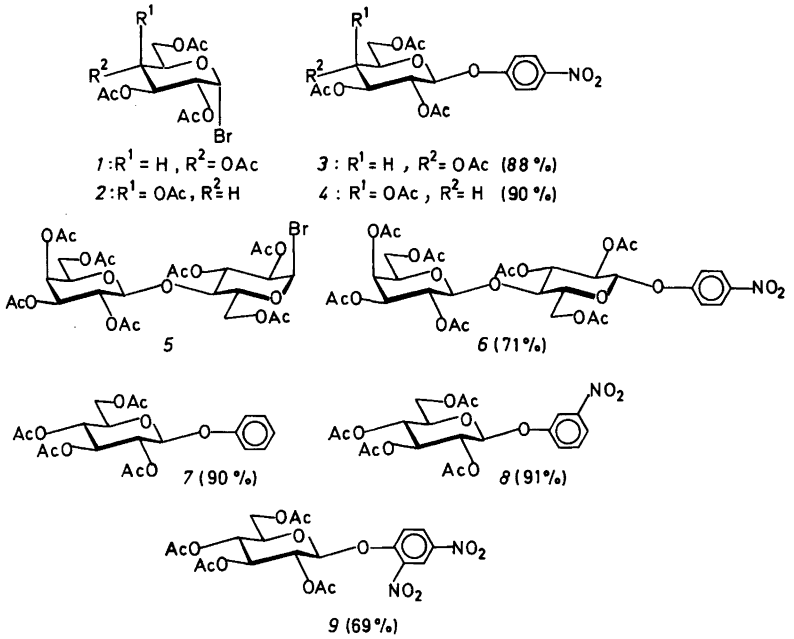

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were 1:1.5:~6:0.75 and the yields of 1,2-*trans*-linked *p*-nitrophenyl glycosides varied from 71 to 90%. Other 1,2-*trans*-linked aryl glycosides (*7*–*9*) were similarly obtained in yields ranging from 69 to 91%. The yields obtained are, with one exception only (compound *4*[5]), higher than those previously reported[5–13] for the synthesis of 1,2-*trans*-linked aryl glycosides from fully acetylated glycopyranosyl bromides.

EXPERIMENTAL

General methods were the same as those reported elsewhere.[4,14]

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranoside* (*3*). A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (*1*) (400 mg, 1 mmol), *p*-nitrophenol (180 mg, 1.5 mmol), silver imidazolate[15,16] (131 mg, 0.75 mmol) and zinc chloride (dry weight 0.8–1 g, ~6 mmol[4]) in dichloromethane (10 ml) containing 3 Å molecular sieves was stirred in the dark at 40 °C for 48 h, when TLC indicated complete reaction. The reaction mixture was diluted with dichloromethane and filtered. The solids were washed with dichloromethane. The combined filtrates were partitioned between toluene (200 ml) and aqueous sodium carbonate (200 ml). The organic phase was washed with water. The solution was dried ($MgSO_4$), filtered and concentrated. Crystallization of the product from ethanol gave *3* (383 mg, 88%), m.p. 175–176 °C, $[\alpha]_D^{22}$ −41° (*c* 1, $CHCl_3$) [lit.[5–7,13] m.p. 174–175 °C, $[\alpha]_D$ −37°, −41° ($CHCl_3$)].

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranoside* (*4*) was obtained from *2* as described above for *3*, in a yield of 90%, m.p. 148–149 °C, $[\alpha]_D^{22}$ −11° (*c* 1, $CHCl_3$) [lit.[5,8] m.p. 145–146 °C, 144–145 °C, $[\alpha]_D$ −11°, −8°, −10° ($CHCl_3$)].

p-*Nitrophenyl 4*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (*6*) was obtained from *5* as described above for *3*, in a yield of 71%, m.p. 131–133 °C, $[\alpha]_D^{22}$ −37° (*c* 1, $CHCl_3$) [lit.[9] 132–133 °C, $[\alpha]_D$ −35° ($CHCl_3$)].

Phenyl 2,3,4,6-tetra-O-*acetyl-β*-D-*glucopyranoside* (*7*) was obtained from the reaction of *1* with phenol as described above for *3*. The reaction mixture was diluted with dichloromethane and filtered. The combined filtrates were washed with aqueous ammonia, 2 M sodium hydroxide, and water, dried ($MgSO_4$), filtered and concentrated. Crystallization from ethanol gave *7*, 90%, m.p. 124–125 °C, $[\alpha]_D^{22}$ −22° (*c* 1, $CHCl_3$) [lit.[10] m.p. 124–125 °C, $[\alpha]_D$ −22° ($CHCl_3$)].

m-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranoside* (*8*) was obtained from the reaction of *1* with *m*-nitrophenol as described above for *7*, in a yield of 91%, m.p. 136–137 °C, $[\alpha]_D^{22}$ −37° (*c* 1, $CHCl_3$) [lit.[5,6] m.p. 136–137 °C, $[\alpha]_D$ −42°, −37° ($CHCl_3$)].

o,p-*Dinitrophenyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranoside* (*9*) was obtained from the reaction of *1* with *o,p*-dinitrophenol as described above for *7*, in a yield of 69%, m.p. 176–177 °C, $[\alpha]_D^{22}$ +35° ($CHCl_3$) [lit.[11,12] m.p. 177–179 °C and 173–177 °C, $[\alpha]_D$ +34.5°, +33°].

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Formation of 1,5-Dideoxy-1,5-iminohexitols on Borohydride Reduction of 2-Amino-2-deoxyhexofuranurono-6,3-lactones

KARIN LEONTEIN, BENGT LINDBERG and JÖRGEN LÖNNGREN

Department of Organic Chemistry, University of Stockholm, S-106 91 Stockholm, Sweden

1,5-Dideoxy-1,5-imino-D-mannitol and 1,5-dideoxy-1,5-imino-L-gulitol are formed on borohydride reduction of 2-amino-2-deoxy-D-mannofuranurono-6,3-lactone and the corresponding D-*gluco*-derivative, respectively. In the assumed mechanism 5-amino-5-deoxy-D-mannose and 5-amino-5-deoxy-L-gulose are first formed by partial reduction of the starting materials, and are then further reduced to the corresponding 1,5-dideoxy-1,5-iminohexitols.

The formation of 1,5-dideoxy-1,5-iminohexitols as artefacts in sugar analysis of complex carbohydrates containing 2-amino-2-deoxyhexuronic acid residues is discussed.

During sugar analysis of the capsular polysaccharide from *Streptococcus pneumoniae* type 12F,[1] involving acid hydrolysis, borohydride reduction and analysis of the resulting alditols by GLC of their acetates, we observed an artefact which, from its mass spectrum, was identified as a 1,5-dideoxy-1,5-iminohexitol. The polysaccharide contained 2-acetamido-2-deoxy-D-mannuronic acid as one of its sugar components and, as this sugar was the probable source of the artefact, the latter was assumed to have the D-*manno*-configuration. We now report further studies on the formation of this substance and of the corresponding L-*gulo*-derivative.

In the assumed reaction route the 2-acetamido-2-deoxy-D-mannuronic acid released during the acid hydrolysis is *N*-deacetylated and lactonized to 2-amino-2-deoxy-D-mannofuranurono-6,3-lactone (*1*). On treatment with aqueous sodium borohydride this lactone should be partially reduced, at C-1 and C-6, to 5-amino-5-deoxy-D-mannose (*2*) (that the amino group is now at C-5 is the result of nomenclature rules.). Analogous reductions of lactones to aldoses have been reported.[2] Compound *2*, in the pyranose form with nitrogen in the ring, is further reduced to 1,5-dideoxy-1,5-imino-D-mannitol (*3*). The corresponding reduction of 5-amino-5-deoxy-D-glucose, nojirimycin, to 1,5-dideoxy-1,5-imino-D-glucitol, has been reported.[3]

In order to test this hypothesis, the crystalline 2-acetamido-2-deoxy-D-mannofuranurono-6,3-lactone, prepared *via* the appropriate glycal by azidonitration,[4] was converted into *1* by acid hydrolysis. The best yields were obtained with strong acid and short reaction time (*e.g.* 4 M HCl, 100 °C, 7 min). Treatment of *1* with aqueous sodium borohydride followed by acidification with acetic acid yielded *3*, isolated as its amorphous hydroacetate (*3* × HOAc) (72 %), $[\alpha]_{578}$ −5°. The sodium salt of 5-amino-5-deoxy-D-mannonic acid (*4*) was also formed in about 16 % yield, demonstrating that part of the lactone was hydrolyzed. However, no 2-amino-2-deoxy-D-mannitol was found, indicating that the formation of *2* in cyclic form is faster than the reduction of the intermediary aldehyde to its alcohol. When *4* was converted into the lactone by treatment with acid and then treated with sodium borohydride, a further amount of *3* was formed. When the reduction of *1* was performed with borodeuteride, two atoms of deuterium were introduced at C-1 and one at C-6 in *3*, as demonstrated by mass spectrometry of the acetylated product. As expected, no *3* but only 2-acetamido-2-deoxy-D-mannitol was obtained when the *N*-acetylated uronolactone was treated with sodium borohydride.

Similar treatment of 2-amino-2-deoxy-D-gluco-furanurono-6,3-lactone (*5*), prepared from the 2-acetamido derivative, yielded 1,5-dideoxy-1,5-imino-L-gulitol (*7*), *via* the assumed 5-amino-5-deoxy-L-gulose (*6*). The yield of *7*, as its amorphous hydroacetate (*7* × HOAc), $[\alpha]_{578}$ +2°, was only

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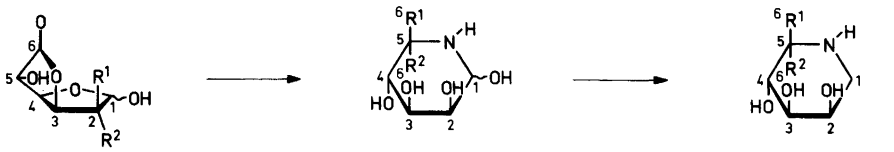
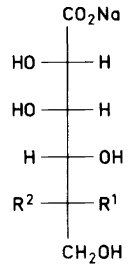


1 $R^1=NH_2$; $R^2=H$ *2* $R^1=CH_2OH$; $R^2=H$ *3* $R^1=CH_2OH$; $R^2=H$

5 $R^1=H$; $R^2=NH_2$ *6* $R^1=H$; $R^2=CH_2OH$ *7* $R^1=H$; $R^2=CH_2OH$



4 $R^1=NH_2$; $R^2=H$

8 $R^1=H$; $R^2=NH_2$

about 30 % but was increased to 61 % by treating the product, containing the sodium salt of 5-amino-5-deoxy-L-gulonic acid (*8*), with acid followed by a second borohydride reduction. The results suggest that the lactone with the D-*gluco*-configuration (*5*) is hydrolyzed more readily than the D-*manno*-isomer. Acidic aqueous solutions of *1* and *5* contain about 9:1 and 1:1, respectively, of the lactone *vs.* free acid at equilibrium, as demonstrated by NMR spectroscopy.

When 2-acetamido-2-deoxy-D-galacturonic acid[4] was treated with strong acid, followed by borohydride reduction, no 1,5-dideoxy-1,5-iminohexitol was formed. This was expected, as 2-amino-2-deoxy-D-galacturonic acid does not lactonize readily.

The mass spectra of the fully acetylated 1,5-dideoxy-1,5-iminohexitols with the D-*gluco*-, D-*manno*- and L-*gulo*-configurations are similar. The molecular ion, *m/z* 373, is observed in these spectra. The ions *m/z* 313 and *m/z* 300 are formed from the molecular ion by loss of acetic acid or the side chain, respectively. The elimination of an acetoxyl radical from the former ion to give *m/z* 254 is a less common reaction. A similar elimination was observed for the acetate of 1,5-dideoxy-1,5-imino-D-xylitol.[5] Other ions are formed by consecutive eliminations of acetic acid and ketene typical for this group of substances. Mass spectra of deuterated analogues of *7*, containing one deuterium atom on C-6 and/or two deuterium atoms on C-1, were consistent with the fragmentation routes indicated. Pertinent ions with some interpretations are given in the Experimental.

In the ^{13}C NMR spectrum of *3* × HOAc in D_2O, signals were observed at δ 24.5 (CH_3CO), 48.7 (C-1), 59.4 (C-6), 61.5 (C-5), 67.1, 67.3, 73.8 (C-2, C-3, C-4), and 182.8 ($C=O$). The corresponding values for *7* × HOAc were 24.5 (CH_3CO), 43.4 (C-1), 56.4 (C-5), 60.1 (C-6), and 63.8, 68.3, 69.6 (C-2, C-3, C-4), and 182.8 ($C=O$).

The ^1H NMR spectra of *3* and *7* as their hydroacetates are given in Experimental. The signals could readily be assigned by spin decoupling experiments, and the results support the assigned structures. It is obvious that *3* is present in the $^4C_1$ conformation while *7* is in the $^1C_4$ conformation. The spectrum of the D-*manno*-isomer (*3* × HOAc) is closely similar to the spectrum of the corresponding hydrochloride.[6]

In sugar analysis of polysaccharides involving acid hydrolysis, removal of the acid by distillation, borohydride reduction, acetylation and GLC of the alditol acetates, 2-amino-2-deoxyhexuronic acids which are readily lactonized may give 1,5-dideoxy-1,5-iminohexitols, as discussed above. Of the 2-amino-2-deoxyhexuronic acids found in Nature,[7] those with the D-*gluco*-, D-*manno*- and *gulo*-configurations lactonize but not those with the D- or L-*galacto* and L-*altro* configurations. On sugar analysis of three bacterial polysaccharides containing 2-acetamido-2-deoxy-D-mannuronic acid residues, from *Streptococcus pneumoniae* type 12F,[1] and *Haemophilus influenzae* types d[8] and e,[9] the yield

of 1,5-dideoxy-1,5-imino-D-mannitol was 60, 32 and 40 %, respectively, compared to those of the alditols from the non-acidic sugar components and estimated from the areas under the peaks on GLC. In the analysis of a fungal polysaccharide, from *Rhinocladiella mansonii*,[10] containing 2-acetamido-2-deoxy-D-glucuronic acid residues, the corresponding yield of 1,5-dideoxy-1,5-imino-L-gulitol was 20 %. The yield of 1,5-dideoxy-1,5-iminohexitols depends on several factors and was optimized only for the *S. pneumoniae* type 12F polysaccharide.

Two 1,5-dideoxy-1,5-iminohexitols, the D-*gluco*-[11] and the D-*manno*-isomer,[6] are natural products and the former has been synthesized.[12] The present work describes the first synthesis of the D-*manno*-isomer. The D-*galacto*-isomer has also been synthesized recently.[13] Nothing is known about the biosynthesis of 1,5-dideoxy-1,5-iminohexitols. A route starting from the corresponding 2-amino-2-deoxyhexuronic acid, analogous to that described above, does not seem to be excluded.

EXPERIMENTAL

General methods. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded in the pulsed Fourier-transform mode using JEOL FX-100 (^{13}C NMR) or Bruker WH270 (^{1}H NMR) instruments. Chemical shifts are given relative to external TMS (^{13}C) and relative to the HDO peak at δ 4.78 (^{1}H). ^{1}H NMR spectra were interpreted on a first order basis. Mass spectra were recorded at 70 eV on a JEOL D-300 instrument connected with a Finnigan Nova-3 computer. For GLC, at 190 °C, a Perkin-Elmer 990 instrument fitted with a glass column (180 × 0.15 cm) containing 3 % OV-17 on Gas Chrom Q was used.

1,5-Dideoxy-1,5-imino-D-mannitol hydroacetate (3 × HOAc) and sodium 5-amino-5-deoxy-D-mannoate (4). 2-Acetamido-2-deoxy-D-mannofuranurono-6,3-lactone (31 mg) was dissolved in 4*M* hydrochloric acid (2 ml) and kept at 100 °C for 7 min. Hydrochloric acid was removed by distillation in a vacuum at 20 °C. Water was added in the beginning of the distillation in order to avoid high concentration of acid. The product was dissolved in water and freeze-dried. The crude product was dissolved in water (2 ml) and sodium borohydride (70 mg) was added. The solution was kept at room temperature overnight, acidified to pH 4 with 50 % acetic acid and boric acid was removed by co-distillation with methanol (3 × 2 ml). Purification of the crude product on a column of Sephadex G-15 (2.5 × 80 cm) irrigated with water gave amorphous *3* × HOAc (23 mg, 72 %) and *4* (5 mg, 16 %). Compound *3* × HOAc showed $[\alpha]_{578}$ −5° (*c* 1.1, water); ^{1}H NMR (270 MHz, D_2O): δ 1.89 (3 H, s, OAc), 2.94 (1 H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.1 Hz, $J_{5,6'}$ 3.3 Hz, H-5), 3.09 (1 H, dd, $J_{1a,1e}$ 13.6 Hz, $J_{1a,2}$ 1.0 Hz, H-1a), 3.28 (1 H, dd, $J_{1e,2}$ 2.9 Hz, H-1e), 3.64 (1 H, dd, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.2 Hz, H-3), 3.80 (1 H, dd, H-4), 3.81 (1 H, dd, $J_{6,6'}$ 12.2 Hz, H-6), 3.94 (1 H, dd, H-6'), and 4.16 (1 H, ddd, H-2). ^{13}C NMR (25.05 MHz, D_2O): δ 24.5 (OAc), 48.7 (C-1), 59.4 (C-6), 61.5 (C-5), 67.1, 67.3, 73.8 (C-2, C-3, C-4), and 182.8 (C=O). Compound *4* showed $[\alpha]_{578}$ −3° (*c* 0.3, water); ^{13}C NMR (25.05 MHz, D_2O): 56.7 (C-5), 59.5 (C-6), 69.0, 72.9 (C-3, C-4), 74.5 (C-2), and 179.6 (C-1).

1,5-Dideoxy-1,5-imino-L-gulitol hydroacetate (7 × HOAc) and sodium 5-amino-5-deoxy-L-gulonate (8). 2-Acetamido-2-deoxy-D-glucofuranurono-6,3-lactone (32 mg) was *N*-deacetylated and treated with aqueous sodium borohydride as described for compound *3*. Purification of the product gave 7 × HOAc (10 mg, 30 %). In an analogous experiment the crude product was dissolved in 0.5 M hydrochloric acid (3 ml) and kept at 100 °C for 5 min. The solution was concentrated to dryness and dissolved in water. Sodium borohydride (60 mg) was added and the solution was kept at room temperature overnight, acidified to pH 4 with 50 % acetic acid and boric acid was removed by co-distillation with methanol. Purification of the crude product on a column of Sephadex G-15 (2.5 × 80 cm) irrigated with water gave amorphous 7 × HOAc (20 mg, 61 %) and *8* (9 mg, 28 %). Compound 7 × HOAc showed $[\alpha]_{578}$ +2° (*c* 1,0, water); ^{1}H NMR (270 MHz, D_2O): δ 1.89 (3 H, s, OAc), 3.05 (1 H, dd, $J_{1a,1e}$ 12.1 Hz, $J_{1a,2}$ 11.3 Hz, H-1a), 3.23 (1 H, dd, $J_{1e,2}$ 5.0 Hz, $J_{1e,3}$ 0.8 Hz, H-1e), 3.45 (1 H, ddd, $J_{5,6}$ 8.5 Hz, $J_{5,6'}$ 5.2 Hz, $J_{4,5}$ 1.8 Hz, H-5), 3.76 (1 H, dd, $J_{6,6'}$ 12.1 Hz, H-6), 3.84 (1 H, dd, H-6'), 4.01 (1 H, dd, $J_{3,4}$ 4.6 Hz, $J_{2,3}$ 2.8 Hz, H-3), 4.07 (1 H, dd, H-4), and 4.20 (1 H, ddd, H-2). ^{13}C NMR (25.05 MHz, D_2O): δ 24.5 (OAc), 43.4 (C-1), 56.4 (C-5), 60.1 (C-6), 63.8, 68.3, 69.6 (C-2, C-3, C-4), and 182.8 (*C*=0). Compound *8* showed $[\alpha]_{578}$ −9° (*c* 0.8, water); ^{13}C NMR (25.05 MHz, D_2O): 56.6 (C-5), 60.3 (C-6), 68.1, 73.7 (C-3, C-4), 74.0 (C-2), and 180.0 (C-1).

Sugar analyses. The polysaccharide (1 − 2 mg) was treated with 4 M hydrochloric acid (2 ml) at 100 °C for 2 h. The solution was worked up as described for *3* × HOAc. The product was dissolved in water (1 ml) and treated with sodium borohydride (~20 mg) at room temperature overnight. The solution was acidified with 50 % acetic acid, concentrated, and worked up as described above. The product was acetylated by treatment with acetic anhydride – pyridine (1:1, 1 ml) at 100 °C for 50 min. After work-up the alditol acetates were analyzed by GLC. The acetates of *3*, 7 and 1,5-dideoxy-1,5-imino-D-glucitol showed T_{Glc} = 1.41, 1.38 and 1.30,

Table 1. Mass spectra of fully acetylated 7. Relative intensities in and some plausible assignments in brackets. A. Non-deuterated. B. Dideuterated at C-1, monodeuterated at C-6. C. Dideuterated at C-1.

A	B	C
373(<0.1)[M^+]	376(<0.1)	375(<0.1)
330(0.3)[$M^+ - Ac$]	333(0.3)	332(0.4)
313(7)[$M^+ - HOAc$]	316(8)	315(8)
300(9)[$M^+ - CH_2OAc$]	302(9)	302(9)
254(24)[313 − OAc]	257(27)	256(25)
240(42)[313 − CH_2OAc]	242(48)	242(44)
212(4)[254 − $CH_2=C=O$]	215(3),214(4)	214(3),213(4)
198(17)[240 − $CH_2=C=O$]	200(19)	200(18)
180(13)[240 − HOAc]	181(9)	181(9)
152(9)	155(5),154(5)	154(7),153(4)
138(100)[198 − HOAc]	140(100)	140(95)
110(8)	113(4),112(4)	112(6),111(4)
96(95)	98(100)	98(100)

respectively (T_{Glc} = retention time relative to glucitol hexaacetate).

Mass spectrometry. Samples (0.5 − 1.0 mg) of *1* and *5* were converted into *3* and *7* as described above. For preparation of deuterated analogs of *3* and *7* sodium borodeuteride was used in the reduction step. In some experiments *8* was transformed into its lactone and then reduced with sodium borodeuteride to the analogue of *7* with two deuterium atoms at C-1. These compounds and 1,5-dideoxy-1,5-imino-D-glucitol were then acetylated and analyzed by GLC − MS. Some pertinent fragments in the mass spectra of fully acetylated *7* and its partially deuterated analogues are given i Table 1.

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The Multiplicity of Reaction Pathways of Cation Radicals Derived from Anthracene Derivatives in Solvents of Low Nucleophilicity

OLE HAMMERICH[a] and VERNON D. PARKER[b]

[a] Department of General and Organic Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark and [b] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

In acetonitrile containing trifluoroacetic acid, the cation radicals of 9-substituted anthracenes either dimerize (a), react with acetonitrile (b) or react with trifluoroacetic acid (c) depending on the nature of the 9-substituent. All three reaction pathways were demonstrated during kinetic and product studies. Pathway (a) is of importance when the intermediate dimeric dication, which has both the substituents and the charges in the 10,10′-positions, is stabilized by virtue of the electron donating properties of the substituent. This pathway was observed exclusively for 9-phenyl and 9-methoxy and to a lesser extent when the substituent was 9-chloro. Pathway (c) predominates when the 9-substituent destabilizes the positive charge as is the case for 9-nitro. The intermediate case (b) is the predominant reaction pathway for the anthracene cation radical and is also observed when the substituent is 9-methyl. The feature of pathway (b) which differs most from (c) is that the intermediate cation radical-nucleophile adduct in (b) is charged and if the substituent is electron withdrawing the oxidation of this species by cation radical is less favorable so that trifluoroacetoxylation (c) can then effectively compete. All three of the reaction pathways were observed to give rise to complex rate laws.

The reactions of the cation radicals of anthracene and substituted anthracenes have been the subject of numerous investigations.[1–45] The reactions usually produce easily characterized products and this has contributed to their use as model substrates. The first indication of the mechanism of anodic substitution emerged from the study of the anodic pyridination of anthracene[1] in which it was observed that the two-electron oxidation of anthracene in acetonitrile in the presence of pyridine was accompanied by the formation of the 9,10-dipyridinium salt. The oxidation of anthracene in acetonitrile containing water was later shown to produce 10,10′-bianthronyl in high yield.[2] The mechanism of this reaction was suggested to involve the reaction of the cation radical with water to give anthrone as the first product which then undergoes air-oxidation during the work-up procedure.[3] Coulometric and products studies[12–14] later showed that anthrone is not an intermediate in the reaction of the cation radical with water and that 10,10′-bianthronyl is the first stable reaction product. Detailed studies were carried out on several reactions of anthracene cation radical including hydroxylation,[15] acetoxylation[16] and methoxylation.[17]

The anthracenes, particularly 9,10-diphenylanthracene (DPA), served as essential substrates to establish the one-electron oxidation pathway of aromatic hydrocarbons in aprotic media. The latter was firmly established by three independent studies in different solvent systems including acetonitrile,[4] dichloromethane[5] and nitrobenzene.[6] The first definitive kinetic studies of the reactions of cation radicals employed DPA as substrate for hydroxylation[7] and pyridination.[9] The so-called "half-regeneration" and ECE mechanisms, were proposed for these reactions, both of which involve the cation radical as the intermediate reacting with the nucleophile. However, it was later observed that chronoamperometric data for the pyridination of

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DPA were more consistent with the theoretical working curve for the disproportionation mechanism than with that for the ECE scheme, an observation which suggested that the dication is the reactive intermediate.[21]

The mechanism dispute, *i.e.* cation radical (eqn. (1))[9] or dication (eqn. (2))[21] was at about the same time emerging for a related reaction, that of thiantrene cation radical with water.[46,47] These two

$$A^{\cdot+} + N \rightarrow A^{\cdot} - N^{+} \quad (1)$$

$$A^{2+} + N \rightarrow A^{+} - N^{+} \quad (2)$$

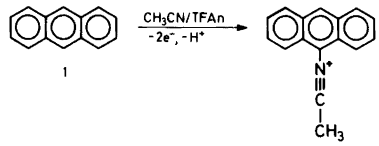
systems and the important mechanistic distinction between primary reactions (1) or (2) set the stage for most of the mechanistic studies of cation radical reactions which were carried out in the 1970's. The proposal of the disproportionation mechanism for the pyridination of $DPA^{\cdot+}$ was shown to be highly unlikely by a kinetic analysis taking into account the small value of the disproportionation equilibrium constant (K_3).[23] It was shown that forward reaction (3) would limit the rate of

$$2\,DPA^{\cdot+} \underset{}{\overset{K_3}{\rightleftarrows}} DPA^{2+} + DPA \quad (3)$$

the overall reaction leading to kinetics independent of the pyridine concentration[23] which is contrary to the experimental observations.[9] The need for reliable data to estimate disproportionation equilibrium constants led to the development of methods to determine the reversible potentials for the oxidation of cation radicals to dications,[26] a highly useful benefit resulting from the mechanism controversy.

Spectroelectrochemical studies,[32,33] apart from minor differences, confirmed the conclusions on the mechanism of the hydroxylation of $DPA^{\cdot+}$. Homogeneous kinetic studies[29,36,37] and spectroelectrochemical[28,36] investigations confirmed the earlier conclusion[9,23] that the cation radical is the reactive intermediate involved in the pyridination of $DPA^{\cdot+}$. Similarly, the much more reactive cation radical derived from 9-phenylanthracene was observed to react with pyridine with a rate constant of the order of 10^7 M^{-1} s^{-1} at 298 K.[49] A number of other nucleophiles have been employed but as yet there is not general agreement on the detailed mechanism of any of the reactions.[43,48]

In a preliminary communication[27] on which this work is based, we observed that when water is effectively removed from acetonitrile solutions anthracene cation radical reacts with solvent to give the nitrilium ion (eqn. (4)). The purpose of this paper is to outline the reaction pathways of cation radicals of anthracene and 9-substituted anthracenes in media with only weak nucleophiles present.

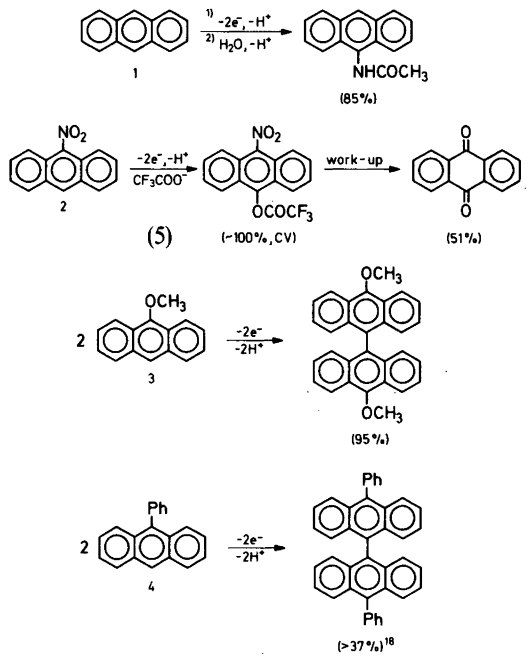


(4)

RESULTS

Product studies. The results of preparative anodic oxidation of anthracene, *1*, and different 9-substituted anthracenes demonstrated that essentially three different reaction pathways were followed dependent on the nature of the substituent as illustrated in Scheme 1. Formation of the acetamido derivative was the major reaction for *1* and was also observed for 9-methyl- and 9-chloroanthracene, although to a lesser extent (30–40 %).

In the case of 9-nitroanthracene, *2*, an apparent inconsistency between the results obtained by cyclic



Scheme 1.

voltammetry and preparative electrolysis was observed. CV analysis demonstrated (see next section) the product to be 9-nitro-10-trifluoroacetoxyanthracene, while the product isolated after preparative electrolysis was found to be anthraquinone. However, it is most likely that the anthraquinone does arise *via* the trifluoroacetoxy derivative which may suffer hydrolysis during work-up in a process similar to the Nef reaction. When the 9-substituent was either methoxy, *3*, or phenyl, *4*, the isolated products were dimers resulting from coupling reactions. The dimers were further oxidized under the reaction conditions which may cause the isolated yield to be low if irreversible follow-up reactions can take place as was observed in the case of *4*. The earlier result [18] that higher oxidation products of the dimer were formed during electrolysis was confirmed in this study. The 10,10′-dimer could be detected as a minor product from 9-chloroanthracene as well.

Trifluoroacetoxylation of 9-nitroanthracene. In acetonitrile containing trifluoroacetic acid and trifluoroacetic anhydride (AN–TFA–TFAn=9:1:1) cyclic voltammetry indicated not only the primary oxidation peak but also one due to the oxidation of a reaction product of the cation radical. In the presence of $CF_3CO_2^-LH^+$ (L=2,6-lutidine), prepared *in situ* by the reaction of TFA with L, the peak due to the reaction product was about the same height as the primary peak indicating nearly complete reaction when the voltage sweep rate (v) was 200 mV/s. Derivative cyclic voltammograms for the solution containing $CF_3CO_2^-LH^+$ (44 mM) with v equal to 200, 2.00 and 0.200 V/s are illustrated in Fig. 1. At 200 V/s (a), the peak due to the reaction product O'_2 is approximately half as intense as that due to the oxidation of substrate O'_1 and the peak due to the reduction of the cation radical derived from substrate, R'_1, is observed on the reverse scan. At 2.00 V/s (b) reaction between the cation radical and $CF_3CO_2^-$ is nearly complete during the time scale of the measurement and the CV peak potentials for oxidation of substrate and the trifluoroacetoxy derivative were observed to be 1.415 and 1.640 V *vs.* $Ag/Ag^+(CH_3CN)$. At $v=0.200$ V/s (c), O'_2 is slightly more intense than O'_1 indicating complete reaction. In analogy with earlier work on the reaction of $4^{\cdot+}$ with nucleophiles,[11,20] it can be deduced from the voltammograms that the product arises from substitution reaction (5) rather than addition of two $CF_3CO_2^-$. The addition reaction gives rise to a derivative in which the central ring is saturated and is not oxidized in the potential range of the experiment.[11,20]

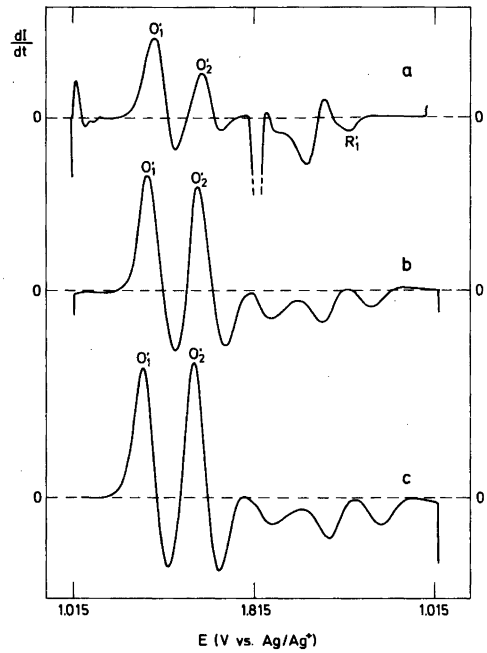


Fig. 1. Derivative cyclic voltammograms for the oxidation of 9-nitroanthracene in acetonitrile–TFA–TFAn (9:1:1) containing $CF_3CO_2^-$ (44 mM) and supporting electrolyte, Bu_4NBF_4 (0.1 M). Voltage scan rates: 200 V/s (a), 2 V/s (b) and 0.2 V/s (c). Switching potential: 1.815 V.

Kinetic studies. Derivative cyclic voltammetry (DCV)[50,51] kinetic studies were carried out on the reactions of the cation radicals derived from *1*, *2* and *4*. The data were treated according to procedures[52] which have been summarized in a recent paper.[53]

The data in Table 1 illustrate the effect of substrate concentration (C_A) and temperature on the apparent

Table 1. DCV kinetic study of the reaction of anthracene cation radical with acetonitrile in AN–TFAn (9:1).

C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A^{0.5}$	T/K
0.25	12.8	810	293.2
0.50	19.3	863	293.2
1.00	28.3	895	293.2
0.50	38.4	–	273.2

Table 2. DCV kinetic study of the reaction of anthracene cation radical with trifluoroacetate ion.[a]

C_A/mM	$C_{CF_3CO_2^-}$/mM	$v_{\frac{1}{2}}$/V s^{-1}	$(v_{\frac{1}{2}}/C_{CF_3CO_2^-}) \times 10^{-3}$
0.313	0	24.6	–
0.313	4.15	44.0	10.6
0.313	8.30	72.2	8.70
0.313	16.6	135.9	8.19
1.25	0	50.0	–
1.25	4.15	93.6	22.6
1.25	6.23	138.3	22.2
1.25	8.30	160.0	19.3
1.25	16.6	217.9	13.1

[a] Measurements at 293.2 K in acetonitrile – TFAn (9:1) containing TFA (5%).

rate constant for the reaction of $1^{\cdot+}$ with acetonitrile as reflected by $v_{\frac{1}{2}}$, the voltage sweep rate at which the derivative peak ratio is equal to 0.500.[52] The reaction order, $R_{A/B}$, which reflects the contributions of both the primary intermediate B ($1^{\cdot+}$ in this case) and the substrate A (*1* in this case) is given by eqn. (6) and the third column in Table 1 shows that

$$R_{A/B} = 1 + z \quad (v_{\frac{1}{2}}/C_A^z = \text{constant}) \qquad (6)$$

a good fit of the data to (6) is obtained for $z = 0.5$ and $R_{A/B} = 1.5$. A comparison of the data in the second and fourth columns provides evidence of a complex mechanism for the reaction with a 20 K decrease in temperature giving rise to a doubling in the apparent rate of the reaction. The mechanistic implications of inverse temperature effects in ion radical reactions have recently been discussed.[54]

The apparent rate constant for the reaction of $1^{\cdot+}$ in AN – TFA – TFAn (9:1:1) was observed to be increased by the presence of $CF_3CO_2^-LH^+$. The last column in Table 2 shows that the reaction order in the salt is very nearly 1 at two different substrate concentrations. At both C_A the rate increased by about a factor of 5 on going from C_{salt} of 0 to 16.6 mM. The temperature dependence of the apparent rate constant (Table 3) was observed to be approximately the same as in the absence of the salt (Table 1) with nearly a two-fold increase accompanying a 20 K temperature decrease.

The rate of decomposition of $2^{\cdot+}$ in AN – TFAn (9:1) was very low in the absence of TFA. The lifetime of the cation radical was greatly decreased in the presence of TFA and the rate of decomposition was observed to be significantly increased in the presence of $CF_3CO_2^-LH^+$. The last column in Table 5 is indicative that at two different substrate concentrations the reaction order in $CF_3CO_2^-$ is 1. This relationship does not hold at low salt concentrations at C_A equal 1.00 mM. Apparent activation energies were obtained from $v_{\frac{1}{2}}$ measured over a 20 K temperature range at C_A equal 0.25 and 1.00 mM (Table 6) using the relationship reported recently[55] which does not require the evaluation of rate constants. Values of 7.6 kcal/mol ($r = -0.997$) and 7.3 kcal/mol ($r = -0.989$) were observed at 1.00 and 0.25 mM, respectively.

Table 3. Effect of temperature on the reaction of anthracene cation radical with trifluoroacetate ion.[a]

T/K	$v_{\frac{1}{2}}$/V s^{-1}
293.2	135.5
282.9	203.5
273.2	261.2

[a] In acetonitrile – TFAn (9:1) containing TFA (5%) and $CF_3CO_2^-$ (16.6 mM).

Table 4. DCV kinetic study of the reaction of 9-nitroanthracene cation radical with trifluoroacetate ion in acetonitrile – TFAn (9:1).[a]

C_A/mM	$v_{\frac{1}{2}}$/V s^{-1}	$v_{\frac{1}{2}}/C_A^{-0.75}$
0.25	180	0.358
0.50	117	0.391
0.75	90.5	0.410
1.00	60.0	0.337
		0.374 ± 0.033

[a] Measurements at 280.4 K in solvent containing TFA (5%) and $CF_3CO_2^-$ (8.3 mM).

Table 5. DCV kinetic study of the reaction of 9-nitroanthracene cation radical with trifluoroacetate ion at different concentrations.[a]

C_A/mM	$C_{CF_3CO_2^-}$/mM	$v_{\frac{1}{2}}$/V s^{-1}	$(v_{\frac{1}{2}}/C_{CF_3CO_2^-}) \times 10^{-3}$
0.25	0	21.0	–
0.25	4.15	101.5	24.5
0.25	6.23	145.4	23.3
0.25	8.30	201.5	24.3
1.00	0	3.7	–
1.00	4.15	9.0	2.17
1.00	8.30	88.8	10.7
1.00	12.45	136.4	11.0
1.00	16.6	179.3	10.8

[a] Measurements at 292.1 K.

Table 6. Effect of temperature on the reaction of 9-nitroanthracene cation radical with trifluoroacetate ion.[a]

C_A/mM	T/K	$C_{CF_3CO_2^-}$/mM	$v_{\frac{1}{2}}$/V s^{-1}
1.00	293.5	16.6	229.7
1.00	281.7	16.6	136.2
1.00	273.2	16.6	80.8
0.25	292.1	8.3	201.5
0.25	282.7	8.3	114.5
0.25	273.2	8.3	78.8

[a] Measurements in acetonitrile – TFAn (9:1) containing TFA (5%).

Table 7. DCV kinetic study of the reaction of 9-phenylanthracene in acetonitrile – TFAn (9:1).[a]

C_A/mM	$v_{3/4}$/V s^{-1}	$v_{3/4}/C_A$	T/K
0.50	0.133	266	287.5
1.00	0.269	269	287.5
1.50	0.433	289	287.5
2.00	0.481	241	287.5
2.00	0.525	–	273.2
2.00	0.542	–	303.4

[a] Measurements in solvent containing TFA (5%) and $CF_3CO_2^-$ (8.3 mM).

Data obtained over an eight-fold range of C_A indicate that $R_{A/B}$ for the reaction of $4^{\cdot+}$ in AN – TFA – TFAn (9:1:1) in the presence of $CF_3CO_2^-LH^+$ (8.3 mM) is very close to 2 (Table 7). Because of the low rate of reaction in this case, $v_{3/4}$, the sweep rate necessary for the derivative peak ratio to equal 0.750 was used instead of $v_{\frac{1}{2}}$. At three temperatures $v_{3/4}/T$ was very nearly constant which indicated an apparent activation energy close to 0.

Linear sweep voltammetry studies. Linear sweep voltammetry studies were carried out on the reactions of $1^{\cdot+}$ and $2^{\cdot+}$. The data in Table 8 can be analyzed using eqns. (7) and (8) where the lower case letters refer to reactions orders in A (*a*), and B (*b*).[56] In AN – TFAn (9:1) the reaction order in $1^{\cdot+}$ was

$$dE^p/d\log v = [1/(b+1)](\ln 10)RT/nF \quad (7)$$

$$dE^p/d\log C_A = [(a+b+i-1)/(b+1)](\ln 10)RT/nF \quad (8)$$

very close to 2 and that in *1* was −1 within experimental error. The theoretical values at 19.3 °C are 19.3 and 0 mV/decade for $dE^p/d\log v$ and $dE^p/d\log C_A$, respectively. The data for the reaction of $1^{\cdot+}$ in the presence of $CF_3CO_2^-$ are most consistent with eqns. (7) and (8) when $b = 1.7$ and $a = 0$. The latter

Table 8. Linear sweep voltammetry kinetic analysis of the reactions of anthracene cation radicals.

Substrate	Conditions	$dE^p/d\log v$	$dE^p/d\log C_A$
Anthracene	*a*	18.4 (0.1)	−0.7 (3.6)
Anthracene	*b*	21.3 (1.2)	−13.5 (0.7)
9-Nitroanthracene	*b*	28.0 (1.7)	+2.3 (2.1)

[a] Acetonitrile – TFAn (9:1) at 292.5 K. [b] Acetonitrile – TFAn (9:1) containing TFA (5%) and $CF_3CO_2^-$ (8.3 mM) at 292.5 K.

result is arrived at by first solving for b in eqn. (7) and then checking eqn. (8) for consistency. The data in the third row of Table 8 indicate that within experimental error, $a=0$ and $b=1$ in the rate law describing the reaction of $2^{\cdot+}$ in AN–TFA–TFAn (9:1:1) containing $CF_3CO_2^-$ (8.3 mM).

DISCUSSION

At the outset of this investigation it was our intention to study the effect of substituents in the 9-position of the anthracene nucleus on the kinetics of the acetamidation of the cation radicals. However, our preliminary results indicated that no two ion radicals in this series undergo precisely the same reactions. We were especially surprised to find what appeared to be an inverse stability–reactivity relationship in that in the absence of added nucleophiles, 9-nitroanthracene cation radical reacts very slowly in AN–TFAn (9:1) while anthracene cation radical is moderately reactive. Our preliminary report on the reaction of anthracene cation radical with acetonitrile[27] naively assumed, as was common to investigations at that stage in the development of ion radical chemistry, that the observed rate constant was a measure of the microscopic rate constant for the first step of reaction (4). More recent work has provided ample evidence that even those reactions which have long been considered to follow simple mechanisms, such as hydrodimerization of anion radicals,[57–60] formation of stable dimer dianions of anion radicals,[61] deprotonation of methylarene cation radicals,[54,62] and the protonation of aromatic hydrocarbon anion radicals[63,64] as well as the pyridination of 9,10-diphenylanthracene cation radical,[43,48] are in fact very complex. With this background, it is perhaps not very surprising to find that a not so subtle change in structure as the nature of the 9-substituent can give rise to an apparent complete change in mechanism of the reaction of cation radicals of substituted anthracenes.

However, we will attempt to show that a manifold of equilibria in which all of the cation radicals can participate is adequate to explain the differences in the products observed. The substituents can be expected to exert their influence on the magnitudes of the various equilibrium constants.

The product studies indicate that the overall reaction pathways can be categorized as dimerization (a), acetamidation (b) and trifluoroacetoxylation (c). The kinetic studies revealed that the rate laws for the reactions of all of the cation radicals are complex and that the evaluation of rate constants is somewhat superfluous in that in no case can a rate constant be assigned to any one microscopic step.

As a starting point, we can consider what appears to be the most simple case encountered, *i.e.* the dimerization of 9-phenylanthracene cation radicals. The kinetic data (Table 7) are consistent with rate law (9). Cation radical dimerization has been observed to have an appreciable activation energy in related cases[65] which suggests that k_{app} in rate law (9) does not apply to the simple irreversible

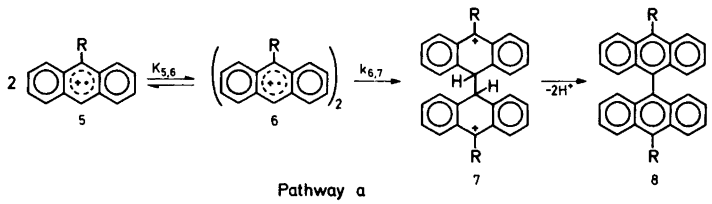
$$\text{Rate}=k_{app}[4^{\cdot+}]^2 \qquad (9)$$

conversion of 5 to 7 (pathway (a)) but rather to the preequilibrium defined by $K_{5,6}$ followed by rate determining $k_{6,7}$ since an apparent activation energy of about 0 was observed for this reaction. Precisely the same situation has been encountered in the dimerization of anion radicals derived from 9-substituted anthracenes[61] and 9-diazofluorene.[66] It is of interest that the dimerization takes place in the presence of the moderately good nucleophile, trifluoroacetate ion.

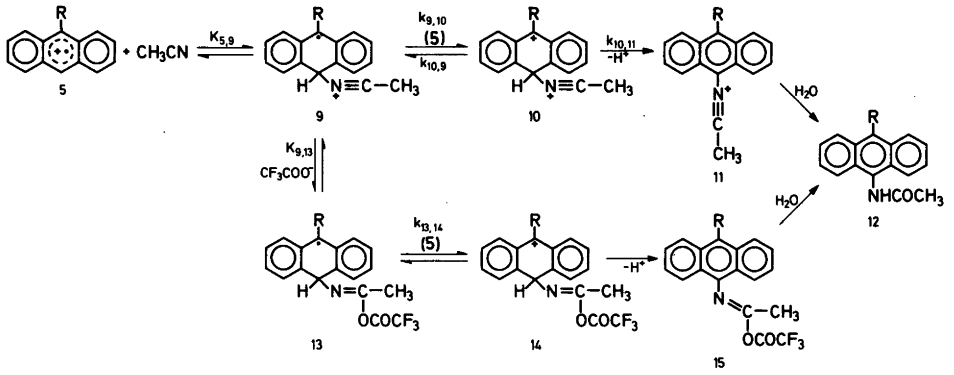
There are a number of noteworthy features of the kinetic data for the acetamidation of anthracene cation radical. In AN–TFAn (9:1), reaction orders in $1^{\cdot+}$ and 1 were observed to be 2 and −1, respectively, (Table 8), which is indicative of rate law (10). In the presence of $CF_3CO_2^-$, there appears to

$$\text{Rate}=k_{app}[1^{\cdot+}]^2/[1] \qquad (10)$$

be a competing first order reaction and the order in 1 goes to 0. These results must be considered in



Pathway a



Pathway b

conjunction with the DCV study (Table 2) which indicates a reaction order of 1 for $CF_3CO_2^-$. The kinetic data can be accounted for by pathway (b). Rate law (10) can be explained by equilibria with constants $K_{5,9}$ and $K_{9,10}$ $(=k_{9,10}/k_{10,9})$ followed by rate determining step $k_{10,11}$ with $k_{app}=K_{5,9}K_{9,10}k_{10,11}$. In the presence of $CF_3CO_2^-$ the reaction is second order in cation radical consistent with rate law (11). This rate law is satisfied by

$$\text{Rate}=k_{app}[1^{\cdot+}]^2[CF_3CO_2^-] \qquad (11)$$

pathway (b) when $k_{app}=K_{5,9}K_{9,13}k_{13,14}$. The catalytic effect of $CF_3CO_2^-$ can be accounted for by the production of neutral *13* which is more easily and rapidly oxidized by *5* than is the cation radical *9*. The inverse temperature effect (Tables 1 and 3) which is the same in the presence or absence of $CF_3CO_2^-$ could mainly be due to the effect of temperature on $K_{5,9}$.

The DCV data in Table 1 were obtained under similar conditions where rate law (10) was observed by LSV (Table 8). This is not an inconsistency. A different regime of cation radical concentrations prevails in the two types of experiments. The data in Table 1 are consistent with rate law (12), differing only from (10) in that back-reaction with $k_{10,9}$ takes place at a rate similar to that with rate constant $k_{10,11}$. The competing reaction which is first order in $1^{\cdot+}$ is most likely the reaction of the cation radical with $CF_3CO_2^-$ (pathway (c)) discussed

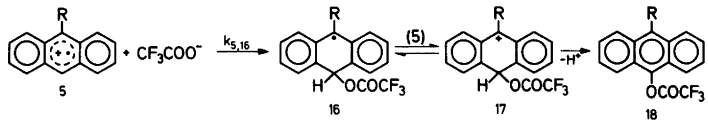
$$\text{Rate}=k_{10,11}K_{5,9}[1^{\cdot+}]^2/(k_{10,9}[1]+k_{10,11}) \qquad (12)$$

below.

The LSV study of the reaction of 9-nitroanthracene cation radical with $CF_3CO_2^-$ provides the strongest evidence for reaction pathway (c) and rate law (13). The reaction order in $CF_3CO_2^-$

$$\text{Rate}=k_{5,16}[2^{\cdot+}][CF_3CO_2^-] \qquad (13)$$

was derived from the DCV data (Table 5). The apparent activation energy of about 7.5 kcal/mol is also consistent with the effect of temperature on $k_{5,16}$. But here we encounter another apparent inconsistency. The data in Table 4 show a remarkable decrease in rate as the substrate concentration is increased. This can be explained by the formation of an inhibitor during the reaction. The only possible inhibitor in pathway (c) is the proton generated in the last step. However, this explanation is not compatible with the observation of rate law (13) in other experiments. An explanation consistent with the entire series of equilibria giving rise to the three pathways is that $2^{\cdot+}$ exists as a dimer complex as *6* or that it associates strongly with substrate. Both of these equilibria are known to be important for related cation radicals.[67] Because of



Pathway c

the presence of electron withdrawing nitro, association with substrate which gives rise to spreading the positive charge over two molecules would be especially favorable. In fact the equilibrium constant for the latter reaction has been observed to be about two orders of magnitude greater than that for cation radical association in some cases.[67] Reaction pathway (c) requires that the dimer dissociate to the reacting intermediate 5. The importance of the side equilibrium is enhanced as the concentration of substrate is increased giving rise to the low value of $R_{A/B}$, 0.25 (Table 4), observed.

The specific influence that a particular substituent has on which of the three reaction pathways will predominate remains to be explained. Pathway (a) predominates or is followed exclusively when the 9-substituent is either phenyl or methoxy. Both of these substituents strongly stabilize the positive charges in the 10,10'-positions of the dimer dication 7.

The discussion in the previous paragraph led to the conclusion that when the 9-substituent is nitro the pre-equilibrium complex 6 may be formed. It is clear that nitro substituents in the 10,10'-positions of 7 will strongly destabilize that structure which prevents pathway (a) from taking place in that case. The reason that the 9-nitro substituent prevents reaction with acetonitrile is also readily explained. The homogeneous electron transfer reaction by which *9* is converted to *10* is expected to be less favorable when R is electron withdrawing NO_2. On the other hand, intermediate *16* is expected to be more easily oxidized than cation radical *9* which allows pathway (c) to channel the 9-nitroanthracene cation radical reaction leading to the trifluoroacetate *18*.

EXPERIMENTAL

The instrumentation, data handling procedures, cells, electrodes and solvent and supporting electrolyte purification were the same as recently described.[43] Anthracene (Fluka, *purum*), 9-nitroanthracene (Fluka, *purum*), 9-phenylanthracene (EGA-Chemie, 98 %) and 9-methylanthracene (EGA-Chemie, 99 %) were used as received. 9-Chloroanthracene[68] and 9-methoxyanthracene[69] were prepared by standard procedures.

Preparative electrolyses. Substrate (1.0 mmol) was dissolved or suspended in acetonitrile–TFAn (40 ml, 9:1) containing Bu_4NBF_4 (0.2 M) and subjected to constant current (100 mA) oxidation for the calculated amount of time (16.1 min for 1 F/mol and 32.2 min for 2 F/mol). The cell was a Metrohm titration vessel, model EA 876, and a sintered glass disk (G 3) served to separate the anolyte and the catholyte (AN/TFAn, 10 ml, 9/1). Electrodes were platinum gauzes. The electrolysis mixture was poured onto crushed ice (~ 150 g) and stirred for 15 min. The resulting solution was extracted with diethyl ether (4 × 40 ml) and the combined organic phases were washed with water (3 × 40 ml) which caused the supporting electrolyte to precipitate. The solid material was removed by filtration and the resulting solution was dried over Na_2CO_3. The solvent was removed at reduced pressure. Yields were determined by GLC (OV 1, 3 %) and the identity of the products was confirmed by comparison with authentic samples.

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Enols of 1,1-Diformylacetone in the Synthesis of 5-Acylpyrimidines

TORE BENNECHE and KJELL UNDHEIM

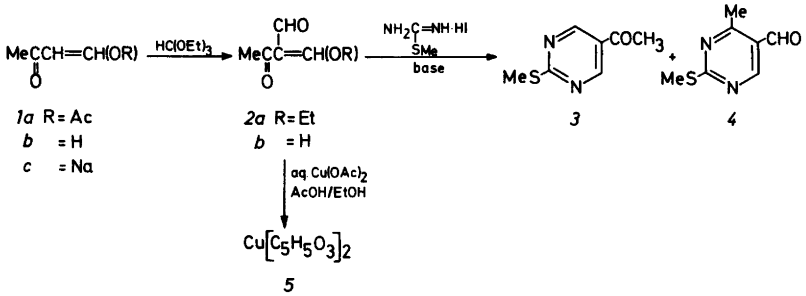
Department of Chemistry, University of Oslo, Oslo 3, Norway

The enol acetate of 3-oxobutanal has been formylated using ethyl orthoformate to provide the enol ether of 1,1-diformylacetone. The latter has been condensed with methylisothiourea to yield isomeric 5-acetyl- and 4-methyl-5-formyl- pyrimidines, the ratio being dependent on the reaction conditions.

Substituted malondialdehydes or their "masked" analogues are useful three-carbon units in the synthesis of 5-substituted pyrimidines.[1] For the preparation of 5-acylpyrimidines without substituents in the 4- and 6-positions, a suitable intermediate acetylmalondialdehyde (1,1-diformylacetone) was required. Thus benzoylmalondialdehyde has been prepared by formylation of 3-oxo-3-phenylpropanal.[2] Other acylmalondialdehydes have been synthesized by the Vilsmeier formylation of ketones and aldehydes.[3,4] In the formylation of acetone by this method, however, the reaction proceeds beyond the 1,1-diformylated acetone *2b* to its triformylated derivative.[5] We describe a synthesis of the desired acetylmalondialdehyde *2b* masked as 4-ethoxy-3-formyl-3-buten-2-one *2a*. This was achieved by formylation of the enol acetate *1a* using triethyl orthoformate and acetic acid catalysis. One half equivalent of acetic acid in the presence of excess ortho ester gave optimal yields. The isomer ratio of (*Z*)- and (*E*)-*2* was close to unity (^{1}H NMR, GLC). The product, an oil, formed a solid adduct with cupric ions (*5*). Formylation as above of the unprotected enol of 3-oxobutanol *1b*, or of its sodium salt *1c*, in the presence of acetic anhydride gave only low yields of the malondialdehyde *2a*.

For the pyrimidine synthesis, 4-ethoxy-3-formyl-3-buten-2-one was condensed with methylisothiourea. A mixture of 5-acetyl-2-methylthiopyrimidine *3* and the isomeric 5-formyl-4-methyl-2-methylthiopyrimidine *4* was obtained, the yields and isomer ratios being dependent on the reaction conditions; in sodium methoxide the yield of isolated product *3* was only 9%, and in DMF using potassium *tert*-butoxide the total yields of *3* and *4* were of the order 50%. The isomer ratio *3*:*4* varied from 45:55 on slow addition of *2a* to a solution of methylisothiourea, to 75:25 for the reverse addition. With acetone as solvent, the ketone *3* was the major isomer (ratio 80:20).

The reaction sequence leading to the cyclic product is rationalized as a Michael type addition of the amino nucleophile with subsequent expulsion of



Scheme 1.

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the RO-substituent and cyclization, the reactivities of the two competitive oxo groups being similar.

By this type of synthesis useful pyrimidine synthons are available due to the ease of substitution of the 2-methylthio substituent and the possibility for transformations of the 5-acyl moiety.

EXPERIMENTAL

(Z)- and (E)-4-ethoxy-3-formyl-3-buten-2-one 2a. *Method A.* Acetic acid (3.0 g, 0.05 mol) was added to a solution of 4-acetoxy-3-buten-2-one[6] (15.0 g, 0.11 mol) in triethyl orthoformate (50 ml) and the mixture heated for 6 h under conditions where the more volatile product distilled from the reaction mixture at 85 °C. When the reaction was over, the excess triethyl formate was removed at reduced pressure and the residue distilled collecting the fraction containing the title compound with b.p. 60 – 68 °C/0.01 mmHg. This fraction was redistilled; yield 6.2 g (40 %), b.p. 62 – 64 °C/0.01 mmHg. GLC gave isomer separation on a 10 % OV-17 column, t = 100 – 250 °C/16 °C per min; ratio 1:1. ^{1}H NMR ($CDCl_3$): δ 1.48 and 4.36 or 4.38 (OEt), 2.32 (COMe), 7.63 (1H,vinyl,s,(*Z*)), 8.00 (1H,vinyl,s,(*E*)), 9.71 (CHO,s), 10.03 (CHO,s). IR (film): 2850 and 2760 (CHO), 1700 – 1660 cm^{-1} (MeCO). MS [70 eV; m/z (% rel.int.)]: 142(0.3,M), 114(6), 100(4), 99(6), 86(12), 43(100).

For elemental analysis the cupric acetate complex was prepared. Thus cupric acetate was added to a solution of a specimen of the above product in ethanol and acetic acid. The complex formed was slowly precipitated in the cold and was recrystallized from methanol, m.p. 211 – 214 °C. Anal. $C_{10}H_{10}O_6Cu$: C,H.

When less than $\frac{1}{2}$ equivalent of acetic acid was used, the yield of *2a* was decreased whereas the yield varied little in the range $\frac{1}{2}$ – 1 equivalent of acetic acid. Invariably, unreacted enol acetate *1a* was recovered (25 – 30 %) from the reaction mixture, but increase in the reaction time also increased polymerization.

Method B: The yields of *2a* were of the order 10 – 15 %, when 3-oxo-butanal[7] or its sodium salt[7] was reacted as above in the presence of one equivalent of acetic anhydride for the *in situ* generation of the enol acetate.

5-Acetyl-2-methylthiopyrimidine 3 *and 4-formyl-4-methyl-2-methylthiopyrimidine* 4. *Method A.* 4-Ethoxy-3-formyl-3-buten-2-one (28.4 g, 0.2 mol) in DMF (100 ml) was added dropwise (105 min) at 5 °C to a stirred mixture from 2-methylisothiouronium iodide (43.4 g, 0.2 mol) and potassium *tert*-butoxide (22.4 g, 0.2 mol) in DMF (200 ml). The mixture was stirred for an additional 1 h at 5 °C, heated gradually to 50 °C over 1 h and stirred at this temperature for 1 h before the solvent was distilled off at reduced pressure. The residue was triturated with water, the solid collected and washed well with water; yield 18.0 g (54 %). GLC analysis on a 10 % IV-17 column at t = 150 – 250 °C/16 °C per min gave composition 47 % of *3* and 53 % of *4*. The isomers were separated by fractional crystallization from methanol, the 5-formyl isomer *4* being the more soluble.

5-Acetyl-2-methylthiopyrimidine: M.p. 133 – 134 °C (MeOH). Anal. $C_7H_8N_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.56 (MeCO), 2.60 (MeS), 8.97 (H-4 and H-6). IR (KBr): 1680 (CO) and 1580 cm^{-1} (Pyr.). MS [70 eV; m/z (% rel.int.)]: 168(100,M), 167(14), 153(36), 125(7), 123(5), 122(19).

5-Formyl-4-methyl-2-methylthiopyrimidine: M.p. 63 – 64 °C (light petrolum). Anal. $C_7H_8N_2OS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.60 (MeS), 2.77 (4-Me), 8.78 (H-6), 10.17 (CHO). IR (KBr): 1690 (CO) and 1580 cm^{-1} (Pyr). MS [70 eV; m/z (% rel.int.)]: 168 (100,M), 167(16), 153(1), 123(5), 122(29), 96(8).

Method B. A mixture from 2-methylisothiouronium iodide (7.0 g, 32 mmol) and potassium *tert*-butoxide (3.6 g, 32 mmol) in DMF (200 ml) was added over 15 min with stirring at room temperature to a solution of 4-ethoxy-3-formyl-3-buten-2-one (4.6 g, 32 mmol) in DMF (100 ml). The mixture was stirred for 1 h at room temperature and for 1 h at 50 °C before the solvent was removed at reduced pressure and the product worked up as above; yield 2.3 g (43 %). The product composition (GLC) was 74 % of *3* and 26 % of *4*.

Method C. A solution of 4-ethoxy-3-formyl-3-buten-2-one (14.2 g, 0.1 mol) in acetone (50 ml) was added over 10 min at 5 °C to a stirred mixture from 2-methylisothioronium iodide (21.7 g, 0.1 mol) in acetone (200 ml) and 1 M potassium *tert*-butoxide in *tert*-butanol (100 ml). The resultant mixture was stirred for 24 h at room temperature before the acetone was distilled off. Part of the 5-acetyl isomer *3* was precipitated and was collected. The filtrate was evaporated at reduced pressure and the remaining *3*-isomer isolated by fractional crystallization of the residue from methanol; yield of *3* 6.7 g (40 %). The methanol solution was subsequently evaporated and the residue subjected to distillation which gave *4* in 12 % (2.0 g) yield; b.p. 88 – 89 °C/0.01 mmHg.

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Electron Transfer Reactions in Organic Chemistry. II.* An Analysis of Alkyl Halide Reduction by Electron Transfer Reagents on the Basis of the Marcus Theory

LENNART EBERSON

Division of Organic Chemistry 3, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund, Sweden

The electron transfer reduction of alkyl halides has been discussed in terms of the Marcus theory as a possible model for an irreversible electron transfer process. An important feature of this treatment is a transition state for electron transfer with a virtually broken C–halogen bond, resulting in a prediction of a large value of the bond and solvent reorganization energy parameter λ of the Marcus equation. For such a mechanism it is logical to use E° values pertaining to the half reaction

$$R-X+e^- \rightarrow R^\cdot + X^-$$

and accordingly E° values were worked out for a series of alkyl halides in a number of solvents of particular interest.

A number of alkyl halide reductions of varying type (by e^-(aq), aromatic radical anions, Co(I), Co(II), Fe(I), Fe(II), Cr(II), and Mo(0)) was found to conform reasonably well to these postulates; for the whole data set a λ value of 46 kcal mol^{-1} could be estimated. Further analysis of different sub-sets revealed a weak trend of increasing λ's in the order RI < RBr < RCl, consistent with a predicted dependence of λ upon the C–halogen bond strength. It was also shown that a typical S_N2 process involving alkyl halides (the Finkelstein reaction) displays a log $k/\Delta G^\circ$ behaviour dramatically different from that of the reactions mentioned above.

In conclusion, the Marcus theory appears to be as well applicable to irreversible as reversible electron transfer in organic systems and hence should constitute a valuable tool in physical organic chemistry.

The Marcus theory was originally adapted for relating kinetic and thermodynamic parameters of *outer-sphere* electron transfer between metal complexes,[1–4] *i.e.* for reactions in which the inner coordination shells of the participating metal ions are intact in the transition state. No *ligand-to-metal bond* is broken or formed in the transition state; on the other hand, an outer-sphere mechanism does not exclude *atom or group transfer, such as hydrogen atom transfer, between ligands* in the transition state.

As one tries to adapt the Marcus theory to organic electron transfer processes, the study of which is now a rapidly developing area,[5] it is found [5a] that the majority of cases conform reasonably well to its conceptual and mathematical framework, as long as the electron transfer step is *reversible* (1). In eqn. (1) R represents an organic species and Ox (Red)

$$R + Ox\,(Red) \rightleftharpoons R^{+(-)} + Red\,(Ox) \qquad (1)$$

an inorganic or organic oxidant (reductant) capable of accepting (donating) one electron from (to) R. The transition state conforms to the Marcus model: An electron is transferred between two species with very little (<1 kcal mol^{-1}) resonance interaction, and with an energy barrier largely originating from the Franck-Condon restrictions, *i.e.* that electron transfer takes place on a time scale in which the positions of all atomic nuclei are frozen. In the terminology of Marcus' theory this means that the height of the barrier is made up from two contributions, namely solvent and bond reorganization energy (λ_o and λ_i, respectively). The total reorganization energy λ, the sum of λ_o and λ_i,

* Part I of this series, see Ref. 5a.

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can either be estimated from extrakinetic quantities or determined experimentally.

Problems arise when the limits of the Marcus model are stretched to include *irreversible* processes, involving simultaneous electron transfer and bond cleavage, of which there are several important classes in organic chemistry.[5a,6] Such reactions are not analogous to the inner-sphere reactions of coordination chemistry, since there is no real analogy to the bridging ligand inherent in this concept. Possible examples of irreversible organic electron processes are the oxidation of carboxylates (3),[7] the reduction of diaroyl peroxides (4)[6,8] and the reduction of alkyl halides (5).[9] In these cases, a general description of the transition state can be given as in eqn. (2), where R is an organic moiety, X

$$R-X+Y \rightleftharpoons [R\cdots X\ Y \longleftrightarrow R^{\cdot}\ X^{+(-)}Y^{-(+)}]^{\ddagger} \rightarrow R^{\cdot}+X^{+(-)}+Y^{-(+)} \quad (2)$$

is a leaving group of some kind and Y represents a one-electron redox species. The following features of the transition state differ from the original Marcus model: In the reactants' transition state the R–X bond is stretched to the point of breaking and the products' transition state is formulated without any bond between R· and $X^{+(-)}$, but with all nuclear positions identical to those of the reactants' transition state. For the three cases mentioned above, the transition states should accordingly be formulated as in eqns. (3)–(5).

$$R-CO_2+Y \rightleftharpoons [(R\cdots CO_2Y)^{-} \longleftrightarrow (R^{\cdot}\ CO_2Y^{-})]^{\ddagger} \rightarrow R^{\cdot}+CO_2+Y^{-} \quad (3)$$

$$ArCO_2-O_2CAr+Y \rightleftharpoons [ArCO_2\cdots O_2CAr\ Y) \longleftrightarrow (ArCO_2^{\cdot}\ {}^{-}O_2CAr\ Y^{+})]^{\ddagger} \rightarrow ArCO_2^{\cdot}+ArCO_2^{-}+Y^{+} \quad (4)$$

$$R-X+Y \rightleftharpoons [R\cdots X\ Y \rightleftharpoons R^{\cdot}\ X^{-}Y^{+}]^{\ddagger} \rightarrow R^{\cdot}+X^{-}+Y^{+} \quad (5)$$

If we postulate this model for the transition state of a simultaneous electron transfer/bond cleavage reaction it immediately follows that the reorganization energy (λ) for the process must be large. We actually invoke cleavage of a single bond in the transition state (a large λ_i), and such a drastic change is also likely to involve a large solvent reorganization energy (λ_o). In addition, the E° of the R–X redox half reaction must be the one pertaining to eqns. (6) and (7) (note that the sign convention for E° values trivially reverses reactions in which RX is oxidized).

$$R-X+e^{-} \rightleftharpoons R^{\cdot}+X^{-} \quad (6)$$

$$R^{\cdot}+X^{+}+e^{-} \rightleftharpoons R-X \quad (7)$$

The *electrochemistry* of carboxylates[10] and alkyl halides[9,11] has been discussed as possible synchronous electron transfer/bond cleavage processes already in the 50's and 60's. Both reactions have all the experimental characteristics of being electrochemically irreversible, *i.e.* the rate-determining step is a slow, heterogeneous electron transfer, and calculated E° values for (6) and (7) were in agreement with this finding. In the case of carboxylate oxidation, the E° of eqn. (8) was far too high to be compatible with irreversibility in the electrochemical sense.[10] However, these ideas were not universally

$$RCOO^{\cdot}+e^{-} \rightleftharpoons RCOO^{-} \quad (8)$$

accepted among practitioners of organic electrochemistry, especially not for carboxylate oxidation. For alkyl halide reduction, the problem of the possible stability of $(R-X)^{-\cdot}$ has been kept open for discussion for a long time,[12] and still presents an experimental as well as theoretical challenge.[13,14] In connection with a review of the possible use of the Marcus theory on organic electron transfer reactions,[5a] it was noted that kinetic data for alkyl halide reduction by different classes of electron transfer reductants could not be completely accommodated within the formalism of the Marcus theory when standard free energy changes were estimated on the basis of Hush's original E° values for alkyl halide reduction.[9] These were calculated for eqn. (6), R = CH_3, X = Cl, Br, I, in aqueous solution. Since we now have good quantitative measures of how ionic solvation changes with solvent, it was deemed of interest to extend calculations of E° for alkyl halide reductions to organic solvents as well, especially those for which data allowing a comparison between experiment and theory are available. In this paper results from such estimates are reported, and pertinent kinetic data for alkyl halide reduction are analyzed on this basis.

RESULTS

In his calculation of standard electrode potentials of methyl halide reductions, Hush[9] split the free

energy change of reaction (9) (R = CH_3), the required quantity for estimating E° in aqueous solution, into two parts, namely reactions (10) and (11). Then ΔG°_9 is simply obtained as the sum of ΔG°_{10} and ΔG°_{11}.

$$RX(aq) + \tfrac{1}{2}H_2(g) \xrightarrow{\Delta G^\circ_9} R^\cdot(aq) + X^-(aq) + H^+(aq) \quad (9)$$

$$RX(g) + \tfrac{1}{2}H_2(g) \xrightarrow{\Delta G^\circ_{10}} R^\cdot(g) + X^-(aq) + H^+(aq) \quad (10)$$

$$RX(aq) + R^\cdot(g) \xrightarrow{\Delta G^\circ_{11}} R^\cdot(aq) + RX(g) \quad (11)$$

The advantage of this treatment is that ΔG°_{10} can be obtained in terms of easily available thermodynamic data, whereas ΔG°_{11} simply is the difference in the standard free energy of solution in water of R·(g) and RX(g), respectively. For the former species, data for RH(g) was used as a reasonable approximation for those of R·(g).

Calculations of E° for $CH_3X/CH_3^\cdot + X^-$ couples in aqueous solution were repeated using modern thermodynamic data[15-18] and are shown in Table 1. The new E° values are slightly more negative than Hush's original ones, which is due to changes in the ΔG°_{10} values. The difference between $\Delta G^\circ_9(CH_3Br)$ and $\Delta G^\circ_9(CH_3I)$ is now -1.7 kcal mol^{-1} (previously -0.8 kcal mol^{-1}), in satisfactory agreement with the experimental value,[19] -1.66 kcal mol^{-1}. Values for ΔG°_{11} were obtained from a recent critical evaluation of the thermodynamics of gas dissolution in water.[20] They are practically identical with those used by Hush.[9]

In Table 2, column 2, this treatment has been extended to ethyl, propyl, isopropyl, butyl, *t*-butyl, allyl and benzyl halides. Almost all data for obtaining ΔG°_{10} are available in standard tables whereas in all cases the ΔG°_{11} values were put equal to those of the methyl halides. For reasons which will be obvious later it is important to establish the *lower* limit of E°, and since ΔG°_{11} is expected to exhibit its largest value for the methyl halides, this assumption affects the E° values in the correct direction.

The aqueous E° values were then converted to E° values for a number of solvents of interest in connection with electron transfer reactions of alkyl halides (Table 2, columns 3–7). This was done using the single ion free energies of transfer from water to other solvents listed by Cox *et al.*[22] For fluoride ion, only $\Delta G^\circ_{tr}(H_2O \rightarrow DMF)$ and $\Delta G^\circ_{tr}(H_2O \rightarrow MeOH)$ are known, and were therefore approximated by those of AcO^- in the other solvents. This approximation has been shown to be a reasonable one in other context.* No ΔG°_{tr} values for THF are available, so these were approximated for the halide ions as those pertaining to DMF and for H^+ that for transfer to MeOH. For acetonitrile, the most positive value of $\Delta G^\circ_{tr}(H^+)$ is 11 kcal mol^{-1} (used for calculation of the values in column 6, Table 2),[24] but it should be noted that lower values of 3.3 and 6.9 kcal mol^{-1} have been estimated by other authors,[25] leading to values of E° that should be higher than those of column 6, Table 2, by 0.3 and 0.18 V, respectively.

In all cases no estimates were made of the free energies of transfer of the neutral species of eqn. (9) from water to the appropriate solvent. Instead, it is assumed that ΔG°_{12} (see eqn. (12)) is 0 which is in

$$RX(solvent) + R^\cdot(aq) \xrightarrow{\Delta G^\circ_{12}} R^\cdot(solvent) + RX(aq) \quad (12)$$

* Ref. 23a; one might also use data [23b] for the "fluoride-like" O_2^- ($\Delta G^\circ_{tr}[H_2O \rightarrow DMF] \approx 13.5$ kcal mol^{-1}, $\Delta G^\circ_{tr}[H_2O \rightarrow DMSO] \approx 12.3$ kcal mol^{-1}) but the result would be almost identical.

Table 1. Calculation of E° values for $CH_3X(aq) + e^- \rightarrow CH_3^\cdot(aq) + X^-(aq)$ according to the method given by Hush. Hush's original values are given within brackets.[9] Data were taken from Refs. 15–18 unless otherwise stated.

X	ΔG°_{10}/kcal mol^{-1} [a]	ΔG°_{11}/kcal mol^{-1}	ΔG°_9/kcal mol^{-1}	$-E^\circ$/V *vs.* NHE
F	18.1[–]	2.2[–]	20.3[–]	0.88[–]
Cl	18.1[15.2]	2.6[2.6]	20.7[17.8]	0.90[0.77]
Br	17.9[14.1]	2.8[2.9]	20.7[17.0]	0.90[0.74]
I	19.3[14.9]	3.1[2.9]	22.4[17.8]	0.97[0.77]

[a] A recent estimate of $\Delta H^\circ_f[CH_3\cdot(g)] = 34.9$ kcal mol^{-1} was used.

Table 2. Estimated E° values for $RX(solv)+e^- \rightarrow R^\cdot(solv)+X^-(solv)$ in different solvents, together with available $E_{1/2}$ values for the cathodic reduction of RX at the dropping Hg electrode. All potentials are given *vs.* NHE.[a]

RX	$-E^\circ$/V in						$-E_{1/2}$/V (solvent)
	H_2O	MeOH	DMF	DMSO	MeCN	THF	
MeF	0.88	1.16	1.33	1.17	1.93	1.59	
MeCl	0.90	1.14	1.23	1.10	1.82	1.49	1.99 (DO – H_2O)
MeBr	0.90	1.12	1.06	0.97	1.71	1.32	1.72 (DMF)
MeI	0.97	1.15	1.07	0.88	1.64	1.28	1.39 (DO – H_2O); 1.15 (50 % EtOH)
EtF	0.75	1.03	1.20	1.04	1.80	1.46	
EtCl	0.82	1.06	1.15	1.02	1.74	1.41	>2.1 (no wave) (DMF)
EtBr	0.74	0.97	0.90	0.81	1.55	1.16	1.89 (DMF)
EtI	0.82	1.00	0.92	0.73	1.49	1.13	1.43 (DO – H_2O)
PrF	0.82	1.10	1.27	1.11	1.87	1.53	
PrCl	0.79	1.03	1.12	0.99	1.71	1.38	
PrBr	0.77	1.00	0.93	0.84	1.58	1.19	1.98 (DMF)
PrI	0.82	1.00	0.92	0.73	1.49	1.13	1.39 (50 % EtOH)
i-PrF	0.74	1.02	1.19	1.03	1.79	1.45	
i-PrCl	0.77	1.01	1.10	0.97	1.69	1.36	
i-PrBr	0.71	0.94	0.87	0.78	1.52	1.13	2.02 (DMF)
i-PrI	0.71	0.89	0.81	0.62	1.38	1.06	
BuCl	0.72	0.96	1.05	0.92	1.64	1.31	
BuBr	0.74	0.92	0.90	0.81	1.55	1.16	1.99 (DMF): 2.03 (DO – H_2O)
t-BuF	0.85	1.13	1.29	1.14	1.90	1.55	
t-BuCl	0.74	0.98	1.07	0.94	1.64	1.33	
t-BuBr	0.66	0.89	0.82	0.73	1.47	1.08	1.95 (DMF); 1.84 (DMSO)
t-BuI	0.67	0.85	0.77	0.56	1.34	1.34	0.98
Allyl-Cl	0.32	0.56	0.65	0.52	1.24	0.87	1.89 (DMSO)
Allyl-Br	0.28	0.51	0.44	0.35	1.09	0.70	0.97 (DMSO)
Allyl-I	0.42	0.60	0.52	0.33	1.09	0.73	
$PhCH_2Cl$[b]	0.32	0.56	0.65	0.52	1.24	0.91	1.70 (DO – H_2O)
$PhCH_2Br$	0.30	0.53	0.46	0.37	1.11	0.72	0.98 (DMF); 0.89 (MeOH)[e]
$PhCH_2I$[c]	0.55	0.73	0.65	0.46	1.22	0.86	
CCl_4[d]	0.25	0.49	0.58	0.45	1.25	0.84	0.54 (DO – H_2O); 0.51 (MeOH –
	(0.05)	(0.29)	(0.28)	(0.25)	(1.0)	(0.64)	0.01 H_2O); (DMF)

[a] Data were taken from Refs. 15 – 18, 22 and 26, unless otherwise stated. See also text. [b] $S^\circ[PhCH_2Cl(g)]$ was estimated to be 3 cal mol^{-1} K^{-1} less than $S^\circ[PhCH_2Br(g)]$. [c] $S^\circ HPhCH_2I(g)]$ was estimated to be 1 cal mol^{-1} K^{-1} greater than $S^\circ[PhCH_2Br(g)]$. [d] Values in parentheses are derived with a value of $\Delta H_f^\circ[CCl_3\cdot(g)] = 14$ kcal mol^{-1}.[27] [e] Ref. 28.

keeping with the goal of establishing the *lower* limits of the E° values. This assumption is equivalent to putting the differences in solvation energy between RX and R· in the non-aqueous solvents (eqn. (13)) equal to those for water (for the X, column 3 of Table 1). Clearly, this treatment establishes an upper limit for ΔG_{13}° since ΔG_{13}° generally must be smaller than ΔG_{11}° due to the weaker interations between neutral organic species and non-aqueous solvent molecules.

The last column of Table 2 gives available half-wave potentials for reduction of alkyl halides, taken from the extensive compilations in Ref. 26.

$$RX(\text{solvent}) + R^{\cdot}(g) \xrightarrow{\Delta G^{\circ}_{13}} RX(g) + R^{\cdot}(\text{solvent}) \quad (13)$$

DISCUSSION

As outlined in the introduction, it is the purpose of this paper to discuss known and/or suspected cases of non-bonded electron transfer between alkyl halides and various reductants in terms of the Marcus theory, assuming that the carbon-to-halogen bond is almost broken in the transition state (eqn. (5)). With this postulate the kinetic form of eqn. (5) is the simple one of eqn. (14), in which k_d and k_{-d}

$$R-X + Y \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} [R^{\cdot}-X\cdots Y] \xrightarrow{k_{el}} \text{products} \quad (14)$$

are the rate constants for diffusing together and apart, respectively, of the reactants, and k_{el} is the rate constant for electron transfer. Using the Marcus expression (eqn. (15)) for $\Delta G^{\neq}$ and converting it to k_{el} *via* the Eyring equation, k_{obs} can be written as in eqn. (16), assuming that electrostatic factors are negligible (as usually done for reactions in which one reactant is neutral). In eqn. (16) A is of the order

$$\Delta G^{\neq} = \frac{\lambda}{4}\left(1 + \frac{\Delta G^{\circ\prime}}{\lambda}\right)^2 \quad (15)$$

$$k_{obs} = \frac{k_d}{1 + A\exp\left[\frac{\lambda}{4}\left(1 + \frac{\Delta G^{\circ\prime}}{\lambda}\right)^2 / RT\right]} \quad (16)$$

of 1 and $\Delta G^{\circ\prime}$ the free energy change for the reaction under the actual conditions of the experiment. For the further treatment A is put equal to 0.2 (a normally used value; calculations are not sensitive to variations of this parameter).[5a] Hence we can express log k_{obs}, the variable of interest, as in eqn. (17), the expression to be used in the analysis of electron transfer reactions of alkyl halides to follow.

$$\log k_{obs} = \log k_d - \log\left(1 + 0.2\exp\left[\frac{\lambda}{4}\left(1 + \frac{\Delta G^{\circ}}{\lambda}\right)^2 / RT\right]\right) \quad (17)$$

Before we embark on this endeavour a comment on the data of Table 2 is necessary. An attempt to discuss alkyl halide reductions[5a] (by metal complexes as well as purely organic reductants) in terms of the Marcus theory led to major inconsistencies, when the original aqueous E° values of Hush (Table 1) were used in eqn. (17). In particular, these rather high values – in addition being contra-intuitively almost independent of the nature of X – imply that alkyl halides are fairly "strong" oxidants, capable of oxidizing many anionic, in particular carbanionic, species in very fast reactions. In spite of this, the by far strongest reductants used, aromatic radical anions, showed far too low experimental rate constants to be compatible with values calculated by the Marcus treatment using Hush's E° values. Even if aqueous E° values come out slightly more negative now (Table 1), it is to be expected that they should not be well suited for estimates of reaction rates in non-aqueous systems. The solvation of halide ions is strongly dependent on the nature of the ion, fluoride ion on the one extreme being strongly destabilized in non-aqueous solvents, especially dipolar aprotic ones, and iodide ion on the other extreme being relatively little affected by a change of solvent.[22] This order of stabilization by solvation is responsible for the invariance of aqueous E° values with the nature of the halide ion; the large differences in bond dissociation energies between C–X bonds are compensated by the solvation energy of X^- operating in the opposite direction.

We are now in a position to explain the need for establishing a *lower* limit for the E° values. Since we want to discuss radical anion reduction of alkyl halides in terms of non-bonded electron transfer, we must use E° values that are biased on the negative side; otherwise one might object that the experimental evidence has been unfairly stacked against using the Marcus theory for what are considered *the most typical* non-bonded electron transfer reactions of the alkyl halides. Thus, as an example, the most positive value of $\Delta G^{\circ}_{tr}(H^+)$ from water to acetonitrile of 11 kcal mol^{-1} was used, in spite of the fact that considerably lower estimates are available.

A comparison between E° values and available $E_{1/2}$ data (last column of Table 2) shows that in all cases but one (CCl_4 in DMF) the cathodic reduction of alkyl halides is electrochemically irreversible, requiring a considerable overpotential to proceed at a measurable rate. The cathodic behaviour of methyl halides in terms of E° values for eqn. (9) (R = Me) has been carefully analyzed by Hush.[9]

Finally, it should be noticed that the crucial assumption that step (3) is dissociative in the case of simple alkyl halides is still under active scrutiny by several groups,[13,14,29–36] and it is by no means

Table 3. Halide (excluding fluorides) reductions by organic and inorganic species, as analyzed by Marcus' theory.

No.	Reductant	Solvent	$E°$/V of reductant	No. of data pairs	λ_5/kcal mol^{-1}	λ_{18}/kcal mol^{-1}	Ref.[a]
1	e^-	H_2O	−2.77	9	53 (40)		38
2	Aromatic radical anions	THF	−(1.08−1.99)	15	54 (4)	90(8)[b]	39; 5a
3	(Anthracene)$^{\cdot-}$	DMSO	−1.07	4			40; 5a
4	Bu_3P−Co(I) cobaloxime	MeOH	−0.51	13	48(4)	66−86	41; 42
5	B_{12s}	MeOH	−0.59	10	46 (3)	62−82	41
6	Co(I)tetraphenylporphin	DMF/PrOH	−0.56	1			43
7	Co(I)salen	DMF	−0.82	2			44
8	Co(I)phthalocyanine	MeOH	−0.39	2			45; 46
9	$Co(CN)_5^{3-}$	MeOH/H_2O	−0.83	7	48 (8)	66−86	47; 48
10	Fe(II)porphins	DMF	−(0.88−0.995)	8	51 (5)	72−92	49
11	Fe(II)deuteroporphine IX	MP/HOAc[c]	0.00[d]	3			50
12	Cr(II)	H_2O/EtOH	−0.41	2			51
13	Cr(II)([15]ane N_4)$^{2+}$	*t*-BuOH/H_2O	−0.58	10	43 (3)	56−76	52
14	$Mo(CO)_2(dmpe)_2$	CH_3CN	0.00	1			53
15	$(C_5Me_5)_2UCl$	THF	−1.06	8	25 (12)		54

[a] First reference to kinetic data, second to source of $E°$ value, unless it is listed in standard tables. [b] λ for self-exchange of aromatic radical anions (ion paired) was set equal to 18 kcal mol^{-1} (see Ref. 5a). [c] *N*-Methylpyrrolidone-acetic acid; data for DMF were used. [d] Set equal to that of Fe(II)tetraphenylporphin.

definitively settled that discrete $(R-X]^{\cdot-}$ do not exist.[12,29] Presently, it seems, however, that most experimental evidence favours dissociative electron transfer for simple alkyl halides[30,31] whereas for certain polyhalogenated methanes the existence of radical anions has been demonstrated.[32,33] Theoretical studies present diverging conclusions,[13,14,34-36] but it is of interest to note for the discussion to follow that $(CH_3F)^{\cdot-}$ is predicted to be capable of finite existence.[14,34] In one *ab initio* study a barrier for solvent assisted bond cleavage of $(CH_3-Cl)^{\cdot-}$ has been inferred,[35] but estimated to be low ($\leq$16 kcal mol^{-1}), whereas another one favours dissociative electron transfer with direct formation of a loose complex of C_{3v} symmetry between $CH_3\cdot$ and Cl.[14] Introduction of a nitro substituent in CH_3Cl removes the barrier for dissociative electron transfer.[13]

Table 3 lists a number of alkyl halide reductions by inorganic and organic species for which kinetic data are available in one or several of the cases listed in Table 2. In most cases the possibility of an electron transfer reduction mechanism has been discussed, although not necessarily favoured as the ultimate mechanistic choice. The reactions cover a wide variety of reducing reagents and reaction conditions, ranging from hydrated electrons to transition metal complexes in acetonitrile. For those reactions where a reasonable number of data pairs is available (1, 2, 4, 5, 9, 10 and 13) a value of λ for the individual reaction was calculated by non-linear regression analysis,* using expression (17) with an adequate value of k_d. These values are given as λ_5 in column 6 of Table 3.

The similarity of the λ values may at first sight seem surprising in view of the widely differing reagents and reaction conditions. However, using the corollary of the Marcus theory that λ for a heteronuclear reaction (*e.g.* eqn. (5)) can be expressed as the mean value of the λ's of the two individual self-exchange reactions (those pertaining to eqn. (5) are formally shown in eqns. (18) and (19)),

$$RX + R^{\cdot}X^{-} \overset{\lambda_{18}}{\rightleftharpoons} R^{\cdot}X^{-} + RX \qquad (18)$$

$$Y + Y^{-\cdot} \overset{\lambda_{19}}{\rightleftharpoons} Y^{-\cdot} + Y \qquad (19)$$

the transition state model postulated in eqn. (5) involves a large λ_{18} value, as discussed in the introduction, and hence will be predominant in determining λ_5. The reagents employed have relatively small λ_{19} values, *e.g.* 15−20 kcal mol^{-1} for

* By Marquardt's method (Hewlett-Packard System 35 Nonlinear Regression Program).

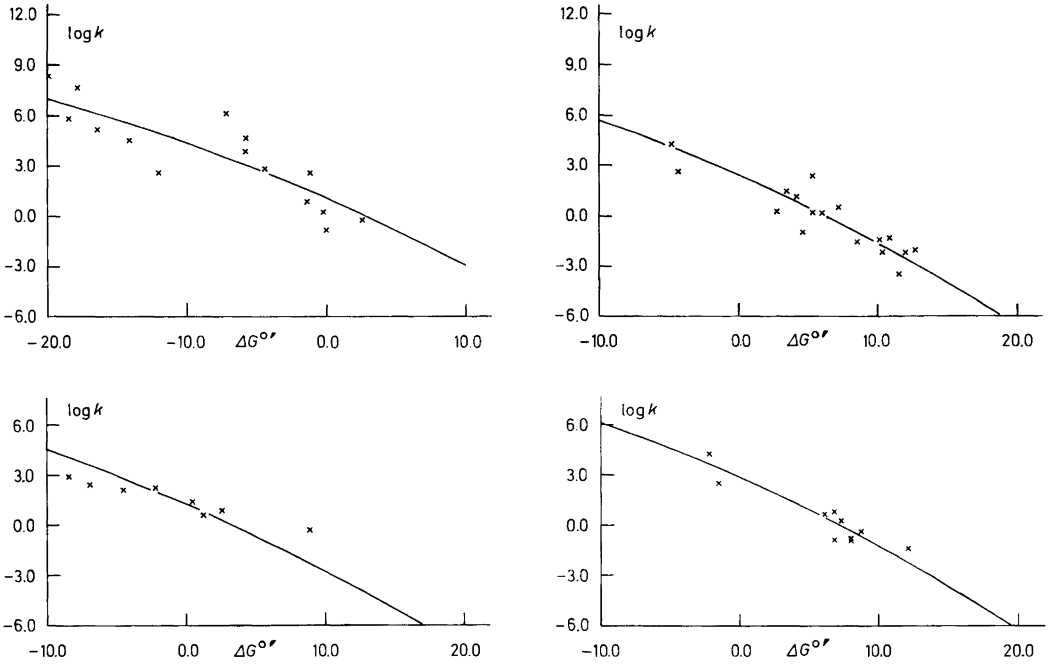

Fig. 1. Marcus plots for the reaction between alkyl halides and a, radical anions; b, Bu_3P-cobaloxime and B_{12s}; c, Fe(I)porphins; and d, Cr(II)([15]ane $N_4)^{2+}$.

ion-paired radical anions,[5a] or can be assumed to have relatively small λ_{19} values.[4,37] Experimental values are unfortunately not available for Co(I)/Co(II) and Fe(I)/Fe(II) couples, but current generalizations regarding structural effects upon λ dictate that the presence of large polarizable ligands should result in low λ values.[37] This means that λ_5 will largely be determined by λ_{18}. With the assumption that λ_{19} lies in the region of 10–30 kcal mol^{-1} we can give limits of λ_{18} for the self-exchange reactions (last column of Table 3) of alkyl halides (eqn. (18)).

Apart from calculating λ values for the individual reactions of Table 3 (a few examples of Marcus plots of these reactions are shown in Fig. 1) the data, except for those of reaction (15) (see below), were analyzed in various aggregated forms to look for possible trends (Table 4). It is first to be noted that one can indeed construct a master plot of all data pairs (Fig. 2) and obtain a reasonable λ_5 of 46(3) kcal mol^{-1}. A closer look at the points that deviate

Table 4. Marcus analysis of aggregated forms of the data referred to in Table 3, except for those of reaction 15.

Compound type	No. of data pairs	λ_5/kcal mol^{-1}
All compounds	87	46 (3)
All compounds, except methyl halides	76	48 (2)
Methyl halides	11	29 (9)
i-PrX + *t*-BuX + $PhCH_2X$ + allyl-X	27	47 (4)
All chlorides	31	50 (4)
All bromides	34	47 (3)
All iodides	22	38 (5)
Established non-bonded electron transfer reactions (1–3, 9 and 14 of Table 3)	36	52 (3)

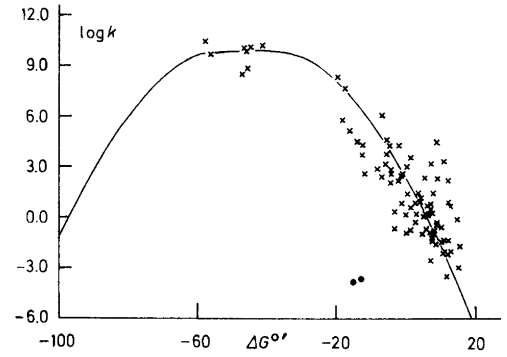

Fig. 2. Marcus plot of all reactions of Table 3, except reaction 15. The two points marked by filled circles refer to alkyl fluoride reactions.

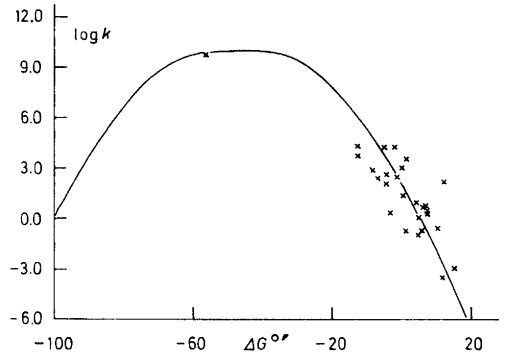

Fig. 4. Marcus plot of all reactions of Table 3, involving isopropyl, tert-butyl, benzyl and allyl halides (except reaction 15).

most strongly from the curve revealed a high percentage of methyl halide reactions with Co(I) complexes (the so-called supernucleophiles).[41,45] Hence *all* reactions of MeX were left out and a second master plot (Fig. 3) was drawn ($\lambda_5 = 48[2]$ kcal mol^{-1}). This is slightly better than the first one, and a plot of the methyl halide data alone considerably worse ($\lambda_5 = 29[3]$ kcal mol^{-1}). Reasons for this behaviour might be that (a) methyl halides have lower $E°$ values and (b) are sterically less hindered than all the other halides of Table 2, both factors combining to make a nucleophilic reaction with the metal center favoured over electron transfer in the case of MeX. To test this possibility, data for i-PrX and *t*-BuX (sterically hindered) and $PhCH_2X$ and allyl-X (low $E°$ values; see Table 2) were combined to give the plot of Fig. 4 (λ_5 is now 47[4] kcal mol^{-1}). It is however hardly possible to claim that this sub-set is more well-behaved than the total set; a more definitive analysis of the problem in these terms must rest on a more homogeneous experimental material. It does differ significantly from the sub-set of MeX reactions, though.

A test for differences according to halogen type (see Table 4) revealed a weak trend in the expected direction, namely that λ_5 should decrease with decreasing bond energy of the R–X bond; again the same reservation as above is valid. A last sub-set collected "established" non-bonded electron transfer reactions, *i.e.* such reactions for which an electron transfer mechanism has been deemed most likely, showing practically no difference in behaviour from that of the whole set (Fig. 5).

It is of interest to see how an established S_N2 process is related to the master plot, just to make

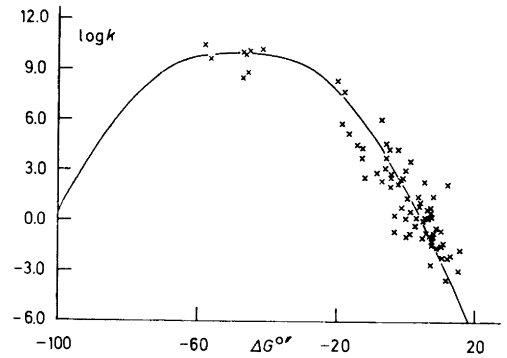

Fig. 3. Marcus plot of all reactions of Table 3, except reaction 15 and excluding all methyl halide reactions.

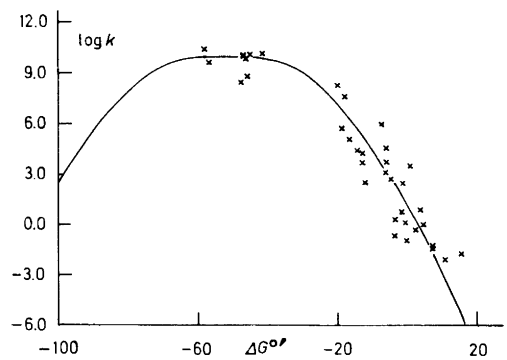

Fig. 5. Marcus plot of "established" electron transfer processes of alkyl halides (reactions 1–3, 9 and 14 of Table 3).

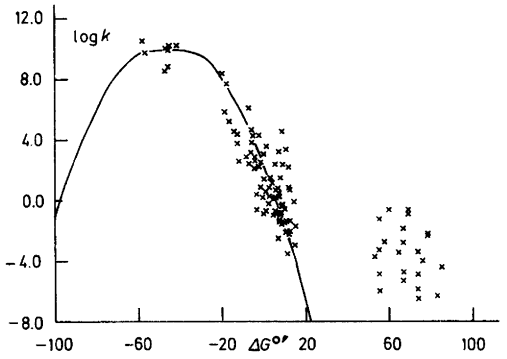

Fig. 6. Master Marcus plot of Fig. 2 with a set of 23 Finkelstein reactions included (domain to the right).

sure that we really are dealing with qualitatively different phenomena. A suitable S_N2 reference process is the Finkelstein reaction ($RX+X^- \rightarrow RX+X^-$) for which a rate constant of 0.6 M^{-1} s^{-1} has been determined in acetone[55] for the exchange of I^- with EtI. From E° of $I^\cdot/I^-$ ($\approx$1.3 V) and of Et–I (estimated at -1.2 V) we obtain $\Delta G^\circ \sim 2.5$ V or 58 kcal mol^{-1}, a situation that is drastically illustrated in Fig. 6 where a set of 23 Finkelstein reactions[55] has been represented in the same plot as Fig. 1, but with a different scale on the x axis.

Another interesting feature is the failure of alkyl fluoride reductions (with aromatic radical anions as reductants) to conform with the Marcus plot, assuming the same reduction mechanism as for chlorides, bromide and halides (see Fig. 2). An obvious explanation is that $(RF)^{\cdot -}$ exists as a relatively stable species and that accordingly the E° values should refer to $RF+e/(RF)^{\cdot -}$ and *not* to $RF+e/R^\cdot+F^-$. Clearly the former half reaction would have a more negative E° value, although it is not possible to make any quantitative estimate. There is thus a reasonable chance that radical anions of simple alkyl fluorides should be stable enough for experimental detection and study.

Reaction (15), reduction of alkyl halides by a U(III) compound, was recently discussed in some detail.[54] An atom transfer mechanism (eqn. (20)) was preferred, partly since it was thought that the reaction would be far too endergonic for non-

$$(C_5Me_5)_2UCl+X-R \rightarrow (C_5Me_5)_2U(X)Cl+R^\cdot \quad (20)$$

bonded electron transfer to occur. This estimate was, however, based on cathodic peak potentials of alkyl halides which generally are much more negative than the corresponding E° values (see Table 2). With E° values from Table 2 reaction (15) actually emerges as a possible borderline case of electron transfer; the kinetic data are themselves badly correlated (see Table 3) with the Marcus equation (17) and inclusion of these 8 data pairs in the total data set of Fig. 2 gives a slightly worse fit to the Marcus parabola (λ_5 is then 46[5] kcal mol^{-1}). Thus it is not possible to dismiss an electron transfer mechanism from this point of view.

Concluding, it has been shown that kinetic data for alkyl halide reduction by electron transfer reagents can be consistently interpreted on the basis of the Marcus theory, provided a transition state structure with an almost completely broken C–halogen bond is postulated. In view of the inhomogeneity and imperfectness of the data base, a fully quantitative treatment has not been possible, but the correlations already established show that the Marcus theory may provide an efficient device for sorting out possible electron transfer mechanisms also in irreversible cases. Similar conclusions, although phrased somewhat differently, were reached in a recent study.[6]

One important difference is to be noted, however, in that Scandola *et al.*[6] excluded the possibility of fixing the zero of the free energy axis due to the difficulty in estimating E° for processes involving almost complete bond breaking in the transition state. As shown above, this seems to be possible, and may be extendable to other systems, *e.g.* alkanecarboxylates, tetraalkyl metals[56] and diacyl peroxides.[57]

Finally, it should be noted that carbon tetrachloride (see Table 2), as judged by its E° value, should be a reasonably strong electron transfer oxidant, as has indeed been very nicely demonstrated by Meyers and coworkers.[58] In general, polyhalogenated organic compounds should resemble carbon tetrachloride in this respect, and this is also qualitatively indicated by the fact that these compounds have half-wave potentials for reduction[11,26] in the region of 0 to -1 V. Assuming that these values have a rather large component of overpotential, polyhalogenated organic compounds would emerge as fairly strong electron transfer oxidants. It is not unreasonable to ascribe the often high but nonselective biological activity of these compounds to their oxidizing properties; in fact, one could build a good case for the view that a polyhalogen compound could act as an excellent

suicide inactivator[59,60] toward a wide range of redox enzymes. Following electron transfer, a very reactive neutral radical would be formed close to the active center, thus opening a pathway for indiscriminate attack on atoms in its vicinity.[61]

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Short Communications

An Alternative Method for Preparing 7- and 9-Methylpurines

JORMA ARPALAHTI and HARRI LÖNNBERG

Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku 50, Finland

N-Alkyl derivatives of purine bases are frequently used as model compounds in the studies of the interactions between metal ions and nucleic acid constituents. However, attempts to prepare a desired *N*-alkylated compound have often met with difficulties. This has particularly been the case with the direct methylation of the unsubstituted purine. Albert and Brown,[1] for example, have reported that conventional alkylating procedures, including treatment with methyl iodide, dimethyl sulfate, methyl *p*-toluenesulfonate or formic acid, are unsuccesful in methylation of purine. Later reaction of thallium(I) salt of purine with methyl iodide in DMF has been shown to yield 9-methylpurine and 7,9-dimethylpurinium iodide.[2] Quite recently *N*,*N*-dimethylformamide dimethyl acetal has been suggested to be an advantageous methylating agent of heterocyclic bases.[3] Treatment of purine with this reagent in refluxing toluene, for example, gives a mixture of 7- and 9-methylpurines in proportion of 3 to 2. We now report that a 1:3 mixture of these compounds can conveniently be prepared in almost 100% yield by allowing purine to react with dimethyl sulfate in acetone in the presence of anhydrous potassium carbonate. The isomers formed can be separated in a preparative scale by passing the methanolic solution of the product mixture through a strong cation exchange resin loaded with magnesium(II) ions. As seen from Fig. 1, the 7-isomer exhibits a considerably larger retention volume (110 cm^3) than 9-methylpurine (70 cm^3), probably due to more efficient complexing with magnesium(II) ion. By this procedure, amounts of 1 g can satisfactorily be fractionated on a column of 2 × 40 cm.

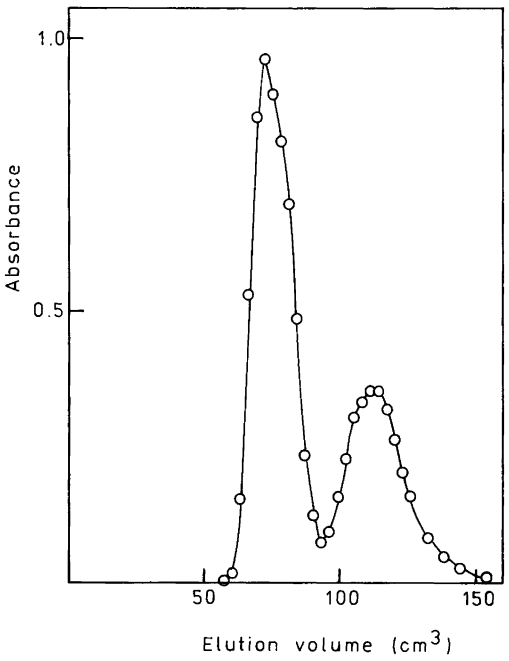

Fig. 1. Elution curve for the separation of 7- and 9-methylpurines on a strong cation exchange resin loaded with magnesium(II).

Experimental. Preparation of the mixture of 7- and 9-methylpurines. A suspension of purine (5 mmol, Sigma Chemical Company) and dimethyl sulfate (5 mmol) in dry acetone (150 cm^3) was agitated at room temperature on anhydrous potassium carbonate (7 mmol) for two days. Evaporation of the filtrated solution to dryness afforded a 1:3 mixture of 7- and 9-methylpurines, as deduced on the basis of the ^1H NMR spectra of the residue. Other products were not detected.

Separation of 7- and 9-methylpurine. The isomeric mixture obtained was applicated in 3 cm^3 of methanol on strong cation exchange column (Dowex 50 W X2, mesh 100 – 200, 2 × 40 cm) loaded with magnesium(II) ions and eluted with dry methanol (15 cm^3 h^{-1}). Fractions of 3 cm^3 were collected and the appearance of the methylpurines was checked by UV-spectroscopy (dilution 1:350). The elution curve obtained is presented in Fig. 1. The pooled fractions were evaporated to dryness

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and the products were crystallized from ethanol and recrystallized from hot carbon tetrachloride.

7-Methylpurine obtained melted at 178 – 180 °C (lit.[3] 181 – 183 °C) and exhibited the following analytical and spectroscopic data. Found: C 53.81; H 4.49; N 41.74. Calc. for $C_6H_6N_4$: C 53.72; H 4.51; N 41.77. UV (log ε): in 0.1 mol dm^{-3} HCl 257.1 (3.85) nm, in water 265.8 (3.93) nm (lit.[4] in pH 0.23 257.5 (3.83) nm, in pH 9.15 266.5 (3.91) nm). ^{1}H NMR (as ppm from DSS in D_2O): δ 3.91 (CH_3,s), 8.76 (H2,s), 8.87 (H6,s), 8.31 (H8,s) (lit.[3] 4.10, 8.95, 9.15, and 8.20 from TMS in $CDCl_3$). ^{13}C NMR (as ppm from DSS in D_2O): δ 34.1 (CH_3), 154.1 (C-2), 161.1 (C-4), 128.3 (C-5), 143.4 (C-6), 153.2 (C-8) (lit.[5] 32.2, 152.1, 159.3, 126.1, 140.9, and 150.7 in water – dioxan mixture.

9-Methylpurine melted at 160 – 161 °C (lit.[3] 160 – 162 °C) and exhibited the following analytical and spectroscopic data. Found: C 53.71; H 4.52; N 41.69. Calc. for $C_6H_6N_4$: C 53.72; H 4.51; N 41.77. UV (log ε): in 0.1 mol dm^{-3} HCl 262.0 (3.76) nm, in water 263.8 (3.90) nm (lit.[4] in pH 0.62 262.5 (3.77) nm, in pH 8.5 264.0 (3.90) nm. ^{1}H NMR (as ppm from DSS in D_2O): δ 3.83 (CH_3,s), 8.74 (H2,s), 8.89 (H6,s), 8.31 (H8,s) (lit.[3] 4.00, 9.00, 9.15, and 8.10 from TMS in $CDCl_3$). ^{13}C NMR (as ppm from DSS in D_2O): δ 32.5 (CH_3), 153.9 (C-2), 153.3 (C-4), 135.2 (C-5), 149.6 (C-6), 150.9 (C-8) (lit.[5] 30.6, 151.9, 150.9, 132.9, 147.4, and 148.8). When external standard was employed each of the ^{13}C shifts diminished by 2 ppm.

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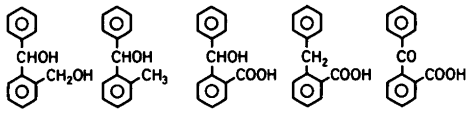
Development of Hydrogen Gas during an Organic Reaction

ENDRE BERNER

Department of Organic Chemistry, University of Oslo, P.O. Box 1033, Blindern, Oslo 3, Norway

Reducing 2-benzoylbenzoic acid by means of $LiAlH_4$ led to 1-(2-hydroxymethylphenyl)-1-phenylmethanol, *1*. In attempts to transfer *1* to other products it was, in one instance, mixed with pulverized potassium hydroxide and heated in a thick-walled glass tube. At about 200 °C melting took place, followed by a lively gas evolution and separation of a slightly discoloured solid substance on top of the fluid alkali. It was first thought that water had been split from the diol, giving 1-phenyl-2-oxaindan as a result, but the latter substance prepared separately did not react with alkali under the same conditions.



1 2 3a 3b 3c

On investigation, the melted mass was found to consist of a neutral substance containing a methyl group, namely 1-(2-methylphenyl)-1-phenylmethanol (*2*), and the potassium salt of a carboxylic acid (*3a*, *3b* or *3c*) referred to below. An explanation of the chemical process taking place during the melting was arrived at when it was suggested, and in fact proved, that the evolved gas was hydrogen. The process actually consists of a reaction between two molecules containing a primary alcohol group, so that one becomes a methyl group and the other a carboxyl group, at the same time leaving one molecule of hydrogen free, as according to reaction (1).

$$-CH_2OH + -CH_2OH \rightarrow CH_3- + -COOH + H_2 \quad (1)$$

As will be seen in *2*, the secondary alcohol group from *1* has remained unchanged. This may be due to the fact that *2* is a neutral substance remaining undissolved in the melted alkali. On the other hand, when coming to the carboxylic acid, three formulae *3a*, *3b* and *3c* have been considered. The reason for this is that separate experiments have shown that diphenylmethanol, on heating in the presence of potassium hydroxide, partly turns into a mixture containing diphenylmethane and benzophenone also. In the present case it is, therefore, probable that the crude product is a mixture from which *3b* and *3c* have been isolated.

Subsequently, it was found that other organic compounds containing a primary alcohol group, on heating with potassium hydroxide, undergo the same reaction with evolution of hydrogen gas. In the case of low-boiling substances, such as benzyl alcohol, the reaction was rather restricted as the required temperature was not reached, but it was obvious that an evolution of gas took place and benzoic acid could be isolated from the molten mass. In the same way, when 3-methylbenzyl alcohol was used, *m*-toluic acid could be isolated.

Using 2-naphthylmethanol the reaction went much easier and the reaction products 2-methylnaphthalene and 2-naphthoic acid were identified.

Finally, it was found that even a higher aliphatic alcohol, such as 1-decanol, to a certain although small degree underwent the same reaction, giving decanoic acid which in the mass spectrometer showed the correct value of *m/z* 172. On standing, the acid crystallized and had a melting point of 32 °C, being the value given for decanoic acid. The corresponding hydrocarbon decane, which should be expected, was obviously too volatile to be isolated in this case.

Experimental. The diol *1* (2 g) was mixed with powdered KOH (8 g) and heated in a thick-walled glass tube, with an arrangement made for collecting the *ca.* 50 ml gas which evolved. By gas chromatography this gas was proved to be hydrogen. After cooling, the melted mass was dissolved in water and a neutral substance extracted with diethyl ether. The substance (0.6 g) was found to be identical with the 1-(2-methylphenyl)-1-phenylmethanol (*2*) prepared synthetically from *o*-methylbenzyl chloride which, after a Friedel-Crafts reaction with benzene followed by a reduction, gave *2* with m.p. 91 °C. Found: C 85.27, H 7.00. Calc. for $C_{14}H_{14}O$: C 84.80, H 7.12. ^1H NMR ($CDCl_3$): δ 7.2 (m, 10 H), 5.47 (1 H,s), 2.25 (3 H,s).

By acidifying the aqueous solution, an organic acid mixture separated, from which in some cases *3b*, (2-benzylbenzoic acid, m.p. 118 °C) in others *3c*, (2-benzoylbenzoic acid, m.p. 126 °C) could be isolated. The authenticity of the two acids was checked by mass spectrometry.

The methyl ester of 2-naphthoic acid was reduced with $LiAlH_4$ to 2-naphthylmethanol, m.p. 80 °C. The latter (1.5 g) mixed with 5 g powdered KOH was heated in a glass tube and at about 210 °C gas began to evolve. When about 50 cm^3 gas had been collected, heating was discontinued and the content of hydrogen in the gas confirmed. The hydrocarbon formed, being rather volatile, was adsorbed in

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diethyl ether and found to be a liquid which, on the mass spectrometer, showed *m/z* 129. The value calculated for methylnaphthalene is 130. Acidifying the alkaline solution, 2-naphthoic acid (0.82 g; m.p. 187 °C) was isolated.

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Characterization of Cytosolic Epoxide Hydrolase in Mouse Liver *

JOHAN MEIJER,[a] SUSANNE MÅNÉR,[a] WINNI BIRBERG,[b] ÅKE PILOTTI[b] and JOSEPH W. DePIERRE[a]

Departments of [a] Biochemistry and [b] Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

In recent years it has become clear that many of the toxic, mutagenic and carcinogenic effects of xenobiotics are not due to the parent substances themselves. In many cases reactive intermediates, often formed *via* the cytochrome P-450 system, are directly responsible for these deleterious effects. One common type of reactive intermediate formed, for example, during the metabolism of polycyclic hydrocarbons by the cytochrome P-450 system, is epoxides. Such epoxides can generally be metabolized further to relatively harmless products by the phase II drug-metabolizing enzymes epoxide hydrolase(s) (E.C.3.3.2.3) and glutathione *S*-transferase(s) (E.C.2.5.1.18).

Originally, it was believed that epoxide hydrolase(s) was localized solely on the endoplasmic reticulum in hepatocytes. However, Hammock and his coworkers [1,2] demonstrated that there is also a cytoplasmic form of this enzyme. The microsomal epoxide hydrolase has received much attention and has been thoroughly characterized and purified to homogeneity.[3,4]

Much less is presently known about the cytoplasmic enzyme and its properties. Until more is known, we will be unable to assess the role of this epoxide hydrolase in the metabolism of different xenobiotics and in protection against the toxic, mutagenic and carcinogenic effects of reactive intermediate epoxides. The goal of the present investigation was to characterize the cytoplasmic epoxide hydrolase in a number of different ways and, in particular, to determine properties of this enzyme which can subsequently be used to design a purification procedure.

These experiments were performed using male NMRI, C57 black, and CBA mice (Anticimex, Stockholm, Sweden) weighing 20–30 g and given free access to food pellets and water until sacrifice by cervical dislocation. The gall bladder was removed and the liver homogenized in 4 volumes of 0.25 M sucrose using 4 up-and-down strokes of a Potter-Elvehjem homogenizer at 440 RPM. The resulting suspension was centrifuged at 10 000 *g* for 10 min and the supernatant from this step then centrifuged at 100 000 *g* for 1 h. The clear high-speed supernatant under the lipid layer, designated cytosol, was carefully sucked off and used in the experiments. The high-speed pellet, the so-called microsomes, was resuspended in 0.25 M sucrose and used for comparison with the cytosolic fraction in one experiment. Time studies performed with NMRI cytosol demonstrated that the epoxide hydrolase activity in this preparation is stable for at least several weeks at −20 °C, for at least 4–6 days at 4 °C and for at least 2 days at 20 °C.

Epoxide hydrolase activity was measured using either styrene oxide,[5] *trans*-[6] or *cis*-stilbene oxide [6] as the substrate. Tritiated *trans*- and *cis*-stilbene oxides of very high specific radioactivity were synthesized according to Dr. B. D. Hammock, University of California at Davis (personal communication). Glutathione *S*-transferase activity was measured with 1-chloro-2,4-dinitrobenzene as the second substrate.[7]

The molecular weight of the cytosolic epoxide hydrolase was determined by ultrafiltration on a column of Sephacryl S-300 (Pharmacia, Uppsala, Sweden) (2.5 × 90 cm) at 4 °C. 3 ml of the cytosol fraction to which standard proteins had been added was applied to this column and eluted with 0.1 M potassium phosphate, pH 7.0, at a flow rate of 14 ml/h.

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Table 1. Epoxide hydrolase activity in microsomes and cytosol from male NMRI mouse liver.

Fraction [a]	Epoxide hydrolase activity [b] (nmol/min mg protein) with different substrates		
	styrene oxide [c]	*trans*-stilbene oxide [d]	*cis*-stilbene oxide [e]
Microsomes	5.25 ± 1.66	0.41 ± 0.24	1.96 ± 0.42
Cytosol	4.79 ± 0.06	1.39 ± 0.48	0.01 ± 0.002

[a] Microsomes and cytosol were prepared as described in the text. [b] The values are means ± S.E.M. for three individual animals. [c] Assayed in 0.1 M potassium phosphate, pH 6.8, for the cytosol and in 0.1 M glycine, pH 9.0, for the microsomes. [d] Assayed at pH 6.8 in the same buffer described in *c*. [e] Assayed at pH 9.0 in the same buffer described in *c*.

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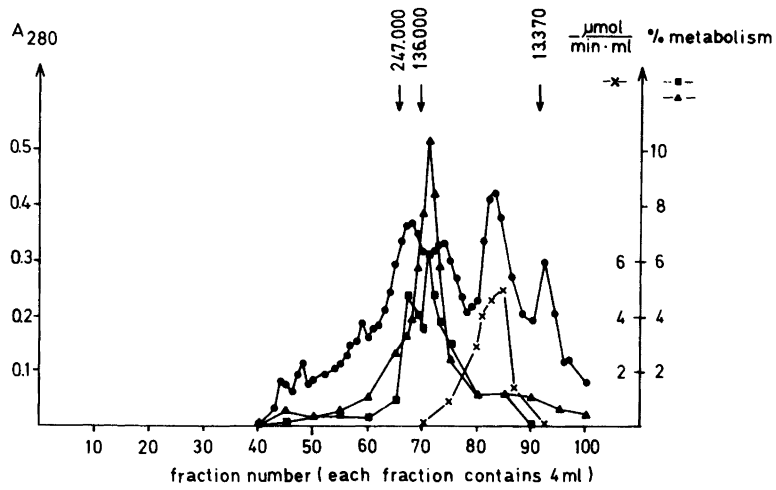

Fig. 1. Gel filtration of the cytosol fraction from mouse liver on Sephacryl S-300.

This experiment was performed as described in the text. The cytosol fraction from C57 black mice was used here, but similar results were obtained with NMRI and CBA mice as well. The symbols used are as follows: ■ = epoxide hydrolase activity measured using *trans*-stilbene oxide as substrate; ▲ = epoxide hydrolase activity with styrene oxide as substrate; × = glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene as substrate; ● = absorption at 280 nm. The elution positions and molecular weights of three standard proteins are indicated in the figure.

The isoelectric point was determined by applying 25 µl of each cytosol fraction to commercially prepared slab gels (purchased from LKB, Bromma, Sweden) and running at 15 mA for 90 min. The enzyme was extracted from the gel with the appropriate buffer for enzyme assay at 4 °C for several hours before being assayed.

Literature reports maintain that *trans*-stilbene oxide is a much better substrate for the soluble epoxide hydrolase than is styrene oxide,[8] but this was not found to be the case in our hands. In the present experiments the cytosol fraction from NMRI mice hydrolyzed styrene oxide at a rate of 4.79 nmol/min mg protein, while the corresponding activity with *trans*-stilbene oxide was only 1.39 nmol/min mg protein (Table 1). It can also be seen from the table that the specific activity of styrene oxide hydrolase for microsomes and cytosol is nearly equal, whereas the cytosol hydrolyzes *trans*-stilbene oxide 3 times faster than the microsomes and the microsomes catalyze the hydrolysis of *cis*-stilbene oxide nearly 200 times more efficiently than the cytosolic fraction. Thus, *trans*- and *cis*-stilbene oxide would seem to be better substrates for distinguishing between the microsomal and cytosolic epoxide hydrolases than is the more commonly used styrene oxide.

As can be seen from Fig. 1, the cytosolic epoxide hydrolase – measured either with *trans*-stilbene oxide or styrene oxide as substrate – demonstrates a molecular weight of approximately 130 000 upon gel filtration. This value agrees well with preliminary results published earlier.[9] The presence of virtually all the epoxide hydrolase activity in a single peak suggests that there is only one form of this enzyme present in mouse liver cytoplasm. The activity peak contains no more than 20% of the total protein applied to the column, so this procedure may prove to be a valuable step in the future purification of the enzyme. It can also be seen from Fig. 1 that cytosolic glutathione *S*-transferase activity can be clearly separated from cytosolic epoxide hydrolase on the basis of molecular weight. The apparent molecular weight of the glutathione *S*-transferase(s) is about 50 000, which agrees with previous reports.[10]

As shown in Fig. 2, the isoelectric point of the cytosolic epoxide hydrolase in mouse liver – using either *trans*-stilbene oxide or styrene oxide as substrate – is approximately 5.6. Also in this case a single peak of activity is seen, indicating the presence of only one epoxide hydrolase. Again, cytosolic glutathione *S*-transferase activity is clearly separated from the cytosolic epoxide hydrolase, focussing as expected [10] at a basic pH above 8, yet another strong piece of evidence that these two enzymes are different proteins.

Finally, the cytosolic epoxide hydrolase in mouse liver demonstrates a pH optimum of around 7.0 and

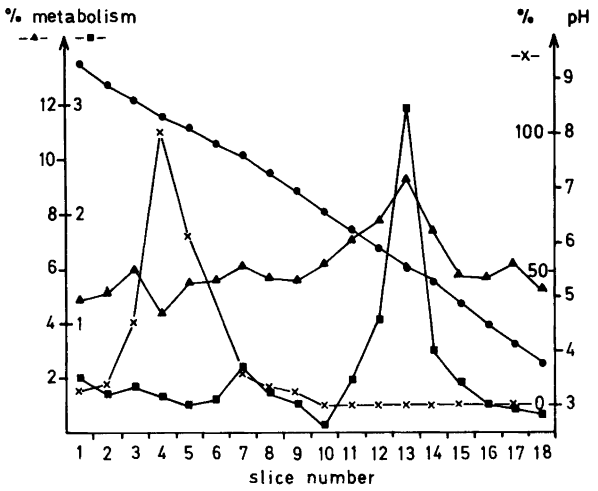

Fig. 2. Isoelectric focussing of the cytosol fraction from mouse liver.

This experiment was performed as described in the text. The cytosol fraction from NMRI mice was used here, but similar results were obtained with C57 black and CBA mice as well. The symbols used are as follows: ■ = epoxide hydrolase activity measured using *trans*-stilbene oxide as substrate; ▲ = epoxide hydrolase activity with styrene oxide as substrate; × = glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene as substrate; ● = pH.

distributes upon subcellular fractionation in the manner expected for a cytosolic enzyme. The next step in our investigations will be purification of this enzyme and subsequent further characterization.

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Lipid Intermediates in Glycosylation Reactions in Preneoplastic Nodules of the Liver*

I. EGGENS, L. C. ERIKSSON, T. CHOJNACKI and G. DALLNER

Department of Pathology at Huddinge Hospital, Karolinska Institutet, S-141 86 Huddinge and Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-10691 Stockholm, Sweden

The protein-bound oligosaccharide chains of various membranes are considered to be important for normal structure and function. The composition of the sugar chain changes during carcinogenesis and several properties of the malignant cell can be related to this modified structure. The intracellular membranes of the rat liver contain almost exclusively *N*-glycosidicly linked oligosaccharide chains, and the biosynthesis of these sugar chains required the participation of the lipid intermediate dolichol phosphate.

The role of dolichol in glycosylation reactions was investigated by using preneoplastic nodules of liver induced by prolonged administration of 2-acetylaminofluorene to rats. The nodules were separated from the unchanged liver tissue and the homogenate or the microsomal fraction was used in the investigations as described earlier.[1] The dolichol content of the liver homogenate in rat was about 50 μg per g liver; this increased greatly in the nodules (Table 1). In the isolated microsomal fraction, the increase was even more dramatic than in the homogenate, which indicated that a large part, if not all, of the increase of the dolichol content in preneoplastic nodules was due to the increase in the microsomal fraction. The two major components in rat liver are dolichols with 18 and 19 isoprene residues while the 17, 20 and particularly the 21 residues large dolichols are present only in smaller amounts. This distribution pattern is unchanged in the case of nodules.

There are three major glycosyl transferases in liver microsomes which in some steps utilize lipid intermediates, and these were investigated here (Table 2). High incorporation of mannose, glucosamine, and glucose into endogenous dolichol phosphate of control microsomes takes place. All three sugars are transferred to endogenous protein acceptors which are present in the microsomes. Transfer of mannose and glucosamine, from the nucleotide activated form to dolichol and protein, decreases greatly when the analyses are performed on microsomes isolated from the nodules.

Dolichol phosphate may represent the rate limiting step in the glycosylation process, and

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Table 1. Distribution of different types of dolichols in control liver and in preneoplastic nodules. The dolichol content and distribution in homogenates and isolated microsomal fractions were measured by high pressure liquid chromatography. Eluates were monitored at 210 nm. In a convex gradient No. 4 (Waters) a solvent system, from the initial 2-propanol – methanol – H_2O (40:60:2) in pump system A to hexan – 2-propanol (70:30) in pump system B was utilized.

Fraction	Amount μg per g liver	μg per mg protein	Control nodules microsomes % of total	
Control				
Total, homogenate	50.2			
Total, microsomes		0.26		
Nodules				
Total, homogenate	143			
Total, microsomes		1.45		
D17, microsomes			14	15
D18, „			41	40
D19, „			32	27
D20, „			10	12
D21, „			3	4

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Table 2. Glycosylation of endogenous lipids and proteins in microsomes prepared from preneoplastic nodules. The incubation mixture contained 30 mM Tris-HCl, pH 7.8; 10 mM $MnCl_2$; 2.4 mM EDTA; 12.5 mM mercaptoethanol; 0.4% Triton X-100; 2 mM ATP and 30 kBq [^{14}C]GDP-mannose or [^{14}C]UDP-GlcNAc or [^{14}C]UDP-glucose and 2.5 mg microsomal protein in a final volume of 1 ml. After 15 min incubation at 30 °C the dol-P, dol-PP−O and protein fraction were prepared as earlier.[2] O = oligosaccharide.

Substrate	Dol-P monosaccharide cpm per mg protein	Dol-PP−O	Protein
Control, microsomes			
GDP-Man	1804	120	560
UDP-GlcNAc	1175	90	868
UDP-Glc	918	44	564
Nodules, microsomes			
GDP-Man	1314	85	493
UDP-GlcNAc	765	68	491
UDP-Glc	910	38	498

Table 3. Glycosidase, kinase and phosphatase activities in microsomes from preneoplastic nodules. Glycosidases, kinases and dolichol monophosphatase were determined as earlier.[2,4,5]

Exp.	Enzyme activity	Microsomes Control	Nodules
1	Glycosidases		
	α-Mannosidase[a]	1,6	1,5
	N-acetylglucosaminidase[a]	5,2	7,4
	Glucosidase[a]	2,7	2,5
2	Kinases		
	Dolichol-(D11)-P[b]	507	420
	Dolichol-(D20)-P[b]	150	80
3	Dolichol-monophosphatase		
	Dolichol-(D17−D21)[c]	3590	5700

[a] μmol *p*-nitrophenyl/min mg protein. [b] Dolichol monophosphate formed (cpm/mg protein. 10 min). [c] Dolichol formed (cpm/mg prot. 10 min).

enzymes which directly regulate the amount of active intermediate are of utmost importance in glycoprotein metabolism.[3] Furthermore, the results obtained in the studies of glycosyl transferases may be influenced by antagonistic enzymes since it has been established that glycosidases are not only lysosomal enzymes but are also present in the microsomal membranes. As shown in Table 3, α-mannosidase, glucosaminidase and glucosidase activities are present in control microsomes, and in the case of nodules the glucosaminidase activity is greatly increased. It is possible that the decrease of sugar transfer to lipid and protein acceptors may be explained by limitation in the amount of dolichol phosphate, since the majority of the dolichol in the liver is present as free alcohol. The CTP-mediated kinase which is specific for dolichol phosphorylation plays a key role in the regulation of the active lipid intermediate.[4] The kinase was measured with both dolichol-D11 and dolichol-D20 as a substrate and the activity in control microsomes was 3 times higher with the former. In the case of nodules the activity decreased with both substrates. Since dolichol phosphate concentration was low in the liver, the phosphatase present must have been

Table 4. Estimation of the amount of available dolichol phosphate for reaction with different sugars. Microsomes were incubated with 10 nmol radioactive sugar nucleotide (30 °C, 15 min) and the amount of lipid associated radioactive sugar in the chloroform extract was determined.[6]

Substrate	Sugar transfer to endogenous dolichol phosphate	
	Control	Nodules
	pmol/mg protein	
GDP-Man	16	12
UDP-GlcNAc	7	3
UDP-Glc	3	4

efficient even under *in vivo* conditions. When the microsomes from nodules were tested, there was a significant increase in monophosphatase activity. These results which demonstrate decreased kinase and increased monophosphatase activities favor the idea that an actual decrease of dolichol phosphate amount occurs in preneoplastic nodules.

In order to estimate the maximal amount of dolichol phosphate which is functionally available as sugar acceptor from the nucleotide activated substrate, we employed an indirect approach[6] (Table 4). Excess of substrate was used in incubation of microsomes, and, after 10 minutes incubation, the sugar transfer reached a plateau value because the amount of phosphorylated lipid intermediate was limited. In control microsomes, the capacity to accept mannose, glucosamine, and glucose was decreased in a proportion of about 4, 2 and 1, respectively. This indicates that different dolichol phosphates probably interact with the various sugars. In the case of nodules the microsomal dolichol phosphate capable for interaction with mannose and glucosamine exhibits a clear decrease.

The experiments described in this paper demonstrate that the amount of dolichol is greatly increased in the early phase of the neoplastic transformation. On the other hand, the results show that the amount of dolichol monophosphate probably decreases because of an increased phosphatase and a decreased phosphorylase activity. The final result is a decreased glycosylation of the endogenous protein.

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Topology of Epoxide Hydrolase in the Membrane of the Endoplasmic Reticulum

JANERIC SEIDEGÅRD,[a] JOSEPH W. DePIERRE,[a] THOMAS M. GUENTHNER[b] and FRANZ OESCH[c]

[a] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden, [b] Department of Pharmacology, University of Illinois Medical Center, Chicago, Ill., U.S.A. and [c] Institute of Pharmacology, University of Mainz, Mainz, Federal Republic of Germany

As has been most clearly demonstrated in the case of mitochondrial inner membranes, chloroplast lamellae, and certain enzymes on the endoplasmic reticulum,[1] the topology of membrane-bound enzymes often has important functional consequences. At present relatively little is known about the topology of epoxide hydrolase in the membrane of the endoplasmic reticulum. A detailed subfractionation study performed earlier in our laboratory[2] suggested that epoxide hydrolase does not form a stoichiometric complex with cytochrome P-448, at least not after induction with 3-methylcholanthrene. The topology of microsomal epoxide hydrolase has also been investigated using proteases[2] and the results have led to the tentative conclusion that little or none of this enzyme is exposed at the outer microsomal surface (=the cytoplasmic surface of the endoplasmic reticulum).

Each of the individual techniques used to investigate the transverse topology of membrane-bound enzymes has its limitations.[1] For instance, epoxide hydrolase may be exposed at the outer microsomal surface and still be resistant to attack by protease. Therefore, we felt that it was important to study the transverse topology of this enzyme using complementary approaches.

In the present experiments microsomal epoxide hydrolase was labelled using lactoperoxidase, H_2O_2, and $^{125}I^-$. This system results in the iodination of proteins, chiefly on tyrosine and histidine residues, exposed at the surface of intact membrane vesicles, since lactoperoxidase is far too large to penetrate across biological membranes.[3] In order to also label proteins exposed at the inner microsomal surface we made the microsomal vesicles permeable to lactoperoxidase with 0.05% deoxycholate.[4] As a control for achieving labelling of all groups in epoxide hydrolase which can be iodinated by lactoperoxidase the isolated lipid and detergent-depleted enzyme was iodinated under similar conditions. Finally, in order to investigate the role of membrane phospholipids in determining the transferase topology of epoxide hydrolase, the enzyme was purified to homogeneity and reincorporated into liposomes composed of phosphatidylcholine or total rat liver microsomal phospholipids. The topology of the enzyme in these liposomes was subsequently compared to its topology in microsomes using the same iodination procedures.

Liver microsomes were prepared from male Sprague-Dawley rats weighing 200–220 g (obtained from Versuchstierzuchtanstalt, WIGA, Sulzfield, Federal Republic of Germany). The liver was homogenized in 3 volumes 1.15% KCl containing 10 mM sodium phosphate, pH 7.4. The homogenates were centrifuged at 10 000 *g* for 10 min and the resulting supernatant fraction was centrifuged at 100 000 *g* for 1 h. The microsomal pellets were subsequently resuspended in 1.15% KCl – 10 mM sodium phosphate, pH 7.4, to give a final protein concentration of 5 mg/ml.

Microsomal epoxide hydrolase was purified to apparent homogeneity from rats treated with *trans*-stilbene oxide essentially according to the procedure of Bentley and Oesch,[5] but with certain modifications designed to achieve higher yields and obtain an enzyme perparation containing smaller amounts of detergent.[6]

Epoxide hydrolase activity towards styrene oxide was determined using the procedure reported earlier.[7]

Total microsomal phospholpids were extracted from rat liver microsomes according to Bligh and Dyer[8] and were stored, as was egg yolk phosphatidylcholine (Sigma Chemical Co., St. Louis, Missouri, USA), in chloroform solution. After evaporation of the chloroform, phospholipids were mixed with purified epoxide hydrolase in a ratio of 10:1, w:w, in the presence of 1% sodium cholate. This mixture was then submitted to two chromatographic steps:[9] both columns were coated with phospholipids and with bovine serum albumin before use. Upon chromatography on Sephadex G-50 virtually all protein and phospholipid eluted in the void volume, while nearly 99% of the cholate present was retarded on the column. Subsequent chromatography on Sepharose 4B revealed that nearly all the protein was associated with the smaller phospholipid vesicles. In a control experiment [^{14}C]-phosphatidylcholine was added to the original mixture and monitored through the two chromatographic steps to further confirm that epoxide hydrolase had indeed been incorporated into phospholipid vesicles by this procedure.

Microsomes, reconstituted lipid vesicles or isolated microsomal epoxide hydrolase were

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Table 1. Iodination of epoxide hydrolase in intact microsomes and after incorporation into liposomes.[a]

System	Intact (%)	Disrupted with deoxycholate (%)
Epoxide hydrolase in intact microsomes	20 – 25	20 – 25
Purified epoxide hydrolase incorporated into liposomes of phosphatidylcholine	40 – 45	80 – 90
Purified epoxide hydrolase incorporated into liposomes of total rat liver microsomal lipids	25 – 30	25 – 30

[a] The figures in the table are the percentages of the total iodinatable groups in epoxide hydrolase (as determined by iodination of the purified, lipid- and detergent-depleted enzyme) labeled in the different systems using lactoperoxidase, H_2O_2, and $^{125}I^-$. For further details see the text.

iodinated [10] in a reaction mixture containing 50 mM potassium phosphate, pH 7.2, 0.2 mg protein/ml, 0.3 μM lactoperoxidase and carrier-free ^{125}I (0.37 – 1.85 MBq/ml). The reaction was started by the addition of H_2O_2 to give a final concentration of 4 μM. This addition was repeated 3 more times during the incubation, which was carried out for 30 min at 20 °C in the dark and terminated by the addition of $Na_2S_2O_3$ and KI to give final concentrations of 0.1 mM and 10 μM, respectively. (The concentrations of lactoperoxidase and H_2O_2 were determined by measuring the optical density at 412 nm and 230 nm, respectively. The absorption coefficients used were 114 mM^{-1} cm^{-1} [11] and 72.4 M^{-1} cm^{-1},[12] respectively).

Iodinated microsomes and phospholipid vesicles, were collected by centrifugation for 30 min at 105,000 g. The pellet was suspended in 50 mM Tris-Cl, pH 7.6, and this procedure repeated three times to remove unbound ^{125}I. The microsomes were then solubilized with 1 % sodium cholate and incubated at room temperature for 3 h with rabbit antibodies against rat liver microsomal epoxide hydrolase (1 mg antibody protein/mg microsomal protein). Goat antibodies against immunoglobulins (1 – 2 mg goat antibodies/40 μg rabbit IgG) were subsequently added to the mixture and the incubation continued overnight at 4 °C.

The epoxide hydrolase – rabbit anti – epoxide hydrolase – goat anti – IgG complex was precipitated by centrifugation at 20 000 *g* for 10 min. This precipitate or reconstituted vesicles precipitated with TCA were then dissolved by boiling for 1 – 2 min in 6% sodium dodecyl sulfate and epoxide hydrolase was separated from the immunoglobulins by SDS-slab gel electrophoresis according to Laemmli.[13] Purified microsomal epoxide hydrolase was separated directly from the reaction mixture used for iodination by SDS-slab gel electrophoresis. The gels were stained with Coomassie blue and the band corresponding to epoxide hydrolase was cut out and the amount of ^{125}I it contained determined by scintillation counting.

It can be seen from Table 1 that when intact microsomes are incubated with lactoperoxidase, H_2O_2, and $^{125}I^-$, 20 – 25 % of the groups in epoxide hydrolase which can be iodinated are labelled. Assuming a random distribution of such groups along the peptide chain of this enzyme, this finding suggests that 1/5 – 1/4 of the epoxide hydrolase molecule is exposed at the outer microsomal surface (= the cytoplasmic surface of the endoplasmic reticulum). There is no increase in the iodination of epoxide hydrolase by lactoperoxidase, H_2O_2, and $^{125}I^-$ when the microsomal vesicles are made leaky with deoxycholate (Table 1), suggesting that little or none of this protein is exposed at the inner microsomal surface (= the luminal surface of the endoplasmic reticulum).

This indicated topology of the microsomal epoxide hydrolase – *i.e.*, the exposure of a small portion of the protein at the cytoplasmic surface and the localization of most of the polypeptide chain in the membrane itself – agrees well with earlier results [14] (see above). Such a topology might provide the microsomal epoxide hydrolase with easy access to epoxides dissolved in the phospholpid bilayer of the endoplasmic reticulum and to epoxides dissolved in the cytoplasm or bound to soluble proteins. It should be remembered that since we used antibodies directed specifically towards a single, homogeneous form of microsomal epoxide hydrolase to determine the extent of iodination (see above), our results are relevant only to this form of the enzyme. It has been reported that there are at least three different microsomal epoxide hydrolases [15] and it may be that the other forms have different transverse topologies in the membrane of the endoplasmic reticulum. However, it may be that the antibody used here can also cross-react with other forms of microsomal epoxide hydrolase.

Table 1 also documents the iodination of purified epoxide hydrolase which has been reincorporated into liposomes composed of phosphatidylcholine or of total rat liver microsomal lipids. After incorporation into liposomes composed of egg yolk phosphatidylcholine 40–45% of the groups that can be iodinated in epoxide hydrolase are labelled by lactoperoxidase, H_2O_2, and $^{125}I^-$. This is considerably more than in the case of intact microsomes. Furthermore, after disruption of these liposomes with deoxycholate almost all of the groups can be labelled (Table 1). Thus, in liposomes formed from egg yolk phosphatidylcholine epoxide hydrolase is almost totally exposed at the membrane surfaces and is also randomly distributed between the inner and outer surfaces. This pattern is very different from that seen with intact microsomes.

On the other hand, after incorporation into liposomes composed of total rat liver microsomal lipids, the topology of epoxide hydrolase as revealed by the approach employed here is essentially the same as that seen in intact microsomes (Table 1). These findings suggest that membrane phospholipids have an important role to play in the transverse topology of epoxide hydrolase in the endoplasmic reticulum.

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Proton NMR Studies of a Tetrasaccharide which is a Receptor for Uropathogenic *E. Coli* Bacteria *

KARL-ERIK FALK, PER-ÅKE JOVALL ** and PAUL WINYARD

Chalmers Institute of Technology and University of Göteborg, Department of Biochemistry and Biophysics, S-412 96 Göteborg, Sweden

Glycosphingolipids occur in the plasma membrane of mammalian cells. The hydrophobic ceramide section is inserted into the external half of the lipid bilayer, whilst the hydrophilic oligosaccharide protrudes from the cell surface.[1,2]

The structures of the carbohydrate chains of glycosphingolipids are extremely variable, allowing them to function, for example, as blood group antigens and as receptors for bacterial toxins.[2] They may also serve as host receptors for adhesion of *E. coli* strains which cause urinary tract infections.[3,4] Pili, single helical coils of protein subunits (approx. M.W. 20 000), have been implicated as the bacterial ligands mediating this binding process.[5]

Preincubation of bacteria with a glycolipid fraction extracted from urinary sediment epithelial cells has been shown to inhibit bacterial attachment.[3] Globotetraosylceramide (*P* antigen, globoside) was the most efficient inhibitor. It has the structure βGalNAc-(1→3)-αGal-(1→4)-βGal-(1→4)-βGlc-(1→1)-Cer.*** The structurally related glycolipid, globotriasylceramide, was also active, but unrelated glycolipids were inactive.[4] The oligosaccharides of active compounds each contain αGal-(1→4)-Gal, binding activity may therefore reside in this disaccharide.[6] The tetrasaccharide (confirmed to be globotetraose in this study), cleaved from ceramide by ozonolysis [7] and alkaline degradation, also reduced the attachment of *E. coli* to human uroepithelial cells.[8]

The aims of this work were twofold: (1) to verify that it was indeed globotetraose that was isolated in the latter study;[8] (2) to assign the ^{1}H NMR spectrum of globotetraose in D_2O, as a prelude to studying its interaction with the protein receptor.

Experimental. A Bruker 270 MHz NMR instrument was used in the Fourier-transform mode. Spectra were typically taken up in 8 K memory with an acquisition time of 1.37 s and a spectral width of 3000 Hz. Chemical shift values are given in ppm relative to 2,2-dimethyl-2-silapentane-5-sulfonate. The time shared mode of homonuclear decoupling was used in some of the experiments. The globotetraose was dissolved in 99.998% deuterium oxide from Stohler Isotope Chemicals.

Results and discussion. In this study, the ^{1}H NMR spectrum of the tetrasaccharide was characterised by homonuclear decoupling experiments and comparison with spectra of glycosphingolipids possessing similar carbohydrate chains.[9,10] The spectrum (Fig. 1) shows five well-resolved resonances in the anomeric region (4.4 – 5.3 ppm). The α-anomeric protons of glucose and galactose have small coupling constants ($J_{1,2} \approx 4$ Hz) and appear at the low-field end of the spectrum, whilst the β-anomeric protons have larger coupling constants ($J_{1,2} \approx 9$ Hz) and appear at a somewhat higher field.

The reducing glucose residue of the tetrasaccharide is present both in α- and β-form, and gives rise to two doublets of lower intensity than the other anomeric resonances. Thus, the doublet at 5.23 ppm is easily assigned to the anomeric proton of α-glucose. The four peaks at 4.6 – 4.7 ppm were shown, by decoupling, to be due to two β-anomer doublets. The doublet to slightly higher field was of lower intensity and was therefore ascribed to the β-anomer of glucose. The percentages of the α- and β-anomers of glucose were found, by integration, to be 30 and 70%, respectively. The anomeric protons of 2-acetamido-2-deoxy-β-D-hexopyranosides appear between the α- and β-anomeric protons of the unsubstituted residues. Thus, the other doublet could be assigned to the 2-acetamido-2-deoxy-β-galactosyl group of the tetrasaccharide. The singlet at 2.05 ppm derives from the acetamido group of this sugar residue.

From their coupling constants and chemical shift values, the doublets at 4.95 ppm and 4.52 ppm were assigned to the anomeric protons of the α- and β-galactosyl residues, respectively.

The ring proton region (3.5 – 4.0 ppm) is very crowded and most resonances are overlapping. However, all H-2's could be assigned by selective irradiation of the appropriate anomer resonance. This was then checked by irradiating the H-2 resonances. The remaining ring proton assignments were made to narrow regions, and some of the H-3 and H-4 resonances could not be identified. A further complication is the small coupling constant between the H-3 and H-4 in galactosyl and 2-acetamido-2-deoxygalactosyl residues ($J_{3,4} \approx 1$ Hz),

* Communication at the Meeting of the Swedish Biochemical Society in Stockholm, 26 – 27th November, 1981.

** Author to whom correspondence should be addressed.

*** Abbreviations used: Gal, galactose; Glc, glucose; GalNAc, 2-acetamido-2-deoxygalactose; Cer, ceramide. All sugars are assumed to exist in the pyranose form, to be of the D series and to have a C1 chair conformation.

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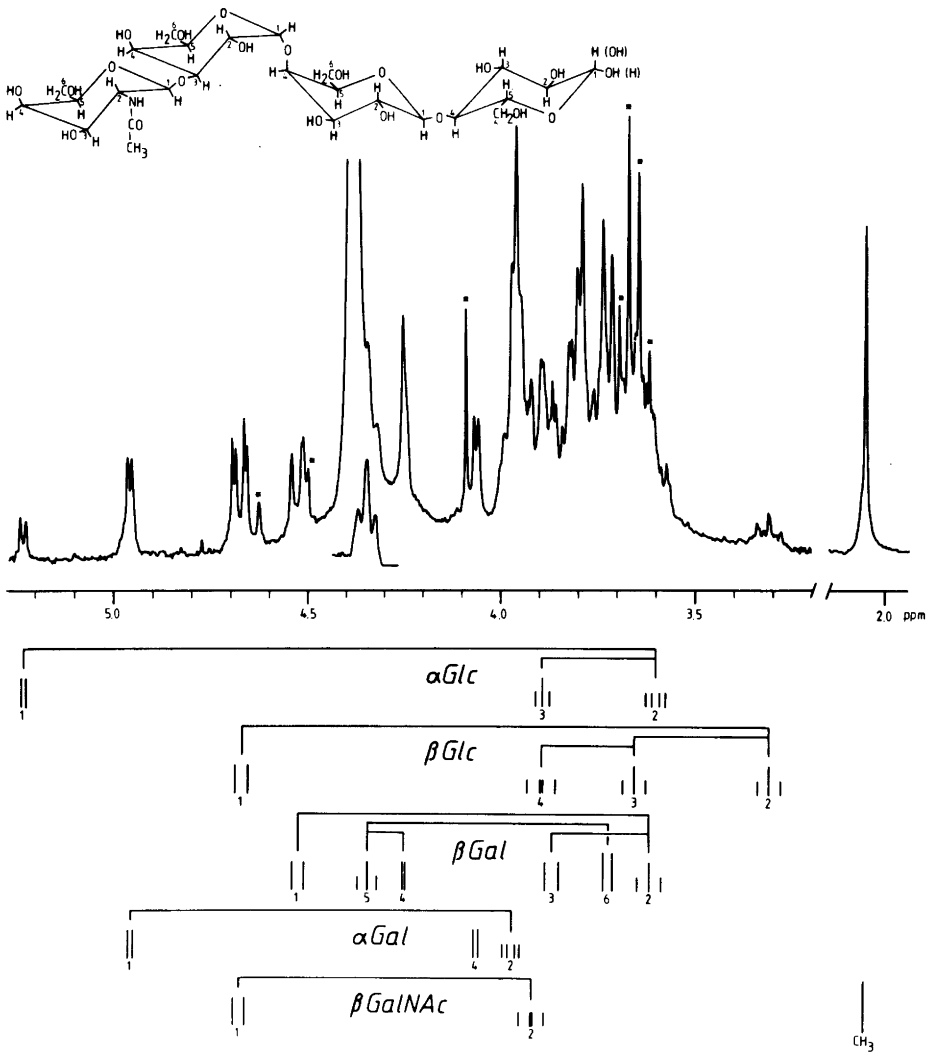

Fig. 1. Assignment of the globotetraose (~2 mg/ml) ^{1}H NMR spectrum at 270 MHz in deuterium oxide at 333 K. This spectrum was recorded in 16 K memory and 1000 transients were collected. Inserted is the triplet-structure at 4.33 ppm recorded at 295 K. The methyl resonance at 2.05 ppm is reduced to half intensity. Resonances marked (*) are due to impurities or spinning sidebands. The structure of globotetraose is indicated above the spectrum, the α-glucose configuration being shown in parenthesis. The C-4, 5 and 6 protons have been tentatively assigned (see text).

which makes it very difficult to detect H-4 signals by irradiation of H-3 signals.

There are three non-anomeric resonances shifted down-field, outside the bulk of the overlapping ring proton resonances. The triplet structure at 4.33 ppm (partly hidden by the HDO resonance at 333 K) is coupled to a resonance in the ring proton region at 3.76 ppm. These resonances arise from H-5 and H-6, respectively, of a galactosyl residue. The triplet also appears to be coupled to the signal at 4.24 ppm, which is a doublet structure, hidden beneath the HDO spinning sideband in Fig. 1. The latter signal and the third of the low-field ring proton resonances, at 4.05 ppm, can be assigned to the H-4's of the two galactosyl residues, since substituted galactosyl H-4's have very low-field shifts compared

to other ring protons.[11]

Dabrowsky *et al.*[11] have suggested that resonances in the spectrum of globoside in dimethyl sulphoxide having corresponding positions to the low-field triplet and doublet structures (4.33 and 4.24 ppm), belong to the α-galactosyl residue. We have not been able to correlate these assignments with the spectrum of globotetraose in D_2O. Neither decoupling experiments nor comparison with other spectra allow unambiguous assignment of these H-4, 5 and 6 signals to the α-galactosyl and/or β-galactosyl residues. One difficulty is that α- and β-galactosyl residues invariably occur together in the glycolipids so far available to us. However, tentative assignments are given in Fig. 1. Further studies are in progress to clarify the origin of these signals. Comparison with the spectrum of the corresponding globoside incorporated into SDS micelles[11] showed the two spectra to be very similar. The differences in the globoside spectrum were due to the reducing glucose residue being bound to ceramide, and to resonances arising from the ceramide itself. The study is also in good agreement with previous studies made in different organic solvents.[9,11] Thus, our study confirms that the isolated tetrasaccharide was globotetraose.

The spectrum of globotetraose has been sufficiently well assigned to allow interaction studies in a physiologically relevant environment. It should now be possible to observe the interaction between purified pili (the bacterial ligand) and the globotetraose (the specific receptor) in D_2O. For these studies, it may be possible to use the isolated sugar in solution, or the intact glycolipid inserted into micelles or vesicles.

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Synthesis and Cathodic Cleavage of an Eight-membered Cyclic Sulfone, 3,4,5,6-Tetrahydro-2*H*-benzo[*b*]-thiocin-1,1-dioxide

BO LAMM * and CARL-JOHAN AURELL

Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

Some years ago, the cathodic cleavage of a series of cyclic sulfones illustrated in Scheme 1 was studied.[1,2] For n = 2 and 3, the upper route was exclusively followed, whereas for n = 4, an 85:15 ratio between the upper and the lower route was observed. It was suggested [2] that the dihedral angle between the sulfonyl group and the benzene ring determines the cleavage mode. In the compound with n = 2, this angle is 90° different from its value in methyl phenyl sulfone, which upon cleavage gives methane, and no benzene.[3] In the seven-membered ring compound, the angle is probably intermediary between the two extremes, and aryl–sulfonyl as well as alkyl–sulfonyl cleavage is observed. Inspection of a Dreiding model of the hitherto unknown member of the series, n = 5, suggested that the conformation would be similar to that of an open alkyl phenyl sulfone. One would then predict that the cathodic cleavage of the eight-membered ring compound should follow the lower route in Scheme 1.

We now report an efficient synthesis of the eight-membered ring compound and its cathodic cleavage at mercury. The route is shown in Scheme 2. The crucial step, reduction of a disulfide and intramolecular displacement of a mesylate under high-dilution conditions, was inspired by recent Japanese work,[4] and gave in the present case 79% yield.

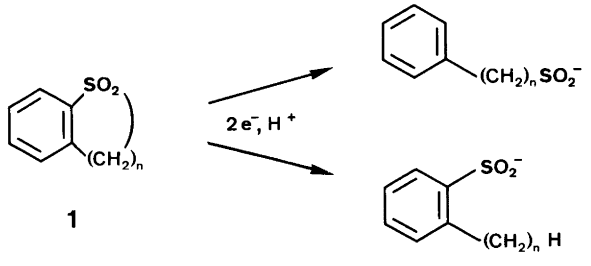
* To whom correspondence should be addressed.

The sulfinate ions formed upon cathodic cleavage were converted into sulfones through reaction with iodomethane. By comparison with authentic material, methyl 5-phenylpentyl sulfone and methyl *o*-pentylphenyl sulfone, respectively, it could be shown that the reaction had taken entirely the lower path in Scheme 1, *i.e.*, alkyl-sulfonyl cleavage.

A single-crystal X-ray diffraction study of the title compound showed [5] the dihedral angle between the benzene ring plane and the C–S–C plane in the thiocin ring to be 71°, not far from the predicted 90°. The present work thus supports the stereoelectronic argument presented earlier [2] to explain the different cleavage mode for cyclic and non-cyclic sulfones.

Experimental. Syntheses. All steps in Scheme 2 represent well-known reactions, the conditions for which were taken from literature procedures for analogous compounds. The structures of all intermediates were verified by ^{1}H NMR spectroscopy. Since the eight-membered ring sulfide and sulfone represent unusual heterocycles, their data are given. For 3,4,5,6-tetrahydro-2*H*-benzo[*b*]thiocin, b.p. 120 °C at 0.1 mmHg (Kugelrohr), ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.29 – 1.41 (2 H, m), 1.65 – 1.77 (4 H, m), 2.70 (2 H, t, *J* 5.7 Hz), 3.13 (2 H, t, *J* 6.4 Hz), 7.07 – 7.29 (3 H, m), 7.58 (1 H, d, *J* 8 Hz). MS IP 50 eV; *m/e* (% rel. int.): 179 (15), 178 (100, M), 177 (91), 149 (14), 137 (18), 135 (54), 123 (20), 121 (13), 117 (18), 91 (34). Mol. wt., obs. 178.0817, calc. for $C_{11}H_{14}S$ 178.0817. The sulfone, 3,4,5,6-tetrahydro-2*H*-benzo[*b*]thiocin- 1,1-dioxide, forms colourless crystals, m.p. 96 – 97 °C (Kofler Hot Stage). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.25 – 1.37 (2 H, m), 1.73 – 1.85 (2 H, m), 1.95 – 2.07 (2 H, m), 3.15 (2 H, t, *J* 6 Hz), 3.37 (2 H, t, *J* 6 Hz), 7.25 – 7.59 (3 H, m), 8.09 (1 H, d, *J* 8 Hz). Anal. $C_{11}H_{14}O_2S$: C, H, S.

Cathodic reduction. An H-type cell with an AMFion® type C-100 ion exchange membrane was used. The electrolyte was 0.5 M tetramethylammonium chloride in methanol, the cathode a 20 cm^2 mercury pool, and the anode a carbon rod. At a potential of –2.5 V *vs.* Ag,0.01 M $AgNO_3$,0.1 M tetraethylammonium perchlorate in DMF,[6] twice the amount of electricity calculated for a two-



Scheme 1. Aryl-sulfonyl (upper path) and alkyl-sulfonyl (lower path) cleavage of cyclic sulfones at mercury.

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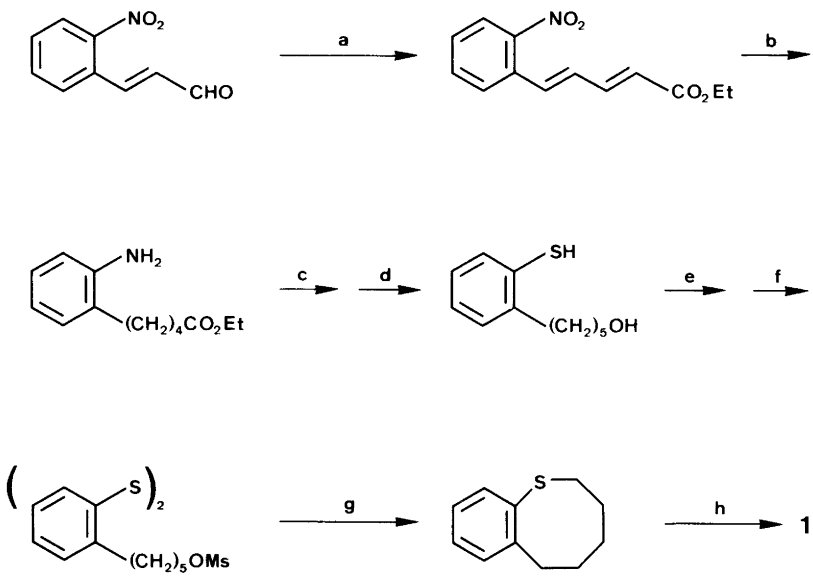

Scheme 2. Synthesis of 3,4,5,6-tetrahydro-2*H*-benzo[*b*]thiocin-1,1-dioxide. Reagents: *a*, NaH, $(MeO)_2P(O)CH_2COOEt$;[7] *b*, H_2/Pd; *c*, HNO_2, then KSC(S)OEt; *d*, $LiAlH_4$;[8] *e*, I_2; *f*, $MeSO_2Cl$, Et_3N;[9] *g*, $NaBH_4$, high dilution;[4] *h*, *m*-$ClC_6H_4CO_3H$.

electron process was introduced. The catholyte was evaporated to a small volume and heated with a tenfold molar excess of iodomethane at reflux for 2 h. The next day, it was worked up through distribution between dichloromethane and water. The sulfone formed was shown to be identical to authentic methyl *o*-pentylphenyl sulfone by comparison of the 270 MHz ^1H NMR spectra, and the absence of methyl 5-phenylpentyl sulfone was demonstrated by GLC analysis; detection limit estimated at 2%.

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Effect of Phthalate Ester Metabolites on Rat Liver *

A. E. GANNING,[a] E. KLASSON,[a] Å. BERGMAN,[a] U. BRUNK [b] and G. DALLNER [c]

[a] Department of Biochemistry and Wallenberg Laboratory, University of Stockholm, Stockholm, [b] Department of Pathology, University of Linköping, Linköping and [c] Department of Pathology, Karolinska Institutet, Stockholm, Sweden

Plasticizers are used extensively in industrial processes, and a relatively high level of contamination is found in food and drinking water.[1] Measurements of the air in cities and analysis of earth also detected a variable content of phthalate esters.[2] In previous investigations we have found prominent induction of peroxisomes and mitochondria in liver of rats obtaining phthalate ester in their diet.[3] Judging from the metabolite pattern excreted from the urine, it is obvious that an extensive metabolism of phthalate esters take place before they are eliminated as water soluble compounds. Consequently, it is very possible that it is not the substrate but some of the metabolites which exert their effects on the liver. For this reason, we have synthesized some of the main known metabolites of di(2-ethylhexyl)phthalate ester (DEHP), which were subsequently fed to rats.

The compounds tested were DEHP, mono-(2-ethylhexyl)phthalate ester (MEHP), 2-ethylhexylbenzoate (EHB), phthalic acid (PA) and 2-ethylhexanol (EH). Rats were fed *ad libitum* with diet containing 2% of the above compounds for two weeks. At the end of this period the liver was removed, pieces were taken for electron microscopy, and the remainder was homogenized and subfractionated to obtain mitochondria and microsomes. Protein and various enzyme activities were measured as described earlier.[3]

The DEHP concentration of 2% in the diet was chosen because this concentration has been shown to give maximal induction within 2 weeks according to previous investigations.[4] Experiments were also performed using diets containing 0.2 and 0.02% DEHP during a 6-month period and the pattern of morphological, chemical and enzymatic changes were very similar to those described below with 2% DEHP. As can be expected, however, the magnitude of the changes was lower.

Induction of peroxisomes can be followed by measuring enzymes known to be localized exclusively in these particles. The mitochondrial β-oxidation of fatty acids is dependent on the respiratory chain and consequently is highly sensitive to KCN. The respiratory chain is not present in peroxisomes, which is the only location for extramitochondrial β-oxidation of fatty acids. This makes it possible to follow the peroxisomal β-oxidation enzymes in the homogenate.[5] As is established in previous experiments, DEHP induces substantially palmitoyl-CoA oxidation (Table 1). MEHP, which is the main metabolite of the ingested substrate, exerts a similar effect. Interestingly, two major metabolites, PA and EH, do not display an effect on peroxisomal β-oxidation. EHB which differs from MEHP by the lack of the carboxyl group is completely ineffective for induction of peroxisomes and peroxisomal enzymes. The specific activity of catalase and urate oxidase are decreased after administration of DEHP whereas the various metabolites tested produced either no change or gave a slight increase in the activities.

One of the unusual effects of DEHP on liver is the pronounced effect on mitochondria which are induced fourfold in the initial two weeks period. The induction involves an increase in the number of mitochondria without changes in the specific

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Table 1. Effect of DEHP and various metabolites on peroxisomal enzymes in homogenates.

Treatment	Palmitoyl-CoA oxidation (nmol NAD reduced) min^{-1} (mg protein)$^{-1}$	Catalase (μmol H_2O_2 decomposed) min^{-1} (mg protein)$^{-1}$	Urate oxidase (nmol urate oxidized) min^{-1} (mg protein)$^{-1}$
Control	4.8	81	37
DEHP	28.8	49	23
MEHP	16.7	113	35
EHB	3.5	65	30
PA	4.2	70	51
EH	3.8	81	40

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Table 2. Effect of DEHP and various metabolites on mitochondrial enzymes measured in isolated mitochondria.

Treatment	Protein mg/g liver	Cytochrome *c* oxidase (μmol cyt *c* ox) min^{-1} (mg protein)$^{-1}$	Carnitine-acetyl transferase (nmol CoA produced) min^{-1} (mg protein)$^{-1}$
Control	18.1	1.2	0.75
DEHP	49.3	1.1	21.8
MEHP	43.0	2.3	7.7
EHB	18.9	0.9	0.7
PA	19.5	2.1	0.8
EH	17.3	1.3	0.9

Table 3. Effect of DEHP and various metabolites on microsomal enzymes measured in isolated microsomes.

Treatment	Protein mg/g liver	Cytochrome P-450 nmol (mg protein)$^{-1}$	NADPH-cytochrome *c* reductase (μmol cyt *c* red) min^{-1} (mg protein)$^{-1}$
Control	18.6	0.60	0.089
DEHP	19.2	0.98	0.142
MEHP	18.6	1.01	0.154
EHB	17.1	0.67	0.092
PA	18.0	0.58	0.095
EH	19.8	0.68	0.101

activities of enzymes in respiration and in energy metabolism. Consequently, the protein content of the mitochondrial fraction is much higher after DEHP administration in comparison with the control but the specific activity of cytochrome *c* oxidase is not elevated (Table 2). An increase of cytochrome *c* oxidase activity is apparent after MEHP and PA administration. The main event occurring in mitochondria when rats were kept on a DEHP-containing diet is the 30-fold increase of carnitine-acetyl transferase determined by a spectrophotometric method. MEHP increases this activity 10 times while the other metabolites have no effect.

The endoplasmic reticulum displays a hypertrophy under the influence of many drugs and chemical reagents and it was interesting to determine if phthalate metabolites affect these membranes. Both enzymes of the NADPH electron transport system, NADPH-cytochrome *c* reductase activity and the amount of cytochrome P-450, increase about 50% with DEHP and MEHP (Table 3). The other three metabolites tested in these experiments did not change the amount or composition of the microsomal membranes. The experiments demonstrate that hydroxylation of phthalate esters performed by microsomal enzymes may have a role in the production of active metabolites influencing biosynthetic processes.

Parallel to the above chemical analyses, all livers were investigated electron microscopically. The results were clear from the point of view of morphological appearance. The pictures were dominated by proliferating peroxisomes and mitochondria in the case of DEHP and MEHP, without induction of the endoplasmic reticulum. On the other hand, no induction of the intracellular membranes could be observed with EHB, PA or EH, and the pictures were in all respects very similar to that of the control.

The experiments described demonstrate that among the metabolites of DEHP studied here only MEHP has a clear effect and induces both peroxisomes and mitochondria. The 2-ethylhexanol side chain and the phthalic acid are ineffective as inducors of membranes and enzymes. Interestingly, the presence of a non-substituted carboxyl group of the monophthalate ester is of basic importance in obtaining the selective and combined effects on peroxisomes and mitochondria. The induction of hydroxylating enzymes in the case of DEHP and MEHP suggests that oxidative metabolism is

probably an important requirement for production of active metabolites and these metabolites should be identified and isolated in the future.

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Thermolysis of 6-Hydroxy-3,4,5-tris-methoxycarbonyl-2*H*-benzo[*b*]thiocin

BO LAMM * and CARL-JOHAN AURELL

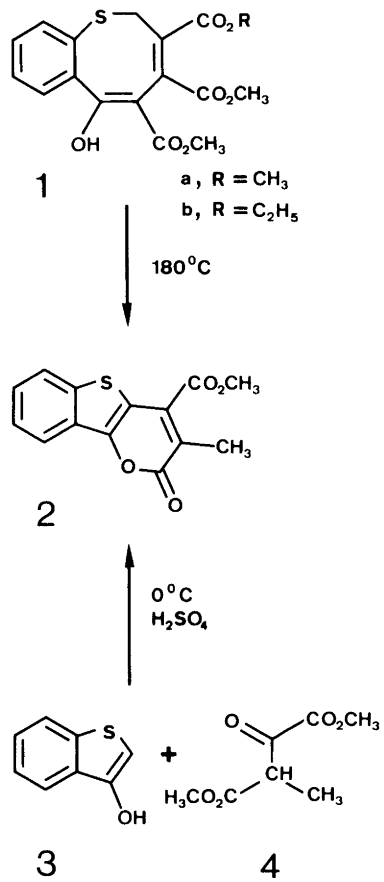
Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

In a previous publication,[1] the syntheses of some eight-membered ring compounds belonging to the benzo[*b*]thiocin system have been described. One of these compounds, 6-hydroxy-3,4,5-tris-methoxycarbonyl-2*H*-benzo[*b*]thiocin, *1a*, melts at 146 – 150 °C.[1] Upon heating to 180 °C, it decomposes, but from the cooled melt, a crystalline, strongly fluorescent compound can be isolated. We were intrigued by this decomposition, particularly since a similarly fluorescent compound had been obtained as a by-product in the reaction between 3-hydroxy-2-methoxycarbonylbenzo[*b*]thiophene and dimethyl acetylenedicarboxylate, and characterized as an α-pyrone, 3,4-bis-methoxycarbonyl-2-oxo-2*H*-[1]benzothieno[3,2-*b*]pyran.[1] We suspected that the present thermolysis product might have a similar structure and decided to identify it.

The present paper will describe the structure determination of the fluorescent compound by spectral methods, verification by independent synthesis and a suggestion of its formation through thermolysis. As an aid in this work, the ethyl dimethyl ester *1b* (Scheme 1) was also synthesized and studied. Heating of *1b* gave the same product as was obtained from *1a*.

The pyrolysis product from *1a* or *1b* was purified through medium-pressure liquid chromatography. Its empirical formula was determined by high-resolution MS to be $C_{14}H_{10}SO_4$, indicating the loss of $C_3H_6O_3$ from *1a*. Since the same compound, *2*, is also obtained from *1b*, it appears that the alkoxycarbonyl or possibly the alkyl group in the 3 position of *1* is split off. Instead of trying to determine the structure of *2* from the MS fragmentation pattern, we next turned to the 270 MHz ^{1}H NMR spectrum. Peaks corresponding to four aromatic hydrogen atoms, one methoxy group (δ 4.05) and one methyl group (δ 2.50) were present. All hydrogens are thus accounted for. The shift of 2.50 is too much upfield for an $-OCH_3$, so we must consider methyl bound to sulfur or an sp^2-hybridized carbon.[2]

Two compounds that fit the above data are 4-methoxycarbonyl-3-methyl-2-oxo-2*H*-[1]benzothieno[3,2-*b*]pyran, *2*, or the 3-methoxycarbonyl-4-methyl isomer. At this point, rather than speculate about which of these is the more probable one from a mechanistic point of view, we decided to make independent syntheses. Luckily, our first choice, the 4-methoxycarbonyl isomer, obtained as shown in Scheme 1, turned out to be identical to the thermolysis product from *1*.



Scheme 1. Formation of 4-methoxycarbonyl-3-methyl-2-oxo-2*H*-[1]benzothieno[3,2-*b*]pyran *via* thermolysis and condensation, respectively.

The analogue of *2* without the 3-methyl group has been synthesized *via* a sulfuric acid-catalyzed reaction between 3-hydroxybenzo[*b*]thiophene and hydroxymaleic acid.[3] We decided to apply this condensation to 3-methyl-2-hydroxymaleic acid, but instead of the free acid, its dimethyl ester was employed. The diester was prepared from dimethyl oxalate and methyl propionate following a method described for the corresponding ethyl esters.[4] The von Pechmann reaction was carried out in conc.

*To whom correspondence should be addressed.

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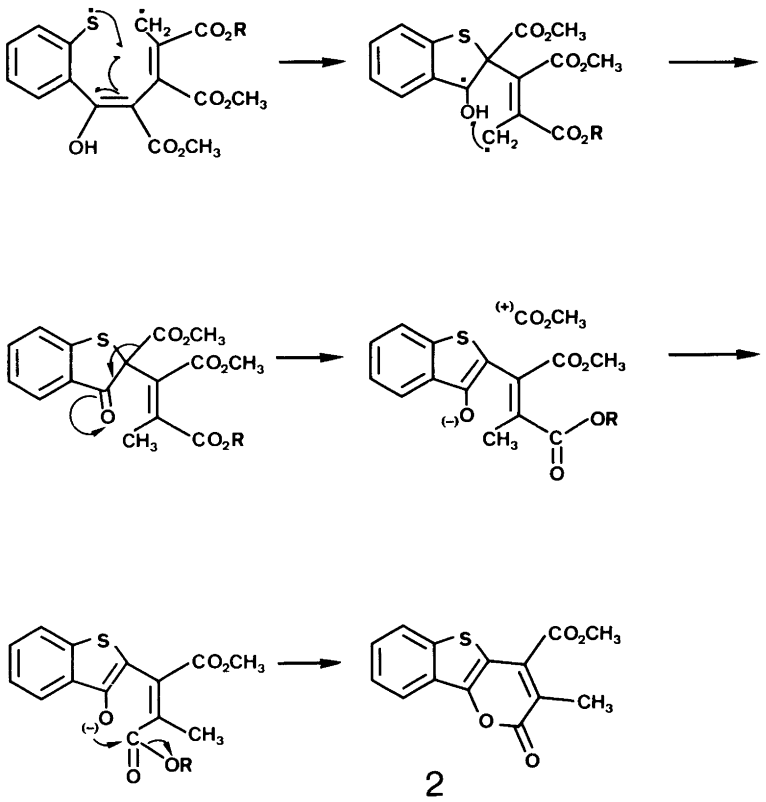

Scheme 2. Proposed mechanism for the thermolysis of 6-hydroxy-3,4,5-tris-methoxycarbonyl-2*H*-benzo[*b*]thiocin.

sulfuric acid at 0 °C and gave 13% yield of a compound that was identical to *2* (270 MHz ^{1}H NMR, m.p., mixed m.p.). The 3-methoxycarbonyl-4-methyl isomer of *2* was not synthesized, since the authenticity of *2* was considered to be established beyond doubt.

Possible mechanism of thermolysis. The driving force for the reaction is no doubt the formation of the non-strained benzothieno[3,2-*b*]pyran system from the eight-membered compound *1*. The origin of the 3-methyl group in *2* is probably neither the 4- nor the 5-methoxycarbonyl methyls of *1*, but rather the ring methylene in 2 position. It was brought to our attention by Professor D. Neckers that certain photochemical rearrangements of [2+2] adducts involve radicals.[5] We argued that initial homolytic fragmentation of the sulfur–methylene bond in *1* would be a comparatively facile process, since a phenylmercapto and an allyl radical, both resonance-stabilized, are formed. The process is outlined in Scheme 2. Attack of the sulfur radical on the 5-carbon atom gives the thiophene ring. Hydrogen atom transfer from the hydroxy group to the allyl radical gives the 3-methyl group of *2*. For the later steps of Scheme 2, an ionic mechanism is suggested, but the details are open to discussion.

Experimental. *6-Hydroxy-3,4,5-tri-methoxycarbonyl-2*H*-benzo*[b]*thiocin* (*1a*) and 3-hydroxybenzo[*b*]thiophene (*3*) were prepared according to the literature.[1,6] Compound *1b* was prepared in analogy with compound *1a*, starting with diethyl oxalate.[7]

3-Methyl-2-oxo-butane-1,4-dioic acid dimethyl ester (*4*). To a heterogeneous mixture of 2.3 g finely divided sodium (100 mmol) in 200 ml of benzene was added 11.8 g dimethyl oxalate (100 mmol) and 9.7 g methyl propionate (110 mmol). The mixture was refluxed for 20 h. Benzene was removed under reduced pressure and ether was added to the crystalline mass. Upon stirring, a yellow salt was left as a precipitate. This salt was filtered off and acidified with dilute sulfuric acid and extracted with ether. The ether solution was washed with water and dried over anhydrous magnesium sulfate. The

residue was distilled to give a dark-yellow oil (b.p. 115 °C at 1 mmHg). The yield was 9.5 g (55%). In deuterochloroform the keto – enol ratio is 6.5 : 1. ^{1}H NMR (Keto) (270 MHz, $CDCl_3$) δ 1.41 (3 H, d, J 7 Hz), 3.74 (3 H, s), 3.89 (3 H, s) and 4.12 (1 H, q, J 7 Hz). ^{1}H NMR (Enol) (270 MHz, $CDCl_3$) δ 2.00 (3 H, s), 3.85 (3H, s), 3.87 (3H, s) and 12.35 (1 H, s).

*4-Methoxycarbonyl-3-methyl-2-oxo-2*H*-[1] benzothieno[3,2-b]pyran (2). Thermolysis.* Compound *1a*, 1 g, was heated in a tube at 180 – 190 °C for 6 h with stirring. The residue was taken up in dichloromethane and chromatographed on silica gel – dichloromethane with medium-pressure equipment to give 150 mg of compound *2* (18%). Yellow needles from ether, m.p. 191 – 192 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.50 (3H, s), 4.05 (3H, s), 7.45 – 7.49 (2H, m), 7.78 – 7.82 (1 H, m) and 7.99 – 8.03 (1 H, m). MS: M^+ 274.0300. Calc. for $C_{14}H_{10}O_4S$, 274.0320. Fragments: *m/e* 274 (100), 247 (5.3), 246 (34.2), 245 (7.8), 231 (19.2), 218 (10.8), 214 (25.6), 187 (39.2), 186 (16.6) and 115 (25.9). Solutions of compound *2* are fluorescent (green), particularly in dilute ethereal solution.

Independent synthesis. To a mixture of 4.2 g of 3-hydroxybenzo[*b*]thiophene (*3*) (28 mmol) and 5.65 g of 3-methyl-2-oxo-butane-1,4-dioic acid dimethyl ester (*4*) (28 mmol) at 0 °C was added 40 ml of conc. sulfuric acid at 0 °C with stirring. The resulting mixture was kept at 0 °C for 1.5 h and poured onto ice-water. After extraction into dichloromethane, the organic phase was washed with water and dried over magnesium sulfate. Evaporation yielded a red oil, which was flash chromatographed on silica gel – dichloromethane to yield 1.0 g of pure compound (*2*) (13%). All physical data are identical with those of the thermolysis product.

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An EPR Study on the Binding of Alcohols to Soybean Lipoxygenase-1 *

STEVEN SLAPPENDEL,[a] ROLAND AASA,[a] BO G. MALMSTRÖM,[a] GERRIT A. VELDINK [b] and JOHANNES F. G. VLIEGENTHART [b]

[a] Department of Biochemistry and Biophysics, Chalmers Institute of Technology and University of Gothenburg, S-412 96 Gothenburg, Sweden and [b] Department of Bio-organic Chemistry, State University of Utrecht, Croesestraat 79, NL-3522 AD Utrecht, Teh Netherlands

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is a dioxygenase catalyzing the reaction between molecular oxygen and polyunsaturated fatty acids with a 1,4-*cis*,*cis*-pentadiene system.[1] Soybean lipoxygenase-1 contains per molecule one non-heme iron atom which switches between the ferric and ferrous states during catalysis as demonstrated by EPR.[2] In the oxidized form the so-called yellow enzyme shows a complex EPR signal around g 6 stemming from high-spin Fe(III) species. The line shape of the EPR spectrum changes substantially upon addition of small amounts of ethanol and other alcohols.[3] This change in line shape corresponds to a change in the relative amounts of species having different environments of iron.[3]

The purpose of this investigation was to obtain more information on the effects of alcohols on the EPR spectrum of yellow lipoxygenase to gain a better understanding of the binding of the alcohols and the natural substrates.

Experimental. Lipoxygenase-1 was isolated from soybeans according to Finazzi Agrò *et al.*[4] The specific activity was 235 μmol O_2 min^{-1} mg^{-1}. The iron content was 0.97 mol per mol enzyme and the amount of contaminating Mn was found to be 0.07 mol per mol enzyme. 13-L- and 9-D-hydroperoxy-octadecadienoic acid (HPOD) were prepared by aerobic incubation of linoleic acid with soybean lipoxygenase-1 at pH 9.0[5] and corn-germ lipoxygenase at pH 6.6,[6] respectively, and purified by HPLC. 13-L- and 9-D-hydroxy-octadecadienoic acid (HOD) were prepared by reduction with $NaBH_4$ of 13-L- and 9-D-HPOD, respectively. These compounds were purified by TLC on 0.50 mm precoated plates in the solvent system hexane – diethylether – acetic acid (50:50:1, v/v/v). The other chemicals were of reagent grade. EPR spectra at 9 GHz were recorded on a Varian E-9 spectrometer with a 100 kHz field modulation (modulation amplitude 2 mT). Spectra presented in the same figure are corrected for small differences in enzyme concentrations and in dimensions of the EPR tubes thus allowing a direct comparison of intensities.

Results. Yellow lipoxygenase-1, obtained by oxidation of the native, colourless and EPR-silent enzyme with one molar equivalent 13-L-HPOD, shows a complex EPR spectrum around g 6 (Fig. 1A). This signal is built up by at least three different high-spin Fe(III) species with different symmetry.[7] The most axial and the most rhombic species have g_x equal to 6.2 and 7.5, respectively. The small signal at g 4.3 has been attributed to contaminating high-spin Fe(III).[2] The presence of contaminating manganese and radical signals near g 2 obscures the g_z-parts of the high-spin Fe(III) resonances (insert Fig. 1). Fig. 1B gives the spectrum of yellow lipoxygenase to which 30 molar equivalents of ethanol (corresponding to 8.5 mM) were added. Similar spectra are obtained upon addition of butanol-1 and hexanol-1. Titration curves of yellow lipoxygenase with ethanol, butanol-1 and hexanol-1 are presented in Fig. 2. The changes in the EPR spectra are measured by the ratio (R) of the amplitudes of the rhombic (at g 7.5) and axial (at g 6.0 – 6.2) parts.

In order to mimic the situation with substrate fatty acid and product hydroperoxide, hydroxy-octadecadienoic acids were also used. EPR spectra obtained upon addition of 3 molar equivalents 9-D- or 13-L-HOD to yellow lipoxygenase are shown in Fig. 3. Titration curves are given in the insert. In contrast to the other alcohols a decrease of the total signal intensity is observed upon titration of yellow enzyme with 9-D- or 13-L-HOD.

Discussion. In a study on the nature and relative amounts of the high-spin Fe(III) species that build up the complex g 6 signal of yellow lipoxygenase, a large effect of alcohols on the relative amounts of high-spin Fe(III) species has been reported.[3] The low concentration of alcohols (*e.g.* ethanol) which is required for a shift to an axial type of spectrum as shown in Fig. 1B for ethanol suggests that alcohols have a specific affinity for binding to the enzyme, probably in the environment of iron. The titration curves of various alcohols (Fig. 2) clearly show a more pronounced effect on the EPR spectrum with increasing carbon chain length. Thus, the affinity of the alcohols for binding on the enzyme increases with chain length. This result is in line with observations of Mitsuda *et al.*[8] who have reported that the degree of inhibition of lipoxygenase by saturated monovalent alcohols increases with an increase of the chain length of the alcohols. A binding of the alcohols at a hydrophobic region

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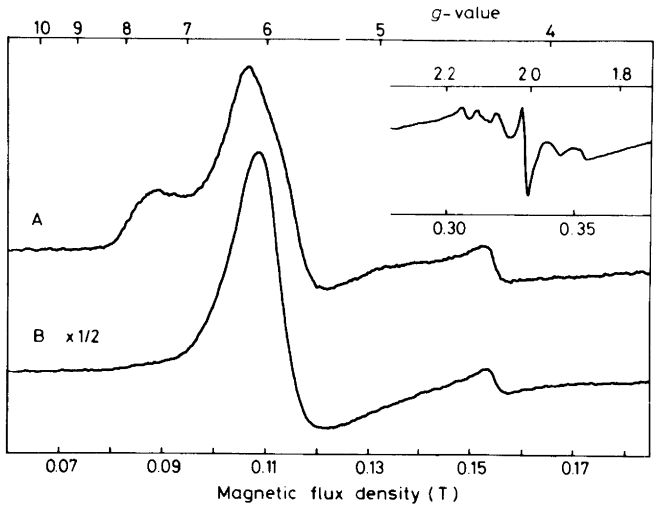

Fig. 1. Effect of ethanol on the line shape of the g 6 signal of yellow lipoxygenase-1. A. A solution of native enzyme (26 g/l) was incubated with a 13-L-HPOD solution (43.3 mM); final concentrations: 0.26 mM for both enzyme and 13-L-HPOD in 0.1 M borate buffer pH 9.0. Insert: High field part of the spectrum. B. 1 μl of an ethanol solution (1.7 M) was added to the sample described for A. Final concentrations: Enzyme 0.26 mM and ethanol 8.5 mM. Microwave frequency 9.256 GHz; microwave power 2 mW; temperature 15 K.

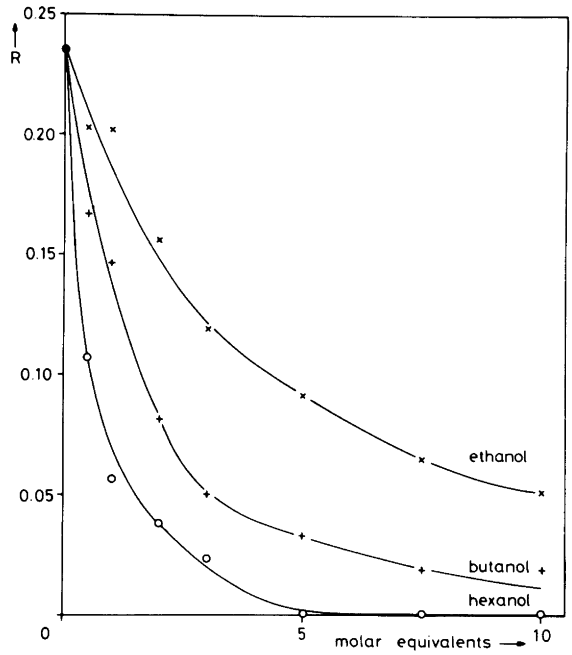

Fig. 2. Titration of yellow lipoxygenase-1 with ethanol, butanol-1 and hexanol-1. On the ordinate the ratio of the rhombic and axial components (R) is given. To yellow enzyme samples prepared as described for Fig. 1A small amounts of alcohol solutions (0.2 % v/v) were added. The amplitude of the rhombic part measured at g 7.5 is corrected for the contribution of the axial part at this g-value.

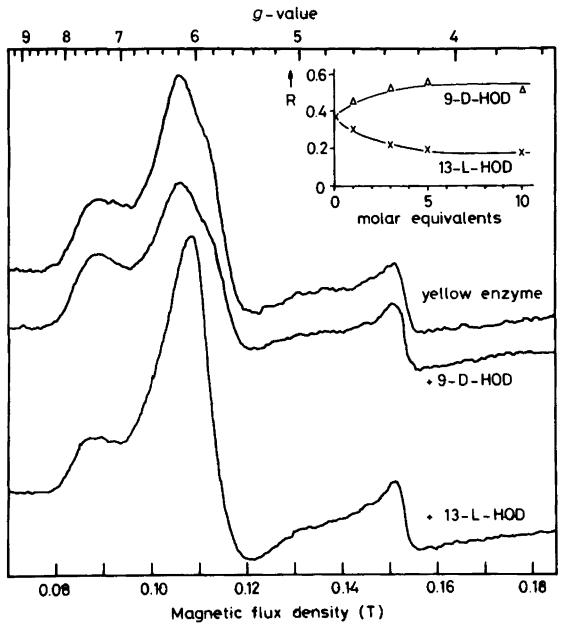

Fig. 3. Effect of hydroxy-octadecadienoic acids on the line shape of the signal around *g* 6 of yellow lipoxygenase-1. A yellow enzyme solution prepared as described for Fig. 1A was incubated with 3 molar equivalents of 9-D-HOD or 13-L-HOD. Final concentrations: Enzyme 0.22 mM and HOD 0.66 mM in 0.1 M borate buffer pH 9.0. Microwave frequency 9.179 GHz; microwave power 2 mW; temperature 15 K. Insert: Titration of yellow lipoxygenase-1 with 9-D- HOD and 13-L-HOD. On the ordinate the ratios of the amplitudes of the rhombic and axial components (R) are given.

which also serves as a binding site for the substrate has been suggested.[8] The effects of hydroxy-octadecadienoic acids, which have a hydrophobic part similar to the product hydroperoxy-octadecadienoic acid, have been investigated. The results are also relevant to the binding of the substrate octadecadienoic acid because the affinity constants of substrate and product are of the same order of magnitude.[9,10] For the two hydroxy-octadecadienoic acids different effects on the line shape are observed (Fig. 3). 13-L-HOD gives a shift to an axial type of spectrum whereas 9-D-HOD induces a rhombic shift. Unlike 13-keto-octadecadienoic acid [3] 9-D-HOD does not cause the appearance of a new rhombic species. This experiment makes clear that besides the chain length other structural elements (*i.e.* positions and configurations of the double bonds and the presence of substituents) are important for binding. Although different effects of 9-D-HOD and 13-L-HOD on the EPR spectrum of yellow lipoxygenase are observed, the titration curves of both compounds (insert Fig. 3) indicate that the changes are almost complete after addition of approx. 5 molar equivalents. This amount is comparable to that for hexanol-1 which has also a saturated chain of five carbon atoms and has the highest affinity for binding to lipoxygenase (Fig. 2). This is consistent with studies on the substrate specificity,[11] showing that the best substrates are polyunsaturated fatty acids with the pentadiene system at the n-6 position, *i.e.* a saturated chain of five carbon atoms.

This EPR study on the binding of monovalent alcohols and hydroxy-octadecadienoic acids to lipoxygenase-1 supports the concept that the hydrophobic part of the substrate is very important for binding to the enzyme.

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Tobacco Chemistry. 57.* Two New Labdanic Compounds from Tobacco

INGER WAHLBERG, CARMEN VOGT, INGRID WALLIN, TOSHIAKI NISHIDA and CURT R. ENZELL

Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62 Stockholm, Sweden

More than forty compounds belonging to the labdane/nor-labdane group have so far been encountered in tobacco.[2] However, as a result of genetic control, their presence is restricted to certain varieties such as Oriental and PB (Bergerac) tobaccos.[3,4] We now report the isolation of two new labdanic compounds (*1*,*2*) from a wax extract of green leaves of Greek tobacco.

Results. The first compound (*1*, $C_{19}H_{32}O_2$) contains an α,β-unsaturated aldehyde group (IR bands at 2710, 1685 and 1640 cm^{-1}), whose allocation to partial structure A (Fig. 1) was determined by proton spin decoupling and spin simulation experiments. Since the remaining oxygen was accommodated by a tertiary hydroxyl group (IR band at 3600 cm^{-1}; ^{13}C NMR singlet at δ 73.6, *cf.* Table 1) and since the ^{13}C NMR spectrum was devoid of signals due to additional sp^2 carbon atoms, it followed that aldehyde *1* is carbobicyclic.

A clue to its structure was provided by the ^{13}C NMR spectrum. Thus, since fourteen of the signals were of appropriate multiplicities and had chemical shift values close to those found for the C-1 to C-10 and C-17 to C-20 signals for (12*Z*)-abienol (*3*), aldehyde *1* was tentatively identified as 15-nor-8-hydroxy-12*E*-labden-14-al.

This assignment was verified by a direct comparison with an authentic sample, which was prepared by oxidative degradation of (12*E*)-abienol (*4*) using osmium tetroxide and sodium periodate.

The IR and ^{13}C NMR spectra demonstrated that the second compound (*2*, $C_{20}H_{34}O_2$) is a diol having a secondary and a tertiary hydroxyl group. It contains a conjugated diene system, which is arranged as shown in partial structure B (Fig. 1),[5] the *Z* geometry also following from a comparison of relevant ^{13}C NMR data with those of the (12*Z*)- and (12*E*)-abienols (*3*,*4*). Since the ^1H NMR spectrum also revealed the presence of four methyl groups attached to fully substituted sp^3 carbon atoms, it

Table 1. Carbon-13 chemical shifts and assignments for compounds *1* – *4*.[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	40.2	18.4	41.8	33.2	56.1	20.5	44.6	73.6	61.9	39.0	25.2	159.5	136.9	195.5		9.3	24.0	33.4	21.5	15.5
2	39.9	18.5	41.7	33.2	53.6	27.8	80.2	78.2	60.3	39.3	22.6	133.7	130.8	133.7	113.7	19.9	17.9	33.5	21.6	15.6
3	40.2	18.6	41.9	33.3	56.2	20.3	44.0	74.3	62.2	39.0	23.2	133.9	130.8	133.7	113.7	19.9	24.4	33.5	21.6	15.5
4	40.1	18.6	41.9	33.2	56.1	20.4	44.1	73.7	62.2	38.9	24.0	136.1	132.1	141.7	110.0	11.8	24.1	33.5	21.6	15.4

[a] δ-Values in $CDCl_3$ relative to TMS.

* For part 56 see Ref. 1.

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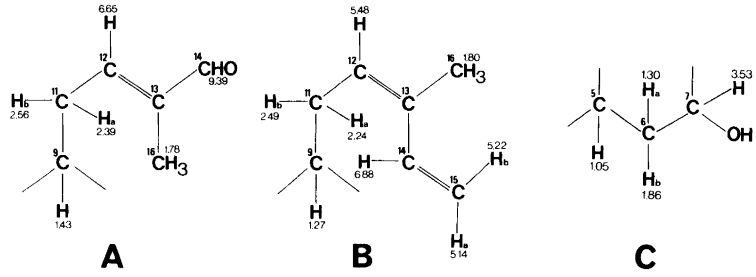


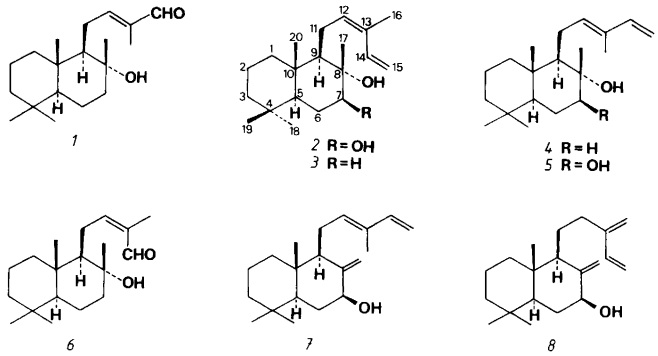
Fig. 1. $^1H-^1H$ coupling constants in Hz.
A. $J_{9,11a}=5.0$; $J_{9,11b}=5.5$; $J_{11a,11b}=-16$; $J_{11a,12}=6.5$; $J_{11b,12}=6.5$.
B. $J_{9,11a}=4.1$; $J_{9,11b}=5.7$; $J_{11a,11b}=-15.6$; $J_{11a,12}=6.4$; $J_{11a,16}=1.2$ $J_{11b,12}=7.9$; $J_{11b,16}=1.2$; $J_{12,14}=0.9$; $J_{12,15a}=1.5$; $J_{12,15b}=0.9$ $J_{12,16}=1.2$; $J_{14,15a}=10.4$; $J_{14,15b}=17.4$; $J_{15a,15b}=1.5$.
C. $J_{5,6a}=12.1$; $J_{5,6b}=1.4$; $J_{6a,6b}=-12.6$; $J_{6a,7}=11.7$; $J_{6b,7}=4.7$.

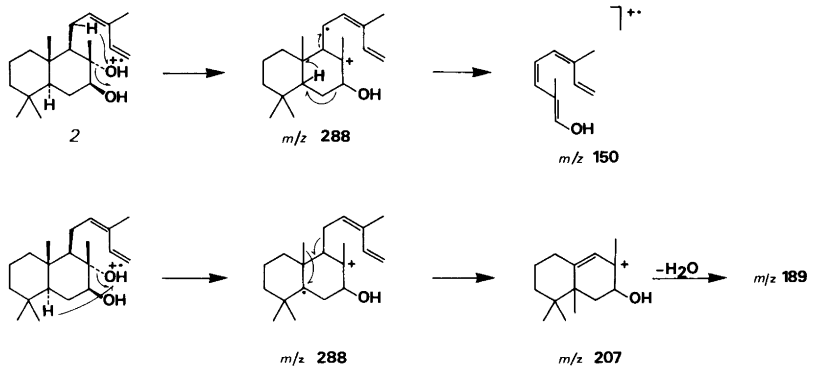
seemed most plausible that diol *2* is a diterpenoid of the labdane type.

This view was reinforced by the ^{13}C NMR data. Thus, eight signals in the spectrum of diol *2*, which are not associated with the side-chain, were virtually superimposable with those due to C-1 to C-4, C-10 and C-18 to C-20 for (12*Z*)-abienol (*3*), thereby showing that ring A is non-oxygenated and that the two hydroxyl groups are present in ring B. Their allocation to C-7 and C-8 followed from the observation that the C-6 to C-8 signals for diol *2* are downfield and the C-5, C-9 and C-17 signals are upfield from the corresponding signals for (12*Z*)-abienol (*3*), a result expected if a hydroxyl substituent is introduced at C-7 in (12*Z*)-abienol (*3*).[6]

In agreement with this assignment the mass spectrum of diol *2* contained diagnostically useful ions at *m/z* 207 and 150, which are analogous to the ions at *m/z* 191 and 134 in the spectrum of (12*Z*)-abienol (*3*)[7] and which are likely to be generated as shown in Scheme 1. Also the ^1H NMR spectrum displayed H-7 as a doublets of doublets at δ 3.53 (*cf.* partial structure C). Since the coupling constants of H-7 ($J=4.7$ and 11.7 Hz) are only consistent with a β-orientation of the hydroxyl group at C-7 and the shielding of C-17 with an *S*-configuration at C-8,[8] the new diol could be formulated as (7*S*,12*Z*)-12,14-labdadiene-7,8-diol (or enantiomer). The corresponding 12*E*-isomer, nidorellol (*5*) has previously been found in a *Nidorella* species.[9]

It may well be that 15-nor-8-hydroxy-12*E*-labden-14-al (*1*), the first C_{19} labdanic compound encountered in tobacco, is formed by oxidative biodegradation of (12*E*)-abienol (*4*).[10,11] However, (12*Z*)-abienol (*3*), which in contrast to its 12*E*-isomer (*4*)[4] is an abundant tobacco constituent and which can be converted to the majority of the tobacco labdanoids by oxidation of its side-chain,[12,13] is a more plausible precursor. This view is supported by the fact that treatment of (12*Z*)-abienol (*3*) with osmium tetroxide/sodium periodate yielded aldehyde *1*, the initially generated 15-nor-8-hydroxy-12*Z*-labden-14-al (*6*) evidently having undergone a facile isomerization.[14]





Scheme 1.

(7*S*,12*Z*)-12,14-Labdadiene-7,8-diol (*2*) is the first tobacco labdanoid encountered, which bears a substituent at C-7. Its generation may be explained by oxidation, *e.g.* microbial hydroxylation, of 12*Z*-abienol (*3*). It is of interest to note that (7*S*,12*E*)-8(17),12,14-labdatrien-7-ol (*7*) and (7*S*),8(17),-13(16),14-labdatrien-7-ol (*8*) have most recently been isolated from *Nicotiana raimondii*.[15]

Experimental. With the exception of accurate mass measurements, which were carried out on a Kratos' MS50-Stereo DS 50 SM/DS 50 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. 16 were used.

Isolation. An extract (24 g) obtained by immersing green leaves of Greek *Nicotiana tabacum* (Basma Drama) in chloroform was distributed between hexane and methanol – water (80:20). The polar material obtained (16 g) was chromatographed over silica gel using a gradient of hexane – ethyl acetate as eluent to give fractions 1 (1 g), 2 (8 g) and 3 (6 g). Fraction 1 was a complex mixture, which was separated further by chromatography over silica gel and HPLC using columns packed with u-Porasil and u-Bondapak/CN to give 3.6 mg of 15-nor-8-hydroxy-12*E*-labden-14-al (*1*) and 10 mg of (7*S*,12*Z*)-12,14-labdadiene-7,8-diol (*2*).

15-Nor-8-hydroxy-12*E*-labden-14-al (*1*) was an oil and had $[\alpha]_D$ +13° (*c* 0.30, $CHCl_3$) (Found: $M^{\cdot+}$ 292.2388. Calc. for $C_{19}H_{32}O_2$: mol. wt. 292.2402); IR (CCl_4) bands at 3600, 3440, 2710, 1685 and 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.81 (s)/0.87 (s)/0.88 (s) (H-18/H-19/H-20) and 1.20 (s, H-17), for other data see Fig. 1. MS [*m/z* (%, composition)]: 292 (M, 3), 277 (18, $C_{18}H_{29}O_2$), 274 (17, $C_{19}H_{30}O$), 259 (8, $C_{18}H_{27}O$), 245 (4, $C_{17}H_{25}O$ and $C_{18}H_{29}$), 227 (3, $C_{17}H_{23}$), 216 (10, $C_{16}H_{24}$), 206 (21, $C_{15}H_{26}$), 191 (60, $C_{14}H_{23}$ and $C_{13}H_{19}O$), 177 (23, $C_{13}H_{21}$ and $C_{12}H_{17}O$), 163 (17, $C_{12}H_{19}$ and $C_{11}H_{15}O$), 150 (20, $C_{11}H_{18}$ and $C_{10}H_{14}O$), 137 (62, $C_{10}H_{17}$ and $C_9H_{13}O$), 123 (43, C_9H_{15} and $C_8H_{11}O$), 109 (77, C_8H_{13} and C_7H_9O), 95 (72, C_7H_{11} and C_6H_7O), 81 (63, C_6H_9 and C_5H_5O), 69 (84, C_5H_9 and C_4H_5O), 55 (72, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7).

(7*S*,12*Z*)-12,14-Labdadiene-7,8-diol (or enantiomer) (*2*) was an oil, which decomposed on standing. It had $[\alpha]_D$ +6.7° (*c* 0.30, $CHCl_3$) (Found: M – 18 $^{\cdot+}$ 288.2458. Calc. for $C_{20}H_{32}O$: 288.2453); IR ($CHCl_3$) bands at 3590 and 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.81 (s)/0.85 (s)/0.89 (s) (H-18/H-19/H-20) and 1.17 (s, H-17); MS [*m/z* (%, composition)]: 306 (M, 1), 288 (18), 273 (4, $C_{19}H_{29}O$), 270 (13, $C_{20}H_{30}$), 251 (12, $C_{16}H_{27}O_2$), 207 (21, $C_{14}H_{23}O$), 189 (18, $C_{14}H_{21}$), 177 (47, $C_{13}H_{21}$), 164 (22, $C_{11}H_{16}O$), 150 (63, $C_{10}H_{14}O$), 137 (33, $C_9H_{13}O$ and $C_{10}H_{17}$), 123 (77, C_9H_{15} and $C_8H_{11}O$), 109 (51, C_8H_{13} and C_7H_9O), 95 (54, C_7H_{11} and C_6H_7O), 81 (78, C_6H_9), 69 (78, C_5H_9 and C_4H_5O), 55 (54, C_4H_7 and C_3H_3O) and 43 (100).

Conversion of the (12E)- and (12Z)-abienols (4, 3) to 15-nor-8-hydroxy-12E-labden-14-al (1). A solution of 50 mg of (12*E*)-abienol (*4*) in 20 ml of dioxane – water (3:1) was stirred with 20 mg of osmium tetroxide at 0 °C for 5 min. After addition of 300 mg of sodium periodate the mixture was stirred at 0 °C for 1 h. Dilution with water, extraction with ether and chromatography over silica gel using a hexane – ethyl acetate gradient afforded 7.7 mg of 15-nor-8-hydroxy-12*E*-labden-14-al, which proved to be identical in all respects to the naturally occurring aldehyde (*1*).

Aldehyde *1* was also obtained by treatment of (12*Z*)-abienol (*3*) with osmium tetroxide/sodium periodate using the same conditions as those described above.

Acknowledgement. We are grateful to Dr. David Jones and Mr. Leif Abrahamsson for recording the mass spectra.

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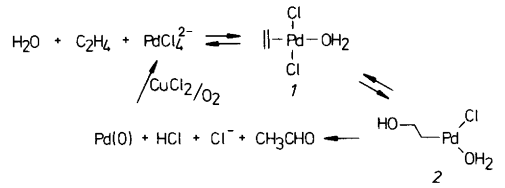
Nucleophilic Addition to π-Olefin-, π-Allyl- and σ-Alkyl-palladium Complexes. Examples of "Umpolung" by the Use of Organometallic Reagents

BJÖRN ÅKERMARK, JAN-E. BÄCKVALL and KRISTER ZETTERBERG

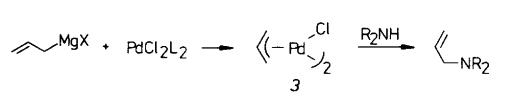
Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden

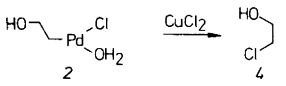
The activation of organic substrates by transition metals has become very important in organic chemistry. Today, a great number of highly selective transition metal-promoted reactions are known. An important feature of the activation is that the reactivity of the organic molecule may be completely altered. For example, simple olefins such as ethene, which are fairly reactive towards electrophiles, may become electron acceptors on coordination to a metal and react with nucleophiles. A well known industrial application is the Wacker process, where ethene bound to palladium(II) is oxidized to acetaldehyde *via* nucleophilic addition of water [1] (Scheme 1).



Scheme 1.

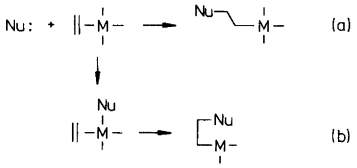
Even groups with a formal negative charge, such as π-allyl systems and carbanions may be converted into electron acceptors: These types of "umpolung" reactions may be illustrated by the formation of allylamine from allylmagnesium chloride *via* the π-allylpalladium complex *3*.[2] Also the transformation of the σ-complex *2*, formally a carbanion attached to palladium, into the chlorohydrin *4* nicely demonstrates the principle.[3]





Our studies on nucleophilic addition to olefins were inspired by the Wacker process and our purpose was to develop an analogous process for amination of olefins.

In principle, the addition of nucleophiles to metal bound π-systems may take two different routes. A free nucleophile may add directly to the π-system (path (a), Scheme 2) or it may coordinate first to the metal and then be transferred internally to the π-system (path (b), Scheme 2). The stereochemical outcome of the two processes will be different in that path (a) leads to *trans* addition of the metal and the nucleophile across the π-system while path (b) results in *cis* addition.



Scheme 2.

The two processes (a) and (b) will probably also show electronic differences. The process (a) would be expected to be strongly dependent on the charge

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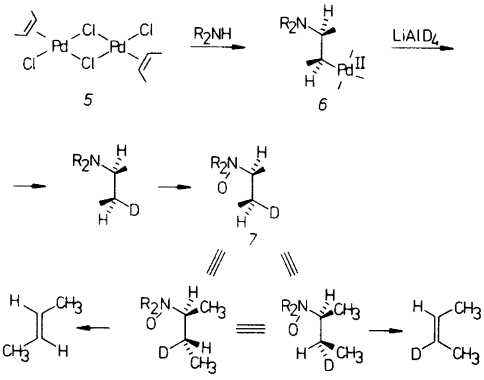
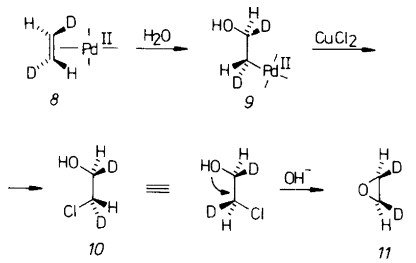
on the olefin and be accelerated by electron withdrawal from the olefin. In contrast, the reaction (b) would in general be expected to be a concerted cyclic process, which does not necessarily require any induction of charge on the olefin.

A study of the stereochemistry of palladium promoted amination revealed that this reaction proceeds *trans* according to path (a).[4] Due to the lability of the intermediate σ-complex *6*, this could not be proven directly by NMR as done for the corresponding mercury compound.[5] Instead, an indirect method was used, (Scheme 3). The *Z*-2-



Scheme 3.

butene palladium complex *5* was reacted with dimethylamine at -70 °C to give the σ-complex *6*. Addition of lithium tetradeuterioaluminate led to the displacement of palladium by deuterium with retention of configuration.[6] The alkylated amine thus formed was oxidized to the *N*-oxide 7, which on moderate heating undergoes *cis*-Cope elimination[7] of hydroxylamine. Elimination of a hydrogen atom gives deuteriated *Z*-2-butene while elimination of a deuterium atom gives non-deuteriated *E*-2-butene. The stereospecificity of the reaction could then be determined by measuring the deuterium content in the individual isomer by mass spectrometry. The same sequence applied to *E*-2-butene gave, as expected, non-deuteriated *Z*-butene and deuteriated *E*-2-butene.[4] The total stereospecificity is $>98\%$ if correction is made for isotope effects and a small amount of deuterium – hydrogen scrambling, which occurs in the reduction step. It thus seems safe to conclude that amination is a completely stereospecific *trans* process according to path (a), Scheme 2.



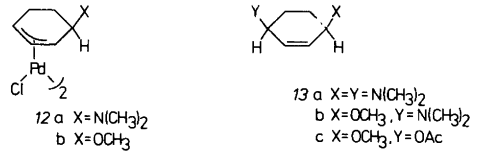
Scheme 4.

Also the Wacker reaction appears to be a *trans* process despite earlier claims to the contrary.[8] This may be shown by examining the stereochemistry of the chlorohydrin *10*, obtained from specifically deuteriated *Z*- or *E*-dideuterioethene. Chlorohydrins are frequently formed as byproducts in the Wacker reactions, and become the major product when the relative concentrations of the oxidant, copper chloride, and chloride ions are increased (Scheme 4). Reaction of *E*-1,2-dideuterioethene gives the chlorohydrin *10*, the stereochemistry of which may be accurately determined as *threo* by microwave spectroscopy. The accuracy is even better if *10* is first converted to the epoxide *11* (Scheme 4). Independent experiments show that the cleavage of palladium – carbon bonds by cupric chloride – chloride ions occurs with inversion at carbon.[10] The primary step, the addition of water, must thus be *trans* to yield the intermediate σ-complex *9*.[3] Again, the stereospecificity is essentially complete.

trans-Hydroxypalladation is also inferred from independent experiments which involve cleavage of the palladium – carbon bond of *9* by carbon monoxide.[11]

It thus appears that amines and water add preferentially as external nucleophiles according to path (a), Scheme 2. This is also true for acetate[8c,12] alcohols[13,14] and stabilized carbanions like β-diketonates.[15] In contrast, non-stabilized carbanions like methyl[16] and phenyl carbanion[17] preferentially add *cis* after coordinating to palladium (path (b)). These results appear to be of general validity and similar conclusions may be drawn from experiments with olefin complexes of platinum[18] and iron.[19]

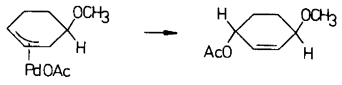
Nucleophilic attack on π-allylpalladium complexes seems to follow the same pattern. Thus the addition of dimethylamine to the π-allyl complexes



12 a X=N(CH3)2
b X=OCH3

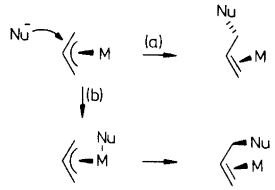
13 a X=Y=N(CH3)2
b X=OCH3, Y=N(CH3)2
c X=OCH3, Y=OAc

12 yields the *cis*-compounds *13*; that is, the nucleophile is added from the face of the π-allyl system opposite to palladium.[20,21] Stabilized carbanions like dialkyl malonates[22] and ketone enolates[23,24] react similarly. Acetate shows an interesting behaviour in that it may be added from either the same or the opposite face, depending on the reaction conditions. Thus in the presence of chloride, the palladium complex *12b* gives exclusively *13c* by *trans* attack where as in the absence of chloride *cis* attack occurs[25] to yield *15*.



14 *15*

Generally however, it appears that the addition reactions follow the trend observed for π-olefin systems, that is hetero atom nucleophiles and stabilized carbanions add *trans* according to path (a) (Scheme 5) while hydride[26] and carbanions add *cis*[27] according to path (b), (Scheme 5). Again, the

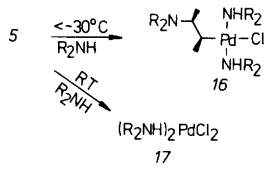


Scheme 5.

results appear to apply to complexes of other metals but palladium, *e.g.* molybdenum.[28]

The early work on amination of π-olefin-[29] and π-allylpalladium[2] systems clearly indicated the importance of cationic intermediates. For the amination of π-olefinpalladium complexes, the importance of the relative charge on the olefin and the metal is also indicated by the keen competition between amination and displacement of the olefin. At temperatures above $-30\,^{\circ}C$ displacement becomes the major reaction (Scheme 6) but it may be noticed also at lower temperatures.

To explore how the formal metal oxidation state and the formal charge on an olefin complex might



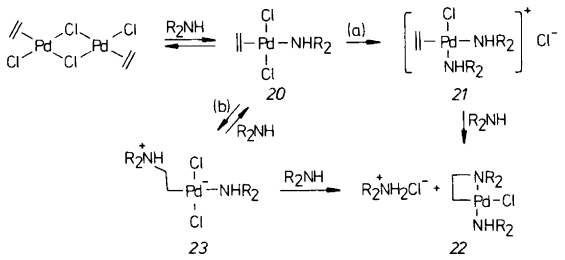
Scheme 6.

effect the charge of the olefin, *ab initio* calculations were performed on a number of model nickel-olefin complexes.[30] As might be expected, the calculations show the metal to carry the major positive charge in the complexes. Thus, from purely electrostatic considerations, the metal would be expected to be the major target for nucleophiles. However, the calculations also indicate that the formal charge of the complexes will have an important effect on the relative metal–olefin charge. In the neutral complex *18* the olefin is essentially neutral and the

18 *19*

metal charge +0.9. In the positively charged complex *19* the nickel charge is increased slightly to +1.1 while the olefin charge is strongly increased to +0.4. The nucleophilic attack on the olefin would thus be expected to be much more favoured in the charged complex *19*.

The fact that three equivalents of amine per palladium are required to complete the amination reaction[29] suggests two reasonable pathways (Scheme 7). In both, the first equivalent serves to generate a mono nuclear complex *20*. In path (a) the second equivalent generates a charged species *21*, which then – in the true amination step – reacts with the third equivalent of amine to yield a σ-complex *22*. Alternatively, path (b), a σ-complex *23* could form directly from *20* by nucleophilic attack



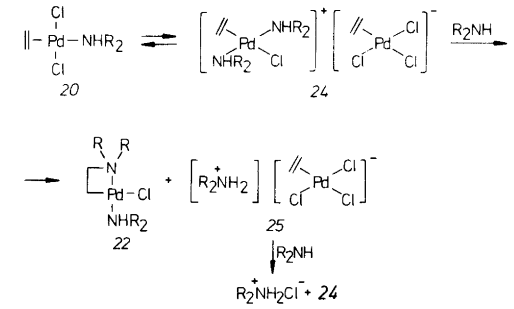
Scheme 7.

by the second equivalent of amine. The function of the third equivalent would then simply be to drive the equilibrium by deprotonating the complex *23*.

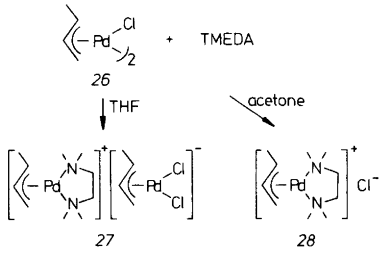
In path (a) the ion pair *21* is formed and should be detectable by conductivity measurements. In contrast, in path (b) the only ionic species formed at any concentration is amine hydrochloride, which is essentially insoluble under the conditions used for the experiments (dimethylamine, THF solution).

The addition of dimethylamine to 1-decene and 1-butene palladium complexes did give rise to a moderate conductivity, but, surprisingly, a maximum conductivity was obtained at an amine – palladium ratio of 1:1 instead of 2:1 as would result from the formation of the complex *21* in path (a).[31] The most reasonable explanation is that an alternative ion pair *24* is formed, perhaps in rapid equilibrium with the mono-nuclear complex *20* as indicated by NMR. NMR also reveals that nucleophilic attack on the cationic part of the complex yields the σ-complex *22*. Compatible with all our data (conductometric titrations, quench studies, ^{1}H- and ^{13}C NMR studies) is the concomittant formation of a fairly insoluble ion pair *25*. This is converted back to *24* in the presence of free amine (Scheme 8).[31]

Indirect support for the mechanism in Scheme 8 is obtained from studies on π-allyl systems. When π-crotylpalladium chloride is treated with *N,N,N',N'*-tetramethylethylenediamine (TMEDA) in THF solution, the conductivity reaches its maximum at an amine – palladium ratio of 1:2 (Fig. 1). This corresponds to the formation of the ion pair *27*, which may be well characterized by NMR at temperatures below -40 °C. For the related ion pair from π-allylpalladium chloride, the definite structure has been established by X-ray crystallography.[32]



Scheme 8.



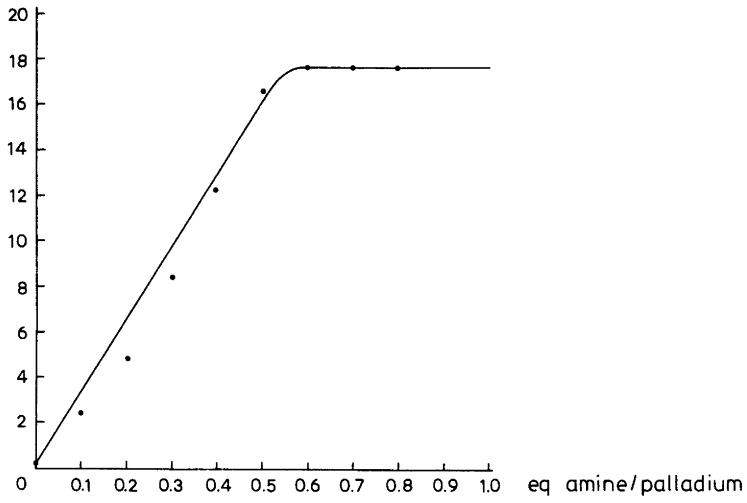
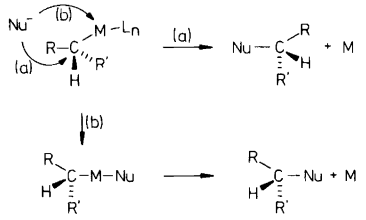


Fig. 1. π-Crotylpalladium chloride and *N*-tetramethylethylenediamine in THF solution.

It is interesting to note that at +40 °C, the exchange of the π-allyl group between the negative and positive halves of *27* becomes rapid relative the NMR time scale. The exact mechanism for this exchange, which appears important also for the olefin complexes[31] is not clear, but an intramolecular exchange of the π-allyl group between the two halves of the ion pair is the most attractive[33] possibility.

The formation of the ion pair serves to illustrate the importance of the solvent in reactions of π-allylpalladium complexes and similar systems. For instance, in acetone, π-crotylpalladium chloride and TMEDA give the simple ion pair *28* instead of *27*.

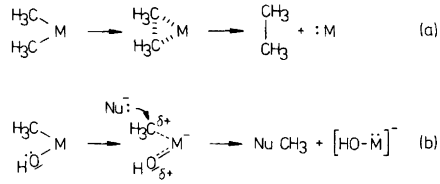
The importance of charge is evident also for the reactions between σ-alkylmetals and nucleophiles. Both nucleophilic substitution[3,10,34] (path (a), Scheme 9) and reductive elimination[35] (path (b), Scheme 9) are promoted by added electron acceptors. Furthermore, stereochemical studies,[3,4,6,25,34,36] which make it possible to distinguish between the two pathways, indicate a similarity between π- and σ-metal systems in their reactions with nucleophiles. Also with σ-alkyl systems external attack is preferred by hetero atom nucleophiles[3,4,34] (path (a)), while carbanions and hydride generally coordinate and then react by reductive elimination[4,6,36] (path (b), Scheme 9).



Scheme 9.

It is interesting to note that the nucleophiles that react according to path (a) may be classified[37] as hard bases and those reacting according to path (b) as soft bases.

In addition to charge, orbital interactions are clearly important in the reactions of π-olefin,[38] π-allyl[39] as well as σ-alkyl[40] metal complexes. Other important factors are the relative oxidation potentials of the reacting ligands in combination with the ability of heteroatom ligands to stabilize charge by using their lone pair electrons.[40e] This idea may be illustrated for alkylmetals in the following way: Reductive elimination may be regarded as a concerted, gradual oxidation of two coordinated methyl carbanions to two methyl radicals which couple to ethane (Scheme 10, path (a)). In contrast, the decomposition of methylmetal hydroxide may take a different route (path (b), Scheme 10) because the methyl carbanion will be more readily oxidized than the hydroxyl group.[40e] Interestingly, it should also quite readily acquire carbonium ion character on account of its higher polarizability.[37] The oxygen will presumably also interact with an electron demanding metal atom, but probably by forming a metal – oxygen double bond rather than by loss of electron density from the metal – oxygen σ-bond. It is conceivable that migration of this strongly coordinated hydroxyl group to the coordinated positive methyl group is less favoured than attack by external nucleophile, as observed experimentally.[3,10,34]

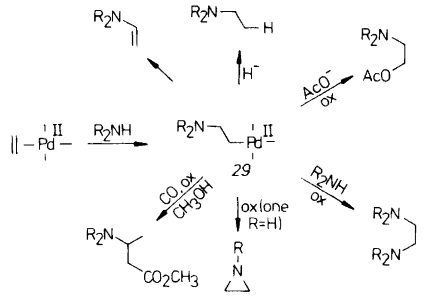


Scheme 10.

This is also in accord with the principle of hard and soft acid and bases (HSAB) in that the more polarizable soft ligand *e.g.* alkyl, aryl and hydride will more readily undergo reductive elimination and also in an unsymmetrical case acquire positive charge.

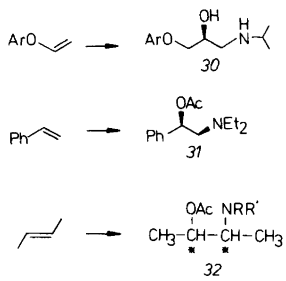
It is not obvious how these ideas apply to π-olefin and π-allylmetals, but it should be born in mind that a π-olefin metal complex may also be regarded as a 3-membered metallacycle.

Synthetic applications. Although a major part of our work has been devoted to mechanistic studies, we have also been concerned with synthetic transformations *via* nucleophilic additions to π-olefin and π-allylpalladium complexes. The σ-complex *29*, obtained from aminopalladation of an olefin, may be transformed into a number of functionalized derivatives (Scheme 11). Reduction with hydrogen or hydride gives amine,[29] oxidation with lead tetraacetate gives acetoxyamines,[34b,41] while the use of other oxidants such as bromine or *m*-chloroperbenzoic acid produces diamines[42] and aziridines.[43] The reactions are highly stereospecific

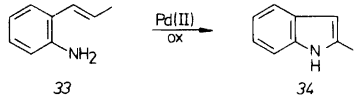


Scheme 11.

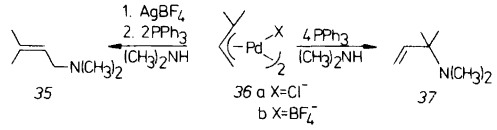
and permit the *cis*-addition of two functional groups to a double bond. The oxyamination reaction has been used to prepare a number of aminoalcohols of pharmaceutical interest, *e.g. 30* and *31*.[41,44] By the use of optically active chiral ligands or nucleophiles an asymmetric induction was obtained in the oxyamination reaction *e.g. trans*-2-butene→*32*.[45] Also the use of a chiral ligand in the analogous palladium-promoted alkylation of 1-hexene resulted in an enantiomeric excess of up to 32% in the alkylated product.[46]



Reaction of the compound *29*, finally, with carbon monoxide and an oxidant gave derivatives of β-aminoacids.[47] In analogy to the Wacker process, catalytic amination of olefins to yield enamines should be possible. Unfortunately, the expected enamines are not stable under the reaction conditions, but readily oxidized by palladium(II).[48] Only in special cases, therefore, has catalytic amination of double bonds been achieved *e.g.* *33*→*34*.[49]



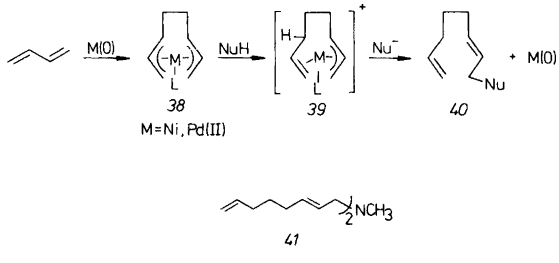
Also the work on nucleophilic additions to π-allyl systems has produced some results that may be of use in synthesis. Amines are readily added to π-allylpalladium complexes.[2] The reaction is stereospecific[20] and the regiochemistry may be controlled by the added ligands.[33] For instance, treatment of the π-allylpalladium chloride complex *36* with dimethylamine in the presence of more than three equivalents of triphenylphosphine produces selectively the allylamine *37*. In contrast, the reaction of the charged complex *36b* (BF_4^- is essentially non-coordinating) with dimethylamine in the presence of 1 to 2 equivalents of phosphine produces exclusively the isomeric allylamine *35* (Scheme 12).



Scheme 12.

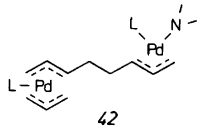
The amination of the π-allyl complexes is stoichiometric but a catalytic reaction, proceeding *via* π-allyl complexes, is possible if palladium(0) is used as catalyst and allylic acetates as substrates.[50] Allyl acetates are versatile synthetic intermediates and the palladium-catalyzed displacement of the acetoxy group by stabilized carbanions such as sodium diethyl malonate has been extensively used in organic synthesis during recent years.[51]

Catalytic telomerizations of 1,3-dienes *via* π-allyl metal complexes is also possible using palladium(0) and nickel(0) catalysts. These transformations proceed *via* bis-π-allyl-metal complexes *38* (Scheme 13). A number of different nucleophiles may be utilized *e.g.* acetate, dialkylmalonates and amines.[51b,52] The mechanism

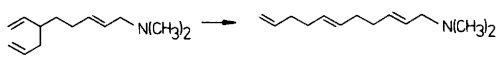


Scheme 13.

of the reaction is not completely understood but in the amination reactions using nickel catalysis, the formation of a charged intermediate *39* is indicated.[53] By the use of this reactions, selective nickel catalyzed formation of bisoctadienyl methylamine *41* is possible.[53,54] If the palladium(0) catalyzed reaction of butadiene is performed in the presence of *e.g.* allyldimethylamine, the latter will participate in the reaction. Thus oxidative addition of the allylic amine to palladium(0), followed by an exchange reaction of the π-allyl complex formed with *38*, would give complex *42*. Selective carbon $-$carbon and carbon$-$nitrogen bond formation would explain the formation of the branched amine *43a*, which may be thermally rearranged to the linear amine *43b*.[54]

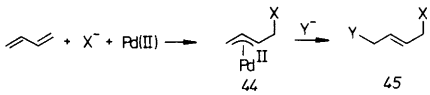


42



43a *43b*

If palladium(II) is used as catalyst or promotor 1,3-dienes may also be difunctionalized. A number of different groups x and y may be added *e.g.* alkoxide,[21] acetate,[25,55] amine,[20,21] dialkyl malonate,[56] enolates[23] and chloride[58] (Scheme 14).

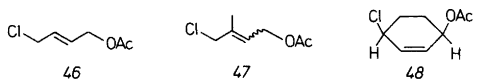


44 *45*

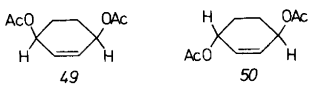
X,Y = Cl, CH_3O, AcO, R_2N, $(RO_2C)_2CH$, $RCOCH_2$, RO_2CCH_2

Scheme 14.

In analogy with difunctionalization of simple olefins, the two groups are generally stereospecifically introduced *cis* in cyclic dienes (for an exception giving *trans*, see below). The *cis*-stereochemistry is a result of two *trans*-additions. Most of these reactions are stoichiometric but recently stereoselective catalytic processes for 1,4-acetoxychlorination[57] and 1,4-diacetoxylation[25] of 1,3-dienes have been developed. For instance, butadiene, isoprene and 1,3-cyclohexadiene were converted to *46*, *47* and 48, respectively, in $\geq 70\%$ yield.[57] The 1,4-diacetoxylation reaction showed a remarkable dual stereoselectivity in that *cis*-acetate (*e.g. 49*), was obtained as expected when chloride ions were present, but



46 *47* *48*



49 *50*

in their absence, the *trans*-diacetate (*e.g. 50*) could be selectively obtained[25] (*cf.* the formation of *13c* and *15*).

The ability of transition metals to selectively activate organic substrates and allow specific transformations has had a strong influence on organic chemistry during the last decade. Today a great number of regio- and stereoselective reactions are known that are promoted or catalyzed by transition metals.

In this brief review we have tried to present some ideas and results on mechanistic pathways in the reactions of π-olefin-, π-allyl- and σ-alkylpalladium complexes. We have also given a few examples of how these reactions may be applied to organic transformations. It is our hope that the results presented demonstrate the usefulness of a mechanistic approach in the development of organic reactions catalyzed by transition metals.

Acknowledgement. This paper is submitted in honour of our teacher and friend Professor Holger Erdtman on the occasion of his 80th birthday in appreciation of his contributions to organic chemistry.

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Chemistry of *gem*-Dihalocyclopropanes. XVII. Cyclopropylidene Insertion. Formation and Ring Opening of Bicyclo[1.1.0]butan-2-olate

NILS O. NILSEN, LARS SKATTEBØL and LEIV K. SYDNES

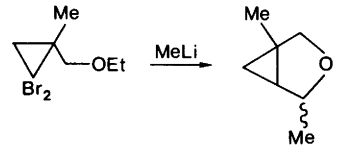
Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, Oslo 3, Norway

Reactions of *gem*-dibromocyclopropanemethanol derivatives *3a–3e* with methyllithium lead to allenic alcohols *4a–4h* and products which derive from ring opening of an intermediate bicyclo[1.1.0]-butan-2-olate (*17*). The latter is formed by insertion of the respective cyclopropylidene into a C–H bond. Evidence for *17* was obtained from deuterium labelling experiments. The ring opening generally occurs by a carbanion mechanism, but in the case of *3d* the intermediate bicyclobutanolate rearranged to the acyclic aldehyde *10*, apparently by a thermal mechanism. The secondary alcohols *3f–3h* reacted with methyllithium to give the corresponding allenes exclusively, which was also the case with ethers *3i–3l* derived from the primary alcohols.

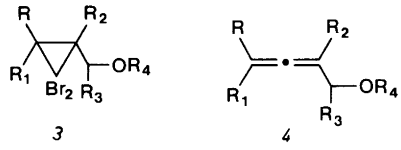
Insertion into σ-bonds and particularly C–H bonds is probably the most characteristic reaction of carbenes.[1] The carbene-like intermediate formed in reactions of *gem*-dihalocyclopropanes and alkyllithium has been shown to undergo both inter- and intramolecular insertion reactions into C–H bonds. In monocyclic systems intramolecular insertion occurs preferentially at carbon atoms three and five relative to the carbenyl carbon, leading to bicyclo[1.1.0]butanes and bicyclo[3.1.0]hexanes, respectively.

Baird[2] has shown that ethers like *1* undergo intramolecular insertion at a C–H bond adjacent to oxygen with formation of the bicyclic ether *2*; bicyclobutanes were not formed in any of these reactions. On the assumption that insertion into C–H bonds adjacent to oxygen is preferred, ethers and alcohols with such bonds available only at position three should yield bicyclobutanes. In a preliminary report[3] we have shown that *gem*-dibromocyclopropanemethanol derivatives react with methyllithium to form the respective bicyclo-[1.1.0]butanolate as intermediate. Such reactions have been previously reported to give only allenyl alcohols.[4] On the other hand, the corresponding *t*-butyl and trimethylsilyl ethers gave allenes as sole products. A full account of this study is reported here.

The *gem*-dibromocyclopropyl alcohols *3a–3h*



1 *2*



3 *4*

a: R=R$_1$=R$_2$=R$_3$=R$_4$=H
b: R=R$_1$=R$_3$=R$_4$=H; R$_2$=Me
c: R$_1$=R$_3$=R$_4$=H; R=R$_2$=Me
d: R$_3$=R$_4$=H; R=R$_1$=R$_2$=Me
e: R=R$_1$=R$_3$=R$_4$=H; R$_2$=Ph
f: R=R$_1$=R$_4$=H; R$_2$=R$_3$=Me
g: R$_1$=R$_4$=H; R=R$_2$=R$_3$=Me
h: R$_2$=R$_4$=H; R=R$_1$=R$_3$=Me
i: R=R$_1$=R$_3$=H; R$_2$=Me; R$_4$=*t*-Bu
j: R=R$_1$=R$_3$=H; R$_2$=Me; R$_4$=SiMe$_3$
k: R$_1$=R$_3$=H; R=R$_2$=Me; R$_4$=SiMe$_3$
l: R=R$_1$=R$_3$=H; R$_2$=Ph; R$_4$=SiMe$_3$

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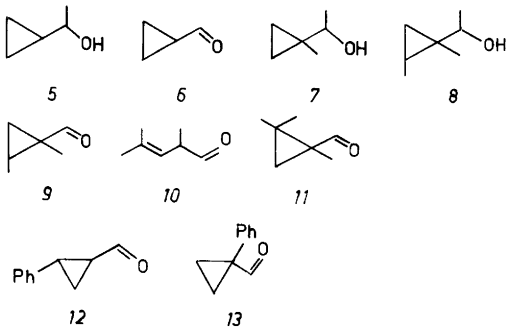
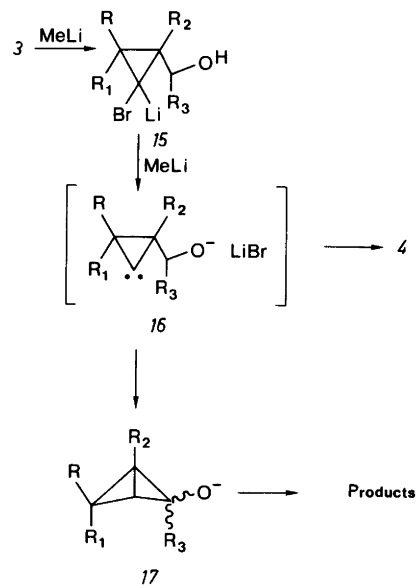
were prepared by addition of dibromocarbene to the respective allyl alcohol according to literature procedures. The ethers *3i* and *3j* were prepared from the respective β-methallyl ethers and dibromocarbene, and the remaining ethers, *3k* and *3l*, were prepared from the corresponding alcohols and chlorotrimethylsilane. Reactions of the alcohols *3a*–*3h* with methyllithium were in most cases carried out at −78 °C using more than two molar equivalents of methyllithium; reactions of the ethers were conveniently carried out at somewhat higher temperatures (−30 to 0 °C) and slightly more than one molar equivalent of the organolithium derivative was used. The reaction mixtures were analyzed by gas liquid chromatography (GLC) prior to work-up. The components of each product mixture were, as far as possible, separated by preparative GLC and identified spectroscopically. From some reactions the isolated yields were low due to extensive polymerization during distillation. Some of the results are recorded in Table 1.

With the exception of *3d*, the alcohols reacted to give some of the corresponding allenes *4*, and from the secondary alcohols *3f*–*3h* they were the sole products isolated in 80–90 % yields. The primary alcohols *3a*–*3c* gave derivatives of cyclopropanemethanol and cyclopropanecarbaldehyde as well. The reaction of the tetraalkylsubstituted dibromocyclopropane *3d* was unique in that the unsaturated aldehyde 2,4-dimethyl-3-pentenal (*10*)[5] was the main product. The alcohol *3e* also afforded aldehydes as major products. The compounds were characterized spectroscopically, in most cases by comparison with those of authentic samples. The aldehyde proton of *9* appeared at δ 8.61 in agreement with the published data for the *trans* isomer,[6] and the carbinol *8*[7] was shown to have the same configuration. The ^{1}H NMR spectrum of the aldehyde *12* is in agreement with that of the *trans* isomer.[8]



The ethers *3i*—*3l* reacted with methyllithium to give the corresponding allenes *4i*–*l* as sole products; however, in the case of *3l* it was necessary to carry out the reaction at 0 °C in order to avoid formation of the monobromide, 2-bromo-1-phenyl-1-trimethylsiloxymethylcyclopropane (*14*), which at −78 °C was actually a major product. Compound *14* was formed as the stereoisomer with the bromine and phenyl group *cis* related. Bicyclobutane derivatives were not obtained from any of these reactions.

The first step in reactions of *gem*-dibromocyclopropanes with alkyllithium is an exchange of bromine with lithium. There is evidence indicating that the bromine exchange is faster than proton abstraction from the hydroxyl group[9] in *gem*-dibromocyclopropanecarbinols; hence, we assume that the α-bromocyclopropyllithium intermediate *15* is formed initially in the present reaction. Whether α-elimination of lithium bromide from *15* precedes or follows anion formation is not clear; consequently, we cannot tell if the subsequent intramolecular insertion takes place on the alcohol or the corresponding anion. Lithium oxygen coordination stabilizes *15*, which suggests that anion formation occurs prior to α-elimination, and the carbene-like intermediate has been for simplicity pictured as *16* (Scheme 1). The stabilization of intermediates like *15* by adjacent oxygen functions has been exten-



Scheme 1.

Table 1. Reactions of 2,2-dibromocyclopropylmethanol derivatives with methyllithium.

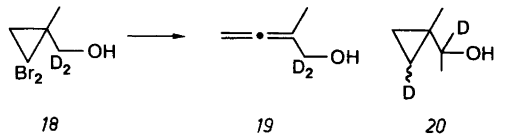
Starting material	Products (%)[a]		
3a	*4a*(56)	*5*(38)	*6*(6)
3b	*4b*(50)	*7*(50)	
3c	*4c*(75)	*8*(17)	*9*(8)
3d	*10*(91)	*11*(3)	
3e	*4e*(45)	*12*(35)	*13*(20)
3f	*4f*(100)		
3g	*4g*(100)		
3h	*4h*(100)		

[a] % of mixture determined by GLC; for yields see Experimental.

sively reported in the literature.[1,10]

Rearrangement to allenes was the expected reaction of the cyclopropylidenes *16*. The other products of Table 1 can all be explained by invoking the bicyclo[1.1.0]butanolate *17*, formed from *16* by intramolecular insertion into a C–H bond adjacent to oxygen. Cyclopropanol derivatives undergo base induced ring opening quite readily,[11] and it is not surprising that the highly strained intermediate *17* behaves similarly.

The cyclopropanecarbaldehydes produced may react further with methyllithium to the corresponding alcohols. The ring opening is apparently quite rapid since we failed in attempts to trap the bicyclobutanolate *17* as the corresponding ether by treating the reaction mixture from *3b* at −78 °C with either methyl iodide or chlorotrimethylsilane. The bicyclobutyl ether was expected to survive the reaction conditions; Hamon and Trenerry[12] have recently reported the isolation of the first example of this type of compound. Benzenethiol is known to react with bicyclobutanes by addition to the central bond,[13] but no phenylthiocyclobutanol derivative was obtained when the reaction mixture from *3b* was quenched at −78 °C with this reagent. However, labelling experiments provided conclusive evidence for the reaction path depicted in Scheme 1. We prepared the carbinol *18* labelled with deuterium at the α-carbon by reducing ethyl 2,2-dibromo-1-methylcyclopropanecarboxylate with lithium aluminium deuteride. Treatment of *18* with methyllithium afforded the expected allene *19* and the cyclopropylcarbinol *20* (Scheme 2). The allene was labelled at the methylene group as shown by the absence of the resonance at δ 3.92 in the ^{1}H NMR spectrum



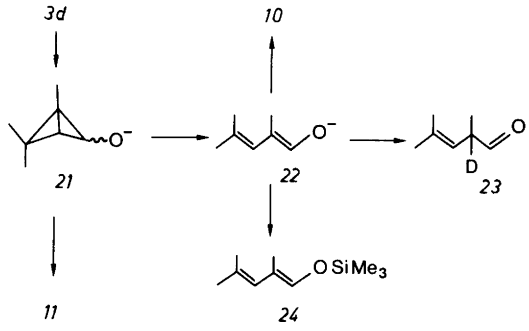
18 *19* *20*

Scheme 2.

and the collapse of the multiplet at δ 4.62 into a quartet. The most striking difference between the spectra of the alcohol *20* and its unlabelled analogue *7* is the complete absence in the former of the quartet at δ 3.08 due to the proton α to the hydroxyl group. With three chiral centres, *20* may be present as a mixture of diastereomers and the ^{1}H NMR spectrum clearly reveals that such a mixture of stereoisomers is indeed formed; the two methyl groups appear as five singlets while the cyclopropyl hydrogens exhibit a complex multiplet centred at δ 0.33. The cyclopropane derivatives formed are in accordance with ring opening of *17* to the most stable carbanion, and the formation of *trans*-2-phenylcyclopropanecarbaldehyde (*12*) indicates that protonation occurs with retention of configuration. Quenching of the reaction mixture from *3b* with D_2O caused no incorporation of deuterium in the ring, which suggests that the proton is abstracted from the solvent or from another proton donator in the reaction mixture.

The result from the reaction of compound *3d* is particularly interesting. It is not surprising that the allene was not encountered since tetraalkyl-substituted cyclopropylidenes seem to prefer insertion to ring opening,[14] and this reaction is no exception; at least 94 % of the product derives from the bicyclobutanolate *21*. Insertion occurs exclusively at the methylene C–H bonds adjacent to oxygen, but only a small amount of the product is formed by the carbanion mechanism while the major reaction path is apparently a thermal rearrangement of the bicyclobutanolate to the dienol anion *22*. Evidence for this mechanism was obtained from two experiments. First, quenching of the reaction mixture with D_2O gave the aldehyde *23* deuterated at the α carbon as shown by the absence of the multiplet centred at δ 3.15 in the parent compound. Secondly, treatment of the reaction mixture at −78 °C with chlorotrimethylsilane gave the dienol ether *24* (Scheme 3).

It is well established that bicyclo[1.1.0]butanes rearrange thermally to the corresponding 1,3-butadienes. The reaction may be a concerted [σ2s+



Scheme 3.

σ2a] process or proceed *via* diradicals, but for alkyl substituted derivatives temperatures of 200 °C or higher are required for the reaction to take place. Our reaction is rapid even at −78 °C and clearly the oxygen anion must exert a profound effect on the thermal stability of the bicyclobutane ring system. The observation is reminiscent of the dramatic rate-enhancing oxyanionic effect of the oxy-Cope rearrangement, first discovered by Evans;[15] unfortunately, in our case a fair comparison is not available since 2-bicyclo[1.1.0]butanol and derivatives are unknown.

According to Hammet $\sigma-\rho$ correlation there seems to be little build-up of charge at carbon in the transition state of cyclopropylidene insertion.[16] It has been reported that the preference for insertion into C−H bonds follow the order: *tert*>*sec*>*prim.*[17] Moreover, there are many examples in the literature which show a preference for both inter- and intramolecular insertion into C−H bonds adjacent to an ether oxygen;[1,2] however, in the case of 1,3-insertion with formation of bicyclobutyl ethers such a preference has not been observed. There are indications that a methoxy group actually retards the 1,3-insertion into the adjacent C−H bond.[18] On the assumption that substituents on C−3 do not significantly affect the rate of ring opening to allene, our results indicate that the oxide ion facilitate insertion while an ether function does not. It is also interesting that a secondary C−H bond is preferred; with tertiary C−H bonds (compounds *3f*−*3h*, Table 1) only allenes were formed. With the data available so far, it is not easy to see any trend in the C−H insertion reactions of cyclopropylidenes which can be simply correlated with electronic and steric effects.

EXPERIMENTAL

The NMR spectra were recorded on Varian EM 360A or Jeol JNM FX60 instruments. Tetramethylsilane was used as internal standard except for the trimethylsilyl ethers for which chloroform was used. Elemental analyses were performed by Ilse Beetz Microanalytical Laboratory, 8640 Kronach, West Germany.

trans-*2,2-Dibromo-1,3-dimethyl-1-(trimethylsiloxymethyl) cyclopropane (3k)* was prepared in 60 % yield from the alcohol *3c* and trimethylchlorosilane using dimethylanilin as base, b.p. 63−66 °C (0.1 mmHg); Anal. for $C_9H_{18}OSi$:C,H. ^{1}H NMR (CCl_4): δ 0.08 (9H,s) 1.07 (3H,d, J = 2.0 Hz) 1.1 (1H,m) 1.12 (3H,s) 3.42 (2H,s).

2,2-Dibromo-1-phenyl-1-(trimethylsiloxymethyl) cyclopropane (3l) was prepared in 52 % yield from the alcohol *3e* and trimethylchlorosilane using dimethylanilin as base, b.p. 76 °C (0.04 mmHg) n_D^{23} 1.5441; Anal. for $C_{13}H_{18}Br_2OSi$: C,H. ^{1}HNMR (CCl_4): δ −0.14 (9H,s) 1.90 (2H,m) 3.87 (2H,d), 7.21 (5H,s).

2,2-Dibromo-1-methyl-1-(trimethylsiloxymethyl) cyclopropane (3j) was prepared by the Doering-Hoffmann procedure[19] in 35 % yield from 2-methyl-1-trimethylsiloxy-2-propene,[20] bromoform and potassium *t*-butoxide, b.p. 65−68 °C (0.4 mmHg); Anal. for $C_8H_{16}Br_2OSi$; C,H. ^{1}HNMR (CCl_4): δ 0.12 (9H,s) 1.41 (3H,s) 1.45 (2H,m) 3.67 (2H,s).

*2,2-Dibromo-1-methyl-1-*t*-butoxymethylcyclopropane (3i)* was prepared in 54 % yield by the phase transfer procedure[21] from 2-methyl-1-*t*-butoxy-1-propene,[22] bromoform, 50 % aq. sodium hydroxide and triethylbenzylammonium chloride as catalyst, b.p. 54−55 °C (0.5 mmHg), n_D^{18} 1.4937; Anal. for $C_9H_{16}Br_2O$; C,H. ^{1}HNMR (CCl_4): δ 1.17 (9H,s) 1.40 (3H,s) 1.5 (2H,m) 3.38 (2H,s).

Reactions of 2,2-dibromocyclopropanemethanol derivatives with methyllithium. General procedure. A 1.5−1.7 M solution of methyllithium in ether (2 molar equivalents) was added dropwise to a stirred and cooled (−30 to −78 °C, bath temp.) solution of the dibromocyclopropane derivative (1.0 molar equivalent) in dry ether. The reaction mixture was stirred for 0.5−3 h at bath temperature after the addition was completed, allowed to warm and decomposed with water. The product was extracted with ether. The dried ($MgSO_4$) extract was evaporated to give the crude product which was purified by distillation and/or preparative gas chromatography.

Reaction of (2,2-Dibromocyclopropanemethanol (3a). A solution of *3a*[23] (3.5 g, 15.2 mmol) in 15 ml of dry ether was treated with 26.5 ml of 1.7 M methyllithium (45 mmol) at −78 °C. Distillation gave 0.45 g of product, b.p. 45−47 °C (18 mmHg)

consisting of 2,3-butadien-1-ol (*4a*; 56 %),[24] 1-cyclopropylethanol (*5*; 38 %)[25] and cyclopropanecarbaldehyde (*6*; 6 %).[6] The spectral data were identical with those reported in the literature. Extensive polymer formation took place during distillation.

Reaction of 2,2-Dibromo-1-methylcyclopropane methanol (3b). A solution of *3b*[26] (112.7 g), 0.46 mol) in 150 ml of dry ether was treated with 650 ml of 1.7 M methyllithium (1.1 mol) at −50 °C. Distillation gave 11.9 g of product, b.p. 54−57 °C (26 mmHg) consisting of 2-methyl-2,3-butadien-1-ol (*4b*; 50 %)[27] and 1-(1-methylcyclopropyl)ethanol (*7*; 50 %).[28] The spectral data were in accordance with those reported in the literature. Extensive polymer formation occurred during distillation of the product.

Reaction of 2,2-Dibromo-1,3-dimethylcyclopropane methanol (3c). A solution of *3c*[26] (6.2 g; 25 mmol) in 25 ml of dry ether was treated with 35 ml of 1.6 M methyllithium (56 mmol) at −30 °C. The crude product (2.8 g) consisted of 2-methyl-2,3-pentadien-1-ol (*4c*; 75%), 1-(*cis*-1,2-dimethylcyclopropane) ethanol (*8*: 17 %)[7] and *trans*-1,2-dimethylcyclopropanecarbaldehyde (*9*; 8 %).[6] These were separated by prep. GLC and the spectral data of *8* and *9* were in accordance with those in the literature. (*4c*): IR (film) 1960 cm^{-1}. ^1H NMR (CCl_4): 1.64 (3H,d, J = 4.0 Hz) 1.72 (3H,s) 2.5 (1H, broad s) 3.90 (2H,d, J = 2.5 Hz) 5.15 (1H,m).

Reaction of 2,2-Dibromo-1,3,3-trimethylcyclopropane methanol (3d). A solution of *3d*[29] (1.9 g, 7.0 mmol) in 10 ml of dry ether was treated with 9.6 ml of 1.5 M methyllithium (14.4 mmol) at −78 °C. The crude product (1.0 g) consisted of 2,4-dimethyl-3-pentenal (*10*; 91 %),[5] 1,2,2-trimethylcyclopropanecarbaldehyde (*11*; 3 %) and two other unidentified components (2 and 4 %). The spectral data of *10* were identical with those in the literature. *11*: ^1H NMR (98 MHz; CCl_4): δ 0.7 (2H,m) 1.29 (3H,s) 1.34 (3H,s) 1.36 (3H,s) 9.30 (1H,s). Hydrolysis of the above reaction mixture with D_2O gave 2-deutero-2,4-dimethyl-3-pentenal (*23*): ^1H NMR (98 MHz; CCl_4): δ 1.3 (3H,s) 1.81 (3H,s), 1.87 (3H,s) 5.02 (1H, broad s) 9.48 (1H,s).

Reaction of 2,2-Dibromo-1-phenylcyclopropane methanol (3e). A solution of *3e*[30] (6.04 g; 20 mmol) in 20 ml of dry ether was treated with 26.0 ml of 1.8 M methyllithium (45 mmol) at −78 °C to give 2.0 g of a liquid, b.p. 78 °C (0.05 mmHg) consisting of 2-phenyl-2,3-butadien-1-ol (*4e*; 45%),[27] *trans*-2-phenylcyclopropanecarbaldehyde (*12*; 35 %)[8] and 1-phenyl-cyclopropanecarbaldehyde (*13*; 20 %).[31] All the compounds exhibited spectral data in accordance with those reported in the literature.

3-Methyl-3,4-pentadien-3-ol (*4f*)[4] was obtained in 83 % yield from 1-(2,2-dibromo-1-methylcyclopropane) ethanol (*3f*)[4] and methyllithium at −55 °C.

3-Methyl-3,4-hexadien-2-ol (*4g*) was obtained in 90 % yield, b.p. 57−58 °C (13 mmHg) n_D^{22} 1.4760, from *trans*-1-(2,2-dibromo-1,3-dimethylcyclopropane)ethanol (*3g*)[27] and methyllithium at −78 °C.

5-Methyl-3,4-hexadien-2-ol (*4h*)[4] was obtained in 90 % yield from 1-(2,2-dibromo-3,3-dimethylcyclopropane)ethanol (*3h*)[4] and methyllithium at −78 °C.

2,4-Dimethyl-1-(trimethylsiloxy)-1,3-pentadiene (*24*). To the reaction mixture from *3d* (0.20 g; 0.74 mmol) and methyllithium (1.65 mmol) kept at −78 °C was added trimethylchlorosilane (0.33 g; 3.0 mmol. After stirring at this temperature for 15 h, the reaction mixture was allowed to attain room temperature. Usual work-up resulted in 0.2 g crude product consisting essentially of *24*: ^{1}HMNR (CCl_4): δ 0.17 (9H,s) 1.25 (3H,s) 1.60 (3H,s) 1.68 (3H,s) 5.37 (1H,s) 5.97 (1H,s). ^{13}C NMR (CCl_4): δ 3.50 (Si−CH_3), 15.84, 21.43, 28.89 (CH_3) 118.50 (olef. C) 127.07 (olef. CH) 131.81 (olef. C) 139.53 (=CH−O−).

2-Methyl-1-t-butoxy-2,3-butadiene (4i). A solution of *3i* (1.5 g; 5.0 mmol) in 5 ml of dry ether was treated with 3.3 ml of 1.7 M methyllithium (5.5 mmol) to give 0.7 g (100 %) of crude *4i*, which was purified by prep. GLC; IR (film): 1955, 880, 845 cm^{-1}. ^{1}HNMR (CCl_4): δ 1.07 (9H,s) 1.67 (3H,t, J = 3.0 Hz) 3.78 (2H,t, J = 2.0 Hz) 4.55 (2H,m).

2-Methyl-1-trimethylsiloxy-2,3-butadiene (4j). A solution of *3j* (3.1 g; 10.0 mmol) in 15 ml of dry ether was treated with 8.8 ml 1.8 M methyllithium (15.0 mmol) at −78 °C to give 1.6 g (100 %) of crude *4j*, which was purified by prep. GLC; IR (film): 1960, 860 cm^{-1}. ^{1}HNMR (CCl_4): δ −0.03 (9H,s) 1.57 (3H,t, J = 3.0 Hz) 3.93 (2H,t, J = 2.0 Hz) 4.48 (2H,m).

2-Methyl-1-trimethylsiloxy-2,3-pentadiene (4k). A solution of *3k* (1.25 g; 3.8 mmol) in 5 ml of ether was treated with 2.5 ml of 1.6 M methyllithium (4.0 mmol) at −30 °C to give 0.46 g (71 %) of *4k*; IR (film) 1960 cm^{-1}; ^{1}HNMR (CCl_4) δ 0.15 (9H, s) 1.62 (3H,d, J = 5.5 Hz) 1.70 (3H,s) 4.06 (2H,d, J = 2.0 Hz) 5.1 (1H, broad m).

2-Phenyl-1-trimethylsiloxy-2,3-butadiene (4l). A solution of *3l* (4.0 g; 11 mmol) in 10 ml of dry ether was treated with 8.0 ml of 1.7 M methyllithium (13.6 mmol) at −78 °C to give a mixture of 2-phenyl-1-trimethylsiloxy-2,3-butadiene (*4l*; 50 %) and 2-bromo-1-phenyl-1-trimethylsiloxymethylcyclopropane (*14*; 50%). The allene decomposed on attempted distillation. *4l*: IR (film): 1960 cm^{-1}. ^1H NMR (CCl_4): δ 0.10 (9H,s) 3.70 (2H,t, J = 2Hz), 4.70 (2H,m). *14*: b.p. 70−75 °C (0.13 mmHg); ^1H NMR (CCl_4): δ 0.07 (9H,s) 1.42 (2H,m) 3.16 (1H,m) 3.66 (2H,s) 7.23 (5H,s).

2,2-Dibromo-1-methylcyclopropane α,α-*dideutero-*

methanol (*18*) was prepared in 86 % yield from ethyl 2,2-dibromo-1-ethylcyclopropanecarboxylate[32] by selective reduction using lithium aluminium deuteride as described for the hydrogen analog;[30] recrystallization from pentane gave the pure compound, m.p. 70 °C; ^{1}H NMR ($CDCl_3$): δ 1.52 (3H,s), 1.55 (2H, ABq, J = 7.5 Hz), 1.94 (1H,s).

Reaction of 2,2-Dibromo-1-methylcyclopropane α,α-dideuteromethanol (*18*). The reaction was carried out as described for *3b*. The product was shown to consist of 1,1-dideutero-2-methyl-2,3-butadien-1-ol (*19*; 59 %) and 1-deutero-1-(2-deutero-1-methylcyclopropane)ethanol (*20*; 41 %). *19*: IR (film) 2090, 1960, 850 cm^{-1}. ^{1}H NMR (CCl_4): δ 1.70 (3H,t, J = 3.5 Hz) 3.43 (1H,s) 4.66 (2H,q, J = 3.5 Hz). *20*: ^{1}H NMR (CCl_4): δ 0.33 (3H,m) 1.2 (6H,m) 3.5 (1H, broad s).

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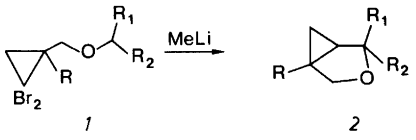
Chemistry of *gem*-Dihalocyclopropanes. XVIII. Reactions of *gem*-Dibromocyclopropylmethyl Sulfides with Methyllithium

JACEK ARCT and LARS SKATTEBØL

Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, Oslo 3, Norway

gem-Dibromocyclopropylmethyl sulfides *3* were prepared in good yields from 1,1-dibromo-2-iodomethylcyclopropane *4* and the appropriate sodium thiolate. Compounds *3* reacted with methyllithium to give products that were separated into low and high boiling fractions. The former contained the allenes *5*, the 3-thiabicyclo[3.1.0]hexane derivatives *6,7* and *8*, and the methylated monobromides *9*. The nonvolatile parts were shown to consist of stereoisomers of the bicyclopropylidenes *10*. The product composition varied with the reaction temperature. The yields of *10* were highest at −78 °C reaching 85 % in the case of *10a*. The amounts of bicyclic sulfides increased with rising temperature. The mechanism of formation of the products is discussed.

The cyclopropylidene intermediate generated from *gem*-dibromocyclopropanes and alkyllithium can undergo a variety of reactions.[1] Insertion into C–H bonds is commonly observed and can also be useful synthetically. Early work by Moore *et al.*[2a] indicated that intermolecular insertion into C–H bonds adjacent to an oxygen function was favoured, and Baird[3] later showed that high yields of the intramolecular insertion products *2* were obtained from the ethers *1* (Scheme 1). Several examples of similar intramolecular insertion reactions have since been reported involving ethers,[4] alcohols[5] and amines.[6] In the present work we want to report that among other reactions insertion also takes place with sulfides of the general structure *3*.



1 *2*

Scheme 1.

After this work was completed, Baird reported[7] a similar study. With one exception, different sulfides have been used in the two studies and more important, our results differ sufficiently to justify publication.

The sulfides were conveniently obtained in high yields by thiolate anion displacement of iodide from 1,1-dibromo-2-iodomethylcyclopropane (*4*), which is readily available from the corresponding chloride.[8] The latter also undergoes displacement with thiolate anions, but as expected, at a considerably slower rate. The preparation of these compounds by addition of dibromocarbene to allyl sulfides, has not been successful,[9] which may explain why very few examples of this potentially interesting class of compounds are described in the literature.

Solutions of the sulfides in ether were treated with methyllithium at two temperatures, −78 and 0 °C. The products consisted invariably of a volatile fraction which was separated by distillation, and a residue which could not be distilled without partial decomposition, but was purified by column chromatography. Most of the compounds constituting the volatile part were isolated by preparative gas liquid chromatography (GLC) some compounds remained unindentified, but more than 85 % of the total product was accounted for in each case. The results are depicted in Scheme 2 and Table 1.

The products from all reactions, except that from *3a*, consisted essentially of four compounds of the structures *5* – *10*. The amount of volatile material,

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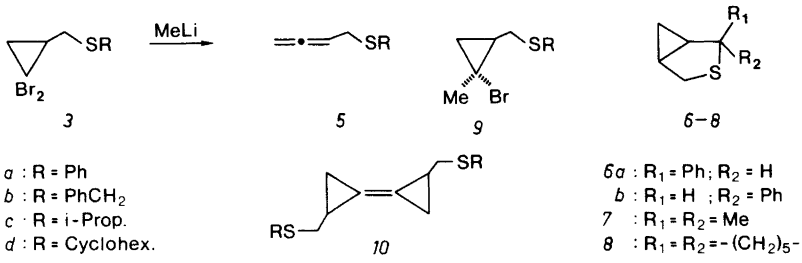
Table 1. Products from reactions of the sulfides *3* with methyllithium.

Starting material	Reaction temp. (°C)	Products (% yields)[a]
3a	−78	*5a* (4), *10a* (86)
	0	*5a* (67), *10a* (20)
3b	−78	*5b* (6), *6* (14), *9b* (9), *10b* (59)
	0	*5b* (21), *6* (17), *9b* (7), *10b* (40)
3c	−78	*5c* (2), *7* (34), *9c* (6), *10c* (48)
	0	*5c* (16), *7* (21), *9c* (7), *10c* (42)
3d	−78	*5d* (2), *8* (15), *9d* (5), *10d* (67)
	0	*5d* (19), *8* (38), *10d* (38)

[a] The values for compounds *5* − *9* are based on GLC analysis of distilled material while the values for compounds *10* are based on material isolated by column chromatography.



Scheme 2.

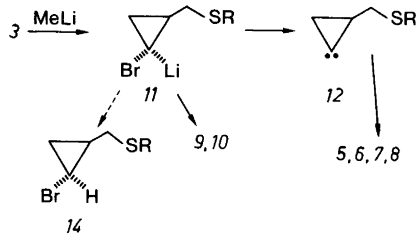
5 − *9*, increased at higher temperature. The structural assignments are based on spectral data, particularly the NMR spectra. The allenes *5* were formed in all reactions, and the IR spectra exhibited absorption at about 1960 and 855 cm^{-1}, characteristic of a terminal allenic linkage.

The bicyclic sulfide *6* appeared homogeneous by GLC, but the ^1H NMR spectrum revealed the presence of two stereoisomers in a ratio of approximately 5:2. The benzylic proton of the major isomer appears as a doublet, $J = 3$ Hz, at δ 4.68, while in the minor isomer the same proton resonates as a singlet at δ 4.31. Unfortunately, there are no conformational studies of the 3-thiabicyclo[3.1.0]-hexane ring system, but the 3-oxa analogue has been investigated using microwave spectroscopy.[10] It has a boat conformation with a torsional angle of the C−2 oxygen bond of 42°. We assume that *6* will have a similar geometry but with a smaller torsional angle to sulfur. According to this model the benzylic proton and the vicinal cyclopropyl proton will be nearly eclipsed in a *cis* configuration, suggesting that the major component of *6* is the *endo* isomer, *i.e.*[6a] Support for the assignment is provided by comparison with the ^1H NMR spectrum of 3-azabicyclo[3.1.0]hexane,[11] which shows a vicinal coupling of 3 Hz for the *exo* and negligible coupling for the *endo* proton. In the case of *9c*, GLC analysis revealed the presence of two isomers in a ratio of ~1:1. It is not apparent from the ^1H NMR spectra, but we assume that all compounds *9* are mixtures of stereoisomers. The methyl group adjacent to bromine appears as a singlet at δ 1.75 ± 0.04 in all three derivatives, in good agreement with data available for similar compounds.[12]

The bicyclopropylidenes *10* constitute the major part of the nonvolatile products. The NMR spectra are in accordance with the assigned bicyclopropylidene structures, but we could not on this basis ascertain which isomer was actually formed. Fortunately, the dimer *10a* was obtained in high yield as a crystalline compound, m.p. 106 − 107 °C, which appeared homogeneous. An X-ray crystallographic determination,[13] established the structure as *trans*-2,3′-bis(phenylthiomethyl)1,1′-bicyclopropylidene (*10a*) with a short, 1.303(2) Å double bond, very similar to that determined recently for another bicyclopropylidene derivative.[14] For symmetry reasons this bond should be inactive in infrared, but active in the Raman spectrum. Surprisingly, the C=C stretching band was absent or very weak in the Raman spectra of all the bicyclopropylidene derivatives

Table 2. ^{13}C NMR chemical shifts for bicyclopropylidene derivatives *10*.

Compound	Chemical shifts (δ ppm)			
	C−1	C−2	C−3	C−4
10a	115.7	15.7	10.9	37.9
10b	115.5	15.7	10.5	34.6
10c	115.4	15.9	10.3	34.0
10d	115.5	16.1	10.5	33.5



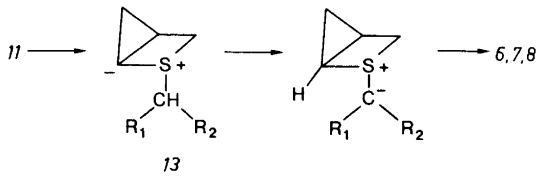
Scheme 3.

investigated so far,[14,15] and *10a* is no exception in this respect. On the other hand, the Raman spectra of both 2,2,3′3′-tetrachloro-2′,2′,3,3-tetramethyl and 2,2,2′,2′-tetrachloro-3,3,3′,3′-tetramethyl-1,1′-bicyclopropylidene exhibit bands of medium intensity at 1851 and 1825 cm^{-1}, respectively, which are assigned to the double bond.[16] The intensity of this band appears to be strongly dependent on the substituents. The ^{13}C chemical shifts in the NMR for the ring carbons of the dimers *10* are recorded in Table 2. The close similarity of the data lead us to assign an *E* relationship of the substituents for compounds *10b*−*10d* as well, but we do not know whether they are *trans*, *cis* or indeed mixtures.

It is established that the first step in reactions of *gem*-dibromocyclopropanes with methyllithium involves an exchange of bromine for lithium with formation of the corresponding α-bromocyclopropyllithium derivative, which for the present reactions will have the general structure *11*. Its formation is normally very fast even at −78 °C and it may react further in several ways. Elimination of lithium bromide leads to an intermediate *12* with chemical properties expected of a carbene. For simplicity we prefer to draw this intermediate as a cyclopropylidene bearing in mind, however, that it is most probably in some way both complexed with lithium bromide and solvated.[17] The rate of lithium bromide elimination from intermediates like *11* is strongly affected by the substituents on the cyclopropane ring. Indeed, with oxygen- or nitrogen-containing substituents that complex with lithium, the formation of *12* becomes slow even at 0 °C and products derive also from the organolithium derivative. Sulfur is also able to interact with lithium and products derived from *11* were expected at low temperature, at least.

The allenes *5* are formed by ring opening of the cyclopropylidene *12*, while intramolecular insertion leads to the bicyclic compounds *6*−*8*. With alkyl-substituted cyclopropylidenes the ratio of ring opening to insertion is strongly influenced by the number of substituents[18] as well as their bulk;[19,20] insertion into the 3,4-related CH bond (1,3 insertion) forming bicyclobutanes has been observed, and normally as the predominant reaction with tetrasubstituted derivatives.[18] On the other hand, the cyclopropylidenes *12* as well as the oxygen[3] and nitrogen[6] analogues undergo insertion exclusively into the 5,6-related CH bond (1,5 insertion). At 0 °C comparable amounts of allenes and insertion products are formed from *3b*−*3d*; the ratio is larger than that found for ethers and amines, but at −78 °C almost negligible amounts of allenic products are formed.

The heteroatom clearly influences the insertion reaction in two ways: (i) the rate is enhanced at the adjacent CH bond and (ii) specifically at the 5,6-related bond. How the heteroatom exerts this effect is not clear. It has been suggested[21] that during insertion a positive charge develops at the carbon from which the hydrogen migrates, and oxygen, nitrogen and sulfur are capable of stabilizing this charge leading to rate enchancement. A kinetic study,[20] however, on intramolecular cyclopropylidene insertion into benzylic methylene groups indicates that only little charge develops in the transition state. The charge stabilizing effect may be important, but it does not explain the regioselective 1,5 insertion. This is particularly demonstrated by the results from the reaction of *3a*; the allene *5a* was obtained as the only volatile product, although 1,3 insertion is available as a reaction path. Conformational effects certainly play an important role in insertion reactions.[4,22] Some kind of interaction between the vacant *p*-orbital of the cyclopropylidene and a filled orbital of the heteroatom could cause the molecule to attain a conformation ideal for 1,5 insertion. The bicyclic components may actually not result from insertion, but rather from a Stevens



Scheme 4.

type rearrangement[23] of an intermediate zwitterion (ylid) *13* as outlined in Scheme 4. This reaction path would accommodate the regioselectivity and evidence in support of such a mechanism has been obtained from reactions of electrophilic carbenes with sulfides,[9,24] ethers[25] and amines.[26]

Most reactions produced low yields of a third volatile compound, *viz.* the monobromides *9*. They are products derived from the α-bromocyclopropyllithium intermediate *11* and methyl bromide, a well documented reaction.[12,27] Some additional volatile products were present in the reaction mixtures and we cannot therefore exclude that the monobromides *14* were formed in small amounts, but we have no evidence for that.

The most remarkable result of the present study is the formation of the bicyclopropylidenes *10* in relatively high yields. These compounds are formally dimers of the corresponding carbene and they were actually encountered in early work on the reactions of *gem*-dibromocyclopropanes with methyllithium.[2b] Only few examples of the dimers have since been reported as products from similar reactions;[14,28,29] with one exception,[29] yields were usually low but highest at low reaction temperature and in the presence of lithium iodide.[28] All evidence point to *11* as the precursor of the dimers *10*. They may form either from reaction of two molecules of the intermediate or by its reaction with a molecule of starting material. Apparently, the intramolecular coordination of lithium in *11* is beneficial for dimerization, but intermolecular complexation may be equally important.

In spite of the complexity of the products compiled in Table 1, the present reaction may be of use synthetically for the preparation of compounds incorporating the 3-thiabicyclo[3.1.0]hexane ring system and derivatives of bicyclopropylidene. No real effort was spent optimizing the yields of *6*, *7* and *8*, but reaction at −78 °C of one fourth the usual concentration of *3c* gave *7* and *10c* in 51 and 31% yields, respectively, and only small amounts of *5c* and *9c*.

The results of the present work agree well with those reported by Baird[7] with regard to formation of the allenes and bicyclic compounds; only the reaction of *3c* was actually carried out in both studies. The discrepancies arise in connection with the methylated monobromides *9* and the dimers *10*, which were not reported by Baird; on the other hand, he reported the formation of *14*. Our reactions were carried out at similar concentrations to those described by Baird, but at lower temperatures; moreover, the methyllithium used in the two studies came from different suppliers and the content of lithium bromide will probably affect the reaction as well. More data is required in order to clarify the influence of these factors on the product composition.

EXPERIMENTAL

NMR spectra were recorded on Varian EM 360A and Jeol JNM FX60 spectrometers. The mass spectra were obtained on an MM 7070 GLC/MS instrument. Elemental analyses were performed by I. Beetz, West Germany.

1,1-Dibromo-2-iodomethylcyclopropane (4). A solution of 23.6 g (0.1 mol) of 2-chloromethyl-1,1-dibromocyclopropane[8] and 30.6 g (0.2 mol) of NaI in 180 ml of acetone was heated under reflux for 30 h. The acetone was evaporated and water was added to the residue. The product was extracted with CH_2Cl_2, dried ($MgSO_4$) and solvent evaporated. Distillation afforded 29.7 g (91 %) of *4*, b.p. 62–63 °C (0.4 mmHg); Anal. $C_4H_5Br_2I$: C,H. ^{1}H NMR (CCl_4): δ1.35 (1H, m) 2.03 (2H, m) 3.28 (2H,m); ^{13}C NMR (CCl_4): δ 4.3 (CH_2–I) 29.4 (CBr_2) 31.0 (cyclopropyl CH_2) 32.5 (cyclopropyl CH).

Preparation of sulfides (3). General procedure. To a solution of the sodium salt of the appropriate thiol (20 mmol) in 30 ml of methanol was added the iodide (*4*) (20 mmol). The mixture was heated with reflux for 2–3 h. MeOH was distilled off, and a mixture of water (10 ml) and ether (50 ml) was added to the residue. The ether layer was separated, washed successively with 10 % aq. NaOH and water and dried ($MgSO_4$). The product was isolated by distillation.

2,2-Dibromo-1-phenylthiomethylcyclopropane (3a), b.p. 110–112 °C (0.01 mmHg). Anal. $C_{10}H_{10}Br_2S$: C.H. ^{1}H NMR (CCl_4): δ 1.28 (1H, m) 1.4–2.0 (2H, m) 3.03 (2H, dq, J=6 and 13 Hz) 7.26 (5H, m).

1-Benzylthiomethyl-2,2-dibromocyclopropane (3b), b.p. 116–118 °C (0.01 mmHg). Anal. $C_{11}H_{12}Br_2S$: C,H. ^{1}H NMR (CCl_4): δ 1.22 (1H, m) 1.3–2.0 (2H, m) 2.53 (2H, m) 3.72 (2H, s) 7.18 (5H, s).

2,2-Dibromo-1-isopropylthiomethylcyclopropane (3c), b.p. 61–62 °C (0.01 mmHg). Anal. $C_7H_{12}Br_2S$: C,H. ^1H NMR (CCl_4): δ 1.3 (6H, d, J=6 Hz) 1.0–3.5 (6H, compl. abs.).

1-Cyclohexylthiomethyl-2,2-dibromocyclopropane (3d), b.p. 100–102 °C (0.01 mmHg). Anal. $C_{10}H_{14}Br_2S$: C,H. ^1H NMR (CCl_4): δ 1.0–2.1 (14H, compl. abs.) 2.65 (2H, m).

Reactions of sulfides 3 with methyllithium. To a stirred solution of the sulfide *3* (5 mmol) in 25 ml of dry ether, kept at −78 °C (method A) or 0 °C (method B), an ethereal solution of methyllithium (6 mmol) was added dropwise. The reaction mixture was stirred at the same temperature for 1 h. Water was added and the ether phase separated, washed with brine and dried ($MgSO_4$). The ether was evaporated and the volatile product collected by distillation under reduced pressure. The residue was purified by column chromatography (neutral Al_2O_3, ether–pentane). The components of the volatile product was separated by preparative GLC. (SE 30, Apiezone L, or OV 17, 3 m).

4-Phenylthio-1,2-butadiene (5a), *b.p.* 47–48 °C (0.015 mmHg); IR (film) 1960, 855 cm^{-1}; ^1H NMR (CCl_4) δ 3.51 (2H, td, J=2.5 Hz, 7.5 Hz) 4.68 (2H, m) 5.20 (1H, m) 7.27 (5H, m); ^{13}C NMR ($CDCl_3$) δ 33.4 (CH_2) 76.1 (=C) 87.5 (=C) 126.3, 128.8, 130.2, 135.8 (Ph) 209.6 (C).

trans-*2,3'-Bis(phenylthiomethyl)-1,1'-bicyclopropylidene (10a)*, m.p. 106–107 °C (from CCl_4); ^1H NMR ($CDCl_3$) δ 1.1 (1H, m) 1.38 (1H, m) 1.8 (1H, m) 2.95 (2H, m) 7.25 (5H, m); ^{13}C NMR, see Table 2. The configuration has been determined by X-ray diffraction.[13]

4-Benzylthio-1,2-butadiene (5b), IR (film) 1960, 855 cm^{-1}; ^1H NMR (CCl_4) δ 2.93 (2H, m) 3.63 (2H, s) 4.73 (2H, m) 5.0 (1H, m) 7.17 (5H, m); MS: *m/e* 176 (M^+).

2-Phenyl-3-thiabicyclo 3.1.0 hexane (6) was formed as a stereoisomeric mixture.

endo-6 (72 %), ^1H NMR (CCl_4) δ 0.3–1.9 (4H, several m) 3.07 (2H, m) 4.68 (1H, d, J=3 Hz) 7.17 (5H, m) ^{13}C NMR (CCl_4) δ 2.9 (cyclopropyl CH_2) 18.8 24.8 (Cyclopropyl CH) 35.2 (CH_2–S) 52.5 (CH–S) 126.3, 126.9, 127.5, 128.0 (Ph); MS: *m/e* 176 (M^+).

exo-6, (28 %): ^1H NMR (CCl_4) δ 0.3–1.9 (4H, several m) 3.07 (2H, m) 4.31 (1H, s) 7.17 (5H, m; ^{13}C NMR (CCl_4) δ 5.6 (Cycloprop. CH_2) 21.1, 26.5 (Cyclopropyl CH) 33.7 (CH_2–S) 53.4 (CH–S) 126.3, 126.9, 127.5, 128.0 (Ph); MS: *m/e* 176 (M^+).

2-Bromo-2-methyl-1-benzylthiomethylcyclopropane (9b), ^1H NMR (CCl_4) δ 0.80 (3H, m) 1.70 (3H, s) 4.26 (2H, m) 3.70 (2H, s) 7.21 (5H, s); MS: *m/e* 272, 270 (M^+).

2,3'-Bis(benzylthiomethyl)-1,1'-bicyclopropylidene (10b), ^1H NMR (CCl_4) δ 0.7–1.4 (6H, compl. abs.) 2.0–2.6 (4H, m) 3.63 (4H, br.s.) 7.11 (10H, s); ^{13}C NMR, see Table 2.

4-Isopropylthio-1,2-butadiene (5c). IR (film) 1950, 840 cm^{-1}; ^1H NMR (CCl_4) δ 1.23 (6H, d, J=6.5 Hz) ~3 and 3.08 (3H, m and dt) 4.7 (2H, m) 5.03 (1H, m): MS: *m/e* 128 (M^+).

2,2-Dimethyl-3-thiabicyclo 3.1.0 hexane (7). ^1H NMR (CCl_4): δ 0.3 (1H, m) 0.7–1.5 (3H, compl.abs.) 1.32 (3H, s) 1.40 (3H, s) 2.93 (2H, ABX, J_{AB}=11 Hz, J_{AX}=3 Hz). ^{13}C NMR (CCl_4) δ 3.8 (cyclopropyl CH_2) 19.7, 26.1 (cyclopropyl CH) 31.3, 32.2 (CH_3) 33.7 (CH_2–S), 53.4 (C–S); MS: *m/e* 128 (M^+).

2-Bromo-1-isopropylthiomethyl-2-methylcyclopropane (9c). Mixture of stereoisomers by GLC (SP 2100, 3m, 140 °C) ^1H NMR (CCl_4) δ 0.92 (3H, m) 1.27 (6H, d, J=6.5 Hz) 1.77 (4H, s) 2.5–3.2 (3H, compl.abs.); MS: *m/e* 224, 222 (M^+).

2,3'-Bis(isopropylthiomethyl)-1,1'-bicyclopropylidene (10c). ^1H NMR (CCl_4) δ 0.6–1.6 (3H, compl.abs.) 1.25 (6H, d) 2.57 (2H, br.d.) 2.97 (1H, m); ^{13}C NMR, see Table 2.

4-Cyclohexylthio-1,2-butadiene (5d). IR (film) 1955, 845 cm^{-1}; ^1H NMR (CCl_4) δ 1.1–2.1 (10H, compl. abs.) 2.65 (1H, m) 3.08 (2H, dt, J=8 Hz, 2.5 Hz) 4.5–5.3 (3H, compl.abs.).

3,4-Methano-1-thiaspiro[5.6]decane (8). ^1H NMR (CCl_4) δ 0.30 (1H, m), 0.87 (1H, m), 1.0–2.0 (12H, compl.abs.), 4.53 (2H, ABX J_{AB}=11 Hz, J_{AX}=4 Hz). ^{13}C NMR (CCl_4) δ 3.1 (cyclopropyl CH_2) 19,3, 23.2 (cyclopropyl CH) 25.7, 29.9, 32.6 (cyclohexyl CH_2) 36.6 (S–CH_2) 39.6 (S–C); MS: *m/e* 168 (M^+).

2-Bromo-1-cyclohexylthiomethyl-2-methylcyclopropane (9d). ^1H NMR (CCl_4) δ 1.75 (3H, s) 1.4–2.1 (13H, compl.abs.) 2.6 (3H, m).

2,3'-Bis(cyclohexylthiomethyl)-1,1'-bicyclopropylidene (10d). ^1H NMR (CCl_4) δ 0.7–2.2 (13H, compl.abs.) 2.2–3.1 (3H, compl.abs.); ^{13}C NMR see Table 2.

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Oxidation of Styrene by Chlorine Dioxide and by Chlorite in Aqueous Solutions

JIRI J. KOLAR and BENGT O. LINDGREN

Swedish Forest Products Research Laboratory, Box 5604, S-114 86 Stockholm, Sweden

In order to get information about the reactions taking place during chlorine dioxide bleaching of pulp the products of the reaction between styrene and chlorine dioxide in aqueous solutions were analyzed. The result indicates that chlorine dioxide epoxidizes styrene. The monochlorine monoxide thereby formed then oxidizes chlorine dioxide to chlorate and is reduced to hypochlorous acid. The hypochlorous acid either reacts with styrene or can be captured with added sulfamic acid.

The amounts of chlorine dioxide formed when styrene reacted with chlorite and mixtures of chlorite and chlorine dioxide were determined. The reactions were carried out both with and without sulfamic acid present. The results indicate that the chlorite oxidation of styrene follows a chain reaction route in which chlorine dioxide, monochlorine monoxide and hypochlorous acid participate.

When cyclohexene and methyl oleate were treated with chlorine dioxide the formation in small yields of the corresponding 1,2-epoxides was observed.[1,2] *trans*-Stilbene yielded the epoxide in rather high yield when the reaction was carried out in a carbon tetrachloride solution.[3] When the reaction was carried out in an aqueous solution the formation of the epoxide was not observed but some products were found which could have been formed *via* the epoxide. Lindgren and Nilsson[4] have suggested that the epoxides are formed from olefines in aqueous solutions according to a reaction route incorporating the reactions A, B and C:

$$ClO_2 + R^1-CH=CH-R^2 \rightarrow ClO + \underset{R^1-CH-CH-R^2}{\overset{O}{/\ \backslash}} \qquad A$$

$$ClO + ClO_2 + H_2O \rightarrow HClO + ClO_3^- + H^+ \qquad B$$

$$HClO + R^1-CH=CH-R^2 \rightarrow R^1-CH(OH)-CHCl-R^2 \qquad C$$

To test this suggestion we have now determined the products formed when styrene is oxidized in aqueous solution with chlorine dioxide. Some of the oxidation experiments were carried out in the presence of sulfamic acid which captures the intermediate hypochlorous acid according to the reaction D.

$$HOCl + H_2NSO_3^- \rightarrow HClNSO_3^- + H_2O \qquad D$$

Sulfamic acid has previously been used to capture hypochlorous acid intermediately formed in the chlorite oxidations of vanillin[5] and of lignin preparations[6] as well as in the chlorine dioxide bleaching of wood pulps.[7] Hypochlorous acid reacts very rapidly with sulfamic acid giving the relatively unreactive *N*-chlorosulfamic acid. The reaction is even more rapid than that between hypochlorous acid and a phenol such as guaiacol.[7]

The rate by which chlorine dioxide delignifies and bleaches wood pulp increases with increasing pH and when chloride is added.[8] In an attempt to explain these effects we have further studied the kinetics of the reaction between chlorine dioxide and styrene.

Because of the close connection between the chlorite and the chlorine dioxide oxidations, the reaction of styrene with chlorite has also been examined.

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Table 1. Inorganic products obtained when chloride dioxide was treated with an excess of styrene in water–*tert*-butyl alcohol (3.4:1 v/v) solution.

Product	Yields[a] pH 2	pH 4	pH 6
Chlorate[b]	41	43	47
Chlorite[c]	0	0	5
Hypochlorous acid[d]	46	47	–[e]

[a] Mol % of chlorine dioxide consumed. [b] Determined according to Eriksson and Sjöström.[9] [c] Determined from the difference between the amounts of available chlorine and of residual chlorine dioxide (UV-determination). No sulfamic acid was added. [d] The amount of intermediately formed hypochlorous acid captured as *N*-chlorosulfamic acid by including sulfamic acid in the reaction mixture. The amount of *N*-chlorosulfamic acid was determined iodometrically. The amount of chlorite was here considered to be insignificant in accordance with the result in the line above. [e] This value cannot be obtained due to the rapid reaction between chlorine dioxide and sulfamic acid at pH 6.

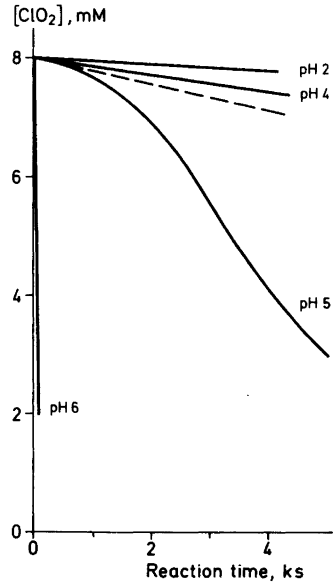


Fig. 1. The consumption of chlorine dioxide by sulfamic acid at different pH-values. The initial concentration of sulfamic acid was 8.8 mM. Temperature was 30 °C. Phosphate buffer solutions (0.28 M). The concentration of chlorine dioxide was determined by following continuously the UV-absorptivity at 357 nm. The dashed line refers to an experiment at pH 6 in the absence of sulfamic acid.

RESULTS

Reaction products. The products formed in the reaction between styrene and chlorine dioxide were analyzed for runs in which styrene was in excess. The yields of inorganic compounds are given in Table 1. Slightly less than half of the chlorine dioxide consumed was oxidized to chlorate and about the same amount was reduced to the intermediate hypochlorous acid, which was captured as N-chlorosulfamic acid. (Chlorine dioxide reacted only slowly with sulfamic acid except when the pH was above 4, see Fig. 1.) Only minor amounts of chlorite were formed.

The organic products were analyzed by gas chromatography (see Fig. 2) and mass spectrometry.

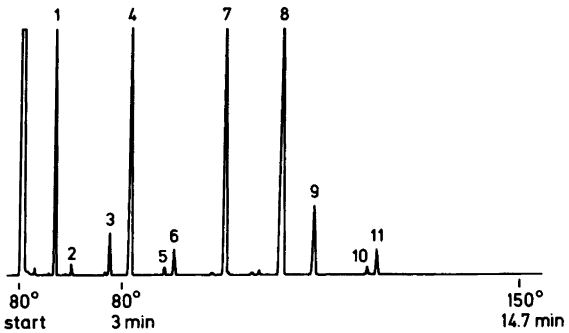


Fig. 2. Gas chromatogram of products from styrene oxidation by chlorine dioxide at pH 6, no sulfamic acid added. The chromatograms were run on an OV 17 fused silica column (25 m) installed in Carlo Erba 4100 gas chromatograph. The program used was 3 min isothermally at 80 °C, then 6 °C/min to 150 °C. Split injection was applied. Naphthalene was used as internal standard. Peak 1 is ascribed to styrene and peak 7 to naphthalene. The designations of the other peaks are given in Table 2.

Table 2. The organic products obtained when chlorine dioxide was treated with an excess of styrene in water – *tert*-butyl alcohol (3.4:1 v/v) solution.

Peak in Fig. 2	Product	Yields[a]				
		Sulfamic acid Absent			Present	
		pH 2	pH 4	pH 6	pH 2	pH 4
Chlorinated products						
8	2-Chloro-1-phenyl-1-ethanol[b]	34	36	34	5	2
6	2-Chloro-1-phenyl-ethene	1.4	1.5	1.7	0.3	0.3
12[c]	α-Chloro-acetophenone	0.8	0.6	0	0.7	0.6
5	1-Chloro-1-phenyl-ethene	0.6	0.5	0.5	<0.1	<0.1
	Total	37	39	36	6	3
Products from the epoxide						
4	Phenyl-oxirane	0.1	0.3	26	0.1	0.2
9	1-Phenyl-1,2-ethanediol[d]	14	27	6	21	22
3	Phenylacetaldehyde[d]	7	0.1	1.1	3	0.2
10, 11	Dimers (total)[d]	8	3.6	2.6	6	2.2
	Total	29	31	36	30	25
Other oxidation products						
2	Benzaldehyde[e]	0.5	0.5	0.5	1.0	0.8

[a] Mol % of chlorine dioxide added (the yields of the dimers were calculated on C_8 basis, *i.e.* twice the mol % yields). The yield figures are only approximate as they were determined from the peak areas of GLC assuming that the response factors were proportional to the number of carbon atoms in the molecule of the compound. [b] Mixed with a small amount of 1,2-dichloro-1-phenyl-ethane. [c] Peak 12 is not presented in Fig. 1 because the corresponding compound is not formed at pH 6. In other cases peak 12 is located between peaks 8 and 9. [d] These compounds were also formed when the epoxide was kept under the reaction conditions used. [e] Benzoic acid was also found in the reaction mixture.

The products and their yields are given in Table 2. These products consisted partly of compounds which are formed when hypochlorous acid reacts with styrene and partly of styrene oxide (= phenyl-oxirane) and compounds which are formed when styrene oxide is kept in aqueous solution. Considerable amounts of styrene oxide were obtained only when the oxidation was carried out at pH 6. When sulfamic acid was added the yields of the chlorinated products decreased greatly.

We conclude from these results that the main reaction route for the reaction between styrene and chlorine dioxide is that shown in reactions A, B and C, $R^1 = C_6H_5$ and $R^2 = H$.

Kinetics. The rate of the reaction between styrene and chlorine dioxide was determined by following the decrease in chlorine dioxide concentration. The styrene concentration was then taken as the initial styrene concentration minus the styrene consumption. In the absence of sulfamic acid (reaction sequence A, B, C) this consumption was assumed to be equimolar with the chlorine dioxide consumption. In the presence of sulfamic acid (sequence A, B, D) it was assumed to be half that of the chlorine dioxide.

We concluded in the following way that the epoxidation (reaction A) is the rate-determining step in both the routes A, B, C and A, B, D. Reaction B must be very rapid due to the instability of monochlorine monoxide. Reaction C was found to be about 100 times more rapid than the reaction between chlorine dioxide and styrene. Reaction D is almost instantaneous as it is more rapid than the chlorination of phenols.[7] The slow step is therefore the epoxidation (reaction A). When one mol of chlorine dioxide epoxidizes styrene another mol of chlorine dioxide is oxidized by monochlorine monoxide (reaction B). The epoxidation rate is therefore half of the rate of chlorine dioxide consumption.

When the reaction time was plotted in a diagram as a function of log [styrene]/[ClO_2], straight lines

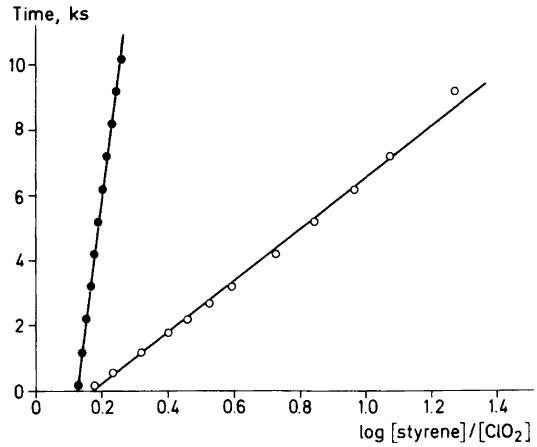


Fig. 3. The reaction time *versus* log [styrene]/[ClO_2] for the reaction between chlorine dioxide and styrene. ○, Solvent: water – *tert*-butyl alcohol 3.4:1 v/v; pH 2; $[ClO_2]_o = 10.16$ mM; $[styrene]_o = 14.05$ mM. ●, Solvent: water – *tert*-butyl alcohol 1:4 v/v; pH 3.5; $[ClO_2]_o = 11.30$ mM; $[styrene]_o = 14.80$ mM.

were obtained up to a consumption of 90 – 95 % of the chlorine dioxide (an example is given in Fig. 3). The kinetics of the reaction was therefore of the second order. The values for the rate constants of the epoxidation were calculated from the gradients of the lines (Table 3). They were about the same for pH 2, 4 and 6 and they did not change significantly when sulfamic acid was added. Chloride (concentration 0.5 M) decreased the rate distinctly.

Table 3. The bimolecular rate constant for the epoxidation of styrene with chlorine dioxide (reaction A). Solvent: water – *tert*-butyl alcohol (3.4:1 v/v).[a]

pH	$10^2 \times k_{II}$ [M^{-1} s^{-1}] Sulfamic acid Absent	Present[b]
2	4.22[c]	4.49:4.21
4	4.13:4.01	4.17:4.16
6	4.34	–[d]

[a] Reactions conditions: $[ClO_2]_o$ about 10 mM; $[styrene]_o$ about 15 mM; 30 °C; the solvent was buffered with phosphate buffers (0.28 M). [b] $[Sulfamic\ acid]_o = 10.3$ mM. [c] The value was 2.98 when NaCl was added, $[Cl^-]_o = 0.5$ M. [d] This experiment was not carried out because sulfamic acid reacted rapidly with chlorine dioxide at this pH.

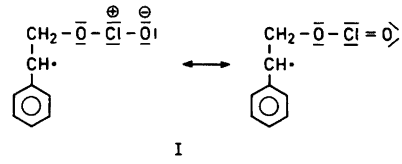
Table 4. The bimolecular rate constant for the epoxidation of styrene with chlorine dioxide (reaction A). Solvent: water – *tert*-butyl alcohol (1:4 v/v).[a]

pH	$[Styrene]_o$ mM	$[ClO_2]_o$ mM	$10^2 \times k_{II}$ M^{-1} s^{-1}
Acid[b]	25.4	1.9	0.45
Acid[b]	37.3	1.7	0.49[c]
3.5	14.8	11.3	0.42:0.45[d]
4	11.8	0.9	0.47
6	37.3	1.7	0.49[c]

[a] Reaction conditions: temperature 33.2 °C except for the run at pH 3.5 where the temperature was 30 °C, phosphate buffers. [b] Unbuffered solvent. [c] When sulfamic acid was added ($[sulfamic\ acid]_o = 3$ mM) the value was unchanged. When LiCl was added ($[Cl^-]_o = 40$ mM) the value was 0.46. [d] The run was carried out at a slightly lower temperature than the others (see *a*).

The solvent in the runs described in Table 3 was water – *tert*-butyl alcohol in the volume proportions 3.4:1. When the proportion was changed to 1:4 the rate was still of the second order (Fig. 3) but the values for the rate constant were only about a tenth of those obtained in the first solvent (Table 4). The influence of the chloride ion (concentration 40 mM) and of sulfamic acid (concentration 3 mM) on the rate was studied in runs with a large excess of styrene. The rate constant did not change significantly when these compounds were added.

The large decrease in the rate when a less polar solvent was used indicates that the transition state for the epoxidation (reaction A) is more polarized than the reactants. A possibility is that the transition state has a structure close to the structure 1. This structure may be more polarized than chlorine



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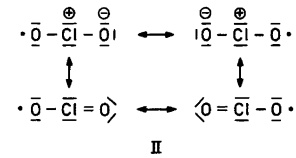

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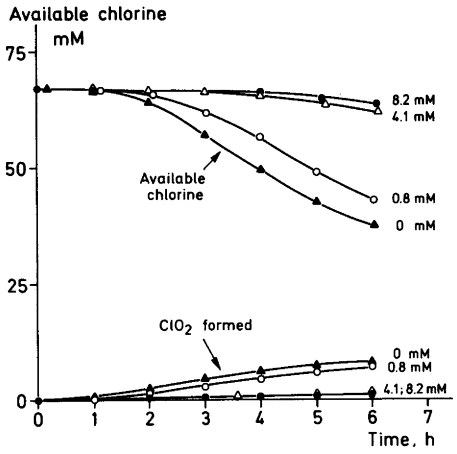

Fig. 4. The concentrations of available chlorine and chlorine dioxide (as available chlorine) *versus* time when styrene was treated with chlorite. Different amounts of sulfamic acid have been added. The figures in the diagram denote its concentration. Reaction conditions: temperature 30 °C, $[ClO_2^-]_o = 33.6$ mM; $[\text{styrene}]_o = 18.3$ mM, solvent: water – *tert*-butyl alcohol (3:1 v/v), pH 4 (phosphate buffer, 0.33 M).

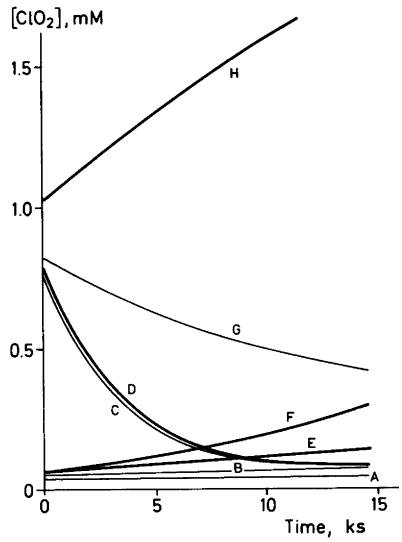

Fig. 5. $[ClO_2]$ *versus* time when styrene was treated with chlorine dioxide, chlorite and a mixture of them. Some runs were also carried out with sulfamic acid added. For comparison, the corresponding curves for the oxido-reduction of chlorite are included. Reaction conditions: pH 4 (phosphate buffer; 0.33 M); temperature 30.0 °C; solvent: water – *tert*-butyl alcohol (3:1 v/v).

Reactants present				Curve
Styrene 10.2 mM	ClO_2^- 20.5 mM	ClO_2 ~1 mM	Sulfamic acid 20.5 mM	
	+			E
	+		+	A
+	+			F
+	+		+	B
+		+		D
+		+	+	C
+	+	+		H
+	+	+	+	G

dioxide, in which the negative charge is distributed between the two oxygen atoms (structure II).

Our kinetic experiments thus show that the chlorine dioxide oxidation of styrene differs from that of lignin in that its rate does not increase either with increase in pH or when chloride is added.

Chlorite oxidation of styrene. The oxidation of styrene with chlorite as judged from the consumption of available chlorine was retarded by the addition of sulfamic acid (Fig. 4). The amount of sulfamic acid needed was small in comparison with the amounts of other reactants. The chlorite oxidation of phenols and lignin preparations is similarly retarded by a comparatively small amount of sulfamic acid.[6] The reason for this behaviour is due to the fact that chlorite itself is not the oxidizing agent. The oxidizing agent is chlorine dioxide which is formed by a chain process from the chlorite. The initiating reaction in this process may be the acid-catalyzed oxido-reduction of chlorite

$$4\ ClO_2^- + 2\ H^+ \rightarrow ClO_3^- + 2\ ClO_2 + Cl^- + H_2O \qquad E$$

(The properties of this complicated reaction are reviewed in Ref. 10.) The chlorine dioxide formed then reacts with the organic material and is thereby partly reduced to hypochlorous acid. The hypochlorous acid regenerates chlorine dioxide by oxidizing chlorite

$$HOCl + 2\ ClO_2^- \rightarrow HO^- + Cl^- + 2\ ClO_2 \qquad F$$

(This reaction is also reviewed in Ref. 10.) The chlorite oxidation of styrene should then consist of

the initiating reaction E and the chain sequence of the reactions A, B and F.

When chlorite was added to a styrene solution at pH 4, chlorine dioxide was however formed (curve F in Fig. 5). This does not fit with the reaction sequence A, B, F the sum of which does not yield chlorine dioxide. Nor could the chlorine dioxide have been formed by acid catalyzed oxido-reduction of chlorite (reaction E) as the formation of chlorine dioxide from chlorite in the absence of styrene was too slow under these conditions (curve E).

A similar formation of chlorine dioxide was obtained when styrene was treated with a mixture of chlorite and chlorine dioxide. Further amounts of chlorine dioxide were formed rapidly (curve H) in spite of the fact that chlorine dioxide when treated with styrene in the absence of chlorite was rapidly consumed (curve D). To obtain a chain mechanism which yields chlorine dioxide we have assumed that the intermediately formed monochlorine monoxide oxidizes not only chlorine dioxide to chlorate (reaction B) but also chlorite to chlorine dioxide.

$$ClO + ClO_2^- + H^+ \rightarrow HClO + ClO_2 \qquad G$$

The sum of the reactions A, G and F yields two mol of chlorine dioxide for every mol of chlorine dioxide which epoxidizes styrene.

The rate determining reaction in the sequence A, G, F should be the reaction A. During chlorine dioxide oxidation of styrene (sequence A, B, C) this reaction is also rate determining. This oxidation consumes two mol of chlorine dioxide; the sequence A, G, F produces the same amount. If, therefore, the oxidation of styrene with a mixture of chlorite and chlorine dioxide strictly followed the sequence A, G, F, the rate of this chlorine dioxide *production* should be equal to the rate of the chlorine dioxide *consumption* when the oxidation is carried out with chlorine dioxide alone. As shown by the gradients of the curves D and H at zero time, that was not the case; the production rate was lower than the consumption rate. This leads to the conclusion that *under the conditions used by us in the H run* the monochlorine monoxide formed reacted both with chlorine dioxide (reaction B) and with chlorite (reaction G).

When the oxidation of styrene with a mixture of chlorite and chlorine dioxide was carried out in the presence of sulfamic acid, a consumption of chlorine dioxide was observed (curve G) but not as great as that in the chlorine dioxide oxidation (curve D). This suggests that the reaction route was a mixture of the sequences A, G, D and A, B, D and thus agrees with our assumption that reactions B and G were competing.

Our results thus indicate that the chlorite oxidation of styrene follows the chain routes described by the reaction sequences A, B, F and A, G, F. The initiating reaction is probably the oxido-reduction of chlorite into chlorine dioxide, chlorate and chloride (reaction E). The chlorine dioxide formed then reacts with styrene. The monochlorine monoxide and the hypochlorous acid intermediately formed react with chlorite giving an increasing concentration of chlorine dioxide which should speed up the oxidation. In agreement with this the gradient of the curve F increases with time, which indicates that the rate really increases. When the chlorine dioxide concentration becomes greater an increasing part of the monochlorine monoxide should oxidize chlorine dioxide to chlorate. In agreement with this, the gradient of the curve H decreases with time.

CONCLUSIONS

The routes of chlorine dioxide reactions. Evidence has earlier been presented which shows that chlorine dioxide is reduced by a one-electron reduction to chlorite when it reacts with phenols.[11,12] This article presents a principally different route for the chlorine dioxide oxidation of organic materials, since the reaction with styrene is a two-electron oxido-reduction by which chlorine dioxide is reduced to monochlorine monoxide. Stilbene and other olefinic compounds may react similarly.

The delignification of pulp lignin. It has been suggested that monochlorine monoxide is formed during the chlorine dioxide bleaching of wood pulps and that it may oxidize chlorine dioxide to chlorate, which should explain a part of the chlorate formation during the bleaching.[4] These experiments have shown that monochlorine monoxide can be formed when olefinic materials react with chlorine dioxide and that it can oxidize chlorine dioxide to chlorate.

Chlorite. Chlorite oxidizes directly neither phenols,[6,12] lignin material[6,12] nor styrene. It must first be transformed into chlorine dioxide. At moderate acidity this transformation is a chain process (at low pH the formation of chlorine dioxide from chlorite by the acid-catalyzed oxido-reduction

may dominate over the chain process). As shown in this paper, more chlorine dioxide can be formed in this process from reactions of chlorite with monochlorine monoxide and hypochlorous acid than is consumed in reactions with the organic material.

EXPERIMENTAL

Styrene oxidation by chlorine dioxide and chlorite. The reaction conditions are given in note *a* to Table 3 and in the legend to Fig. 4.

The analyses of the inorganic products obtained from the chlorine dioxide oxidation of styrene. The concentration of chlorine dioxide was determined from the UV-absorptivity at 357 nm ($\varepsilon = 1250\ M^{-1}\ cm^{-1}$). The determinations of the other compounds are described in Table 1.

The analyses of the organic products obtained from the chlorine dioxide oxidation of styrene. When nearly all the chlorine dioxide was consumed, the reaction mixture was extracted with dichloromethane (3 times). The extract was dried with sodium sulfate and analyzed by gas chromatography and mass spectrometry. The gas chromatography conditions are given in the legend to Fig. 2. The peaks were identified by mass spectrometry and compared with authentic samples of the compounds.

The kinetics of the chlorine dioxide oxidation of styrene. The reaction was carried out in cuvettes kept thermostated in a Cary 118 spectrophotometer. The absorptivity at 357 nm was recorded continuously. The UV-light did not affect the rate of the reaction.

The rate of hypochlorous acid reaction with styrene. A solution of hypochlorous acid (10.35 mM) and styrene (13.94 mM) in water – *tert*-butyl alcohol (3.4:1 v/v, buffered to pH 6.4) was prepared and kept at 30 °C. The chlorine concentration determined iodometrically was 0.90 mM after 1 min.

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The Stereochemistry of Tetrahydroalstonine and Related Indole Alkaloids *

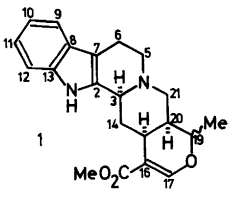
ERNEST WENKERT,** BÖRJE WICKBERG *** and CURTIS L. LEICHT

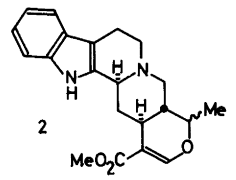
Department of Chemistry, Iowa State University, Ames, Iowa 50010, U.S.A.

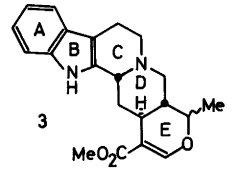
A chemical degradation of tetrahydroalstonine and correlation of a degradation product with one derived from ajmalicine has led to a reassignment of the C(20) stereochemistry of these and related alkaloids. ^{1}H NMR spectral analysis of these bases has yielded their full configurations.

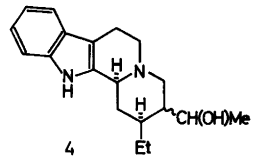
Preliminary experiments on model compounds during the period of active investigation of the stereochemistry of reserpine and related indole alkaloids in the middle 1950s showed that reactions designed to test the ease of electrophilic attack on the non-indolic nitrogen or ease of hydrogen abstraction from C(3) of natural bases of the yohimbine, ajmalicine and corynantheine types might reflect the stereochemistry of such substances.[2] Whereas differences of N_b reactivity were noticeable in pK_a measurements and N_b-oxide preparations, these observations remained unexploited up to the time of the development of an elegant method of stereochemistry analysis based on differences of N_b methiodide formation.[3,4] The observation of differences of ease of H(3) abstraction in palladium–maleic acid dehydrogenation of stereochemically different ring systems[2] was applied to a study of the configuration of ajmalicinoid alkaloids and led to the assignment of structures *1*, *2* and *3* to ajmalicine, tetrahydroalstonine and akuammigine, respectively.[5]

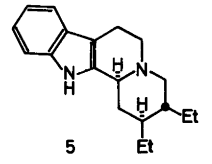
The involvement of heterogeneous catalysis in the H(3) abstraction process and the inherent difficulty of rigorous interpretation of reactions of such complexity made an alternative check of the configurational assignment of the ajmalicinoid alkaloids highly desirable. As a consequence, a degradation of tetrahydroalstonine, in a manner analogous to that of ajmalicine which had revealed the absolute configuration of the latter,[6] was contemplated but had to await the acquisition of sufficient quantity of the alkaloid. In the meantime, the conversion of the ajmalicine degradation product ajmaliciol *4*[6] to dihydrocorynantheane (*5*) by hydrogen bromide treatment and subsequent hydrogenolysis[7] shed serious doubt on the proposed relative configuration of the ajmalicinoid alkaloids[5] and made a reinvestigation of the











* For a preliminary account of this work see Ref. 1.

** Present address: Dept. of Chemistry (D-006), University of California-San Diego, La Jolla, CA 92093, U.S.A.

*** Present address: Division of Organic Chemistry 2, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund, Sweden.

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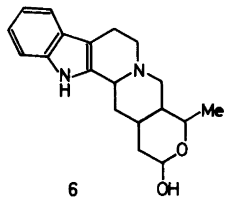
problem mandatory.*

Alkaline hydrolysis of tetrahydroalstonine, prepared by reduction of alstonine supplied kindly by Dr. J. Harley-Mason, and subsequent, short aqueous acid treatment yielded tetrahydroalstonial (*6*).** Wolff-Kishner reduction of the latter gave an isomer of the ajmalicine degradation product ajmaliciol *4*. Oppenauer oxidation of the isomeric alcohol under mild conditions produced a ketone which was isomeric with an analogue derived from ajmalicine. However, sodium methoxide-induced equilibration of the oxidation product transformed it into the ajmalicine-derived ketone [6] isomer. Since the latter thus had been shown to be the more stable 19-ketone, a substance expected to possess conformation *7*, it could be assigned the 18,19-dihydro-19-corynantheone (*8a*) structure and the tetrahydroalstonine-derived ketone the 19-corynantheidone (*8b*) formulation. As a consequence ajmaliciol (*4*)[6] could be attributed the 18,19-dihydro-19-corynantheol (*9a*) configuration, a structural detail reinforced by the observation of the isolation of this alcohol and its 19-epimer as products of sodium borohydride reduction of the stable 19-ketone. Finally, these results showed conclusively that tetrahydroalstonine possesses stereostructure *1* and ajmalicine *2*, in contrast to the previous structure assignments,[5] and that tetrahydroalstonine belongs to the H(15α) indole alkaloid family.[6]



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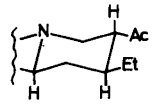

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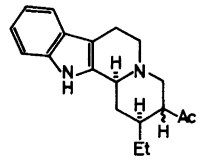

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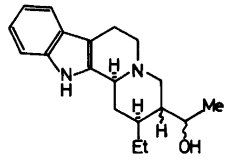
a, H(20β)
b, H(20α)



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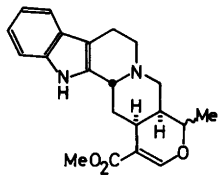
In the previous structure study [5] the ajmalicinoid alkaloid akuammigine had been oxidized to a ring C, tetradehydro product, which had appeared to be identical with alstonine. As confirmation of this correlation akuammigine was dehydrogenated again and the product exposed to Raney nickel-induced hydrogenation at high pH. This two-step procedure led to tetrahydroalstonine, corroborating the suggestion of akuammigine being 3-isotetrahydroalstonine and thus now possessing structure *10*.

Upon completion of the assignments of a *normal* configuration to ajmalicine (*2*) (and hence a *pseudo* structure to 3-isoajmalicine, *3* [5]), an *allo* configuration to tetrahydroalstonine (*1*) and an *epiallo* structure to akuammigine (*10*) only the C(19) stereochemistry of these alkaloids remained undetermined. Since a rapid solution of this problem appeared to reside in a ^{1}H NMR spectral analysis of the alkaloids, such a study on deuteriochloroform solutions of the bases was undertaken.

The *normal* and *pseudo* configurations of ajmalicine (*2*) and 3-isoajmalicine (*3*), respectively, limit these compounds to a rigid D/E *trans* framework possessing an axial H(20) conformation. Furthermore, the *pseudo* 3-isoajmalicine (*3*) has its H(3) limited to an equatorial conformation, a fact confirmed by the ^{1}H NMR spectrum of the compound exhibiting an H(3) multiplet at 4.45 ppm, a strongly downfield signal characteristic of equatorial H(3) substances.[10] The spectra of ajmalicine (*2*) and its 3-epimer (*3*) revealed H(19) pairs of quartets centered at 4.44 and 4.38 ppm, respectively, with H(19)-methyl coupling patterns of 6.0 and 7.0 Hz, respectively, and H(19)–H(20) coupling constants of 2.7 and 1.8 Hz, respectively. The *J* values for the H(19)-methyl interactions were obvious also from the splitting patterns of the 19-methyl doublets centered at 1.16 and 1.19 ppm, respectively. The weak coupling between H(19) and H(20) indicated the former hydrogen to be oriented equatorially and consequently the configuration of ajmalicine (*2*) and its 3-epimer (*3*) to be as depicted in

* At the end of their discussion of the stereochemistry of heteroyohimbine alkaloids Shamma and Richey [4] refer to a comment by van Tamelen at a 1961 American Chemical Society meeting on the total synthesis of ajmalicine purporting to prove a *trans* D/E ring fusion of the alkaloid. The absence of discussion of the stereochemistry in the later description of the ajmalicine synthesis [8] and the yet later proof of the presence of a mixture of C(20) epimers of a crucial, stereochemically determinant ketoester intermediate [9] in the total synthesis indicate this view to have been premature.

** This substance, reported m.p. 177 °C, was produced first by J. Harley-Mason and W. R. Waterfield.[15]



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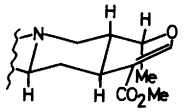

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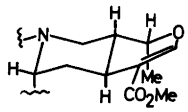

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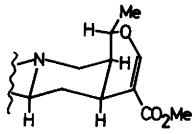

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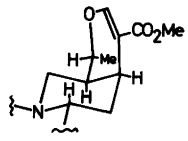

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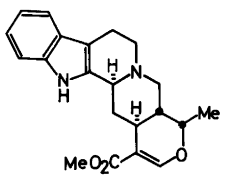
conformations *11* and *12*, respectively.

In view of the absence of a low-field signal, attributable to an equatorial H(3) substituent, in the ^{1}H NMR spectra of the D/E *cis*, 3-epimeric alkaloids tetrahydroalstonine (*1*) and akuammigine (*10*) their D and E ring conformations are limited to those portrayed in formulas *13* and *14*, respectively. The H(19) signals appeared as pairs of quartets centered at *ca.* 4.40 ppm, tetrahydroalstonine exhibiting H(19) – H(20) coupling of 10.2 Hz and akuammigine 5.9 Hz. These facts are compatible with a *trans* diaxial relationship of H(19) and H(20) in the former alkaloid and a *trans* diequatorial orientation in the latter base, as illustrated also in structures *13* and *14*, respectively.

The chemical degradations and ^{1}H NMR special interpretations led to stereostructures *15*, *16a* and *16b* for ajmalicine, tetrahydroalstonine and



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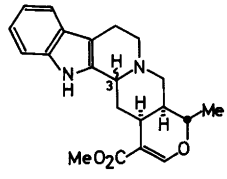

16a, H(3α)
b, H(3β)

akuammigine, respectively, in accord with data from recent syntheses of these natural substances.[11] The identity of the non-aromatic region of the ^{1}H NMR spectrum of tetraphylline[12] (with the exception of the signal of an extra *O*-methyl group) with that of ajmalicine (*15*) established this ajmalicinoid base as 11-methoxyajmalicine. Similar spectral comparison revealed aricine[13] to be 10-methoxytetrahydroalstonine and reserpinine[14] 11-methoxytetrahydroalstonine.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 60 MHz on a Varian V-4300 B instrument equipped with a Varian V-4365 field homogeneity control unit. The measurements were made at 24 °C using $CDCl_3$ as a solvent and tetramethylsilane as an internal standard. Melting points were determined on a micro hot stage (Reichert) and are uncorrected. All reactions were carried out under nitrogen; concentrations of solutions were performed under reduced pressure or under nitrogen.

Tetrahydroalstonial (6). A solution of tetrahydroalstonine (750 mg) and KOH (2.0 mg) in absolute ethanol (10 ml) was refluxed for 5 h. The solution was adjusted to pH 10 with dilute HCl, water was added to dissolve precipitated salts, and non-acidic impurities were removed by two extractions with chloroform. After concentration of the aqueous phase, aqueous HCl was added to give a solution (50 ml) which was 0.8 M with respect to excess acid. The mixture was refluxed for 5 h, then made alkaline with excess K_2CO_3, extracted with chloroform and the chloroform solution concentrated to dryness. The amorphous residue (600 mg) was dissolved in hot benzene and the solution immediately filtered through alumina (1 ml, act. IV). Crude *6* (407 mg) crystallized upon cooling. It dissolved readily in hot benzene (6 ml), but upon continued heating pure tetrahydroalstonial (*6*) separated as prisms (280 mg), m.p. 210 – 214 °C, $[\alpha]_D^{28}$ −122° (*c* 1.5, chloroform). Reported[15] m.p. 177 °C, $[\alpha]_D$ −137°. Found: C 72.34, H 7.78, N 8.83. Calc. for $C_{19}H_{24}N_2O_2$: C 73.04; H 7.74, N 8.97. IR ($CHCl_3$): OH 3620 (w), NH 3480 (m), no band attributable to C=O.

The high-melting modification (or 19-epimer) obtained from hot benzene could be transformed into the low-melting form by dissolving a sample in a small volume of methanol, quickly removing the methanol by evaporation with benzene, dissolving the amorphous residue in cold benzene and seeding it with a sample of m.p. 177 °C (kindly provided by Dr. Harley-Mason). Tetrahydroalstonial now separated as needles, m.p. 173 – 177 °C with rapid resolidification and new m.p. 209 – 213 °C. No

mutarotation attributable to hemiacetal epimerization was observed.

*19-Corynantheidol (*9b*).* A solution of tetrahydroalstonial (340 mg), anhydrous hydrazine (2.9 ml) and acetic acid (0.15 ml) in diethylene glycol (11 ml) was refluxed for 30 min. After addition of KOH (1.16 g) refluxing was continued for 30 min, then water and excess hydrazine were distilled off until the temperature in the solution reached 200 °C and this temperature was maintained for 3 h. Dilution with water and repeated extractions with chloroform afforded crude 19-corynantheidol (300 mg) as an oil, after concentration of the extracts. On adding a slight excess of picric acid in methanol (10 ml), crystalline 19-corynantheidol picrate (370 mg) separated; m.p. 216–222 °C, d. Found: C 57.07, H 5.62, N 13.63. Calc. for $C_{25}H_{29}N_5O_8$: C 56.92, H 5.54, N 13.28. IR (Nujol): OH 3570, NH 3360. After liberation from its pure picrate, 19-corynantheidol (*9b*) failed to crystallize, $[\alpha]_D^{25}$ −121° (*c* 2.0, pyridine).

*19-Corynantheidone (*8b*).* A solution of 19-corynantheidol (250 mg) and cyclohexanone (14 ml) in benzene (50 ml) was made anhydrous by distilling off *ca.* 30 ml of benzene through a Vigreux column. Aluminum phenoxide (1.7 g) was then added and the clear solution kept at 100 °C for 8 h. The gelatinous reaction mixture was triturated with 1 M H_2SO_4 (2 × 45 ml), the extract was washed repeatedly with small portions of ether to remove cyclohexanone, then neutralized with K_2CO_3, made alkaline with NH_4OH and filtered with Celite. After dehydration of the filter cake with a small volume of methanol, both the filter cake and the combined filtrates were extracted with chloroform and the residue after concentrating the extracts was chromatographed on an alumina column (13 × 190 mm). Crude crystalline 19-corynantheidone (45 mg) and unreacted starting material (87 mg) were obtained by elution with benzene–chloroform (99:1 and 1:1, respectively). Recrystallization from benzene–cyclohexane and aqueous ethanol afforded pure 19-corynantheidone (*8b*); m.p. 152–153 °C, $[\alpha]_D^{28}$ −61° (*c* 1.5, chloroform). Anal. $C_{19}H_{24}N_2O$: C, H, N. IR (chloroform): NM 3480 (m), C=O 1710 (s). Mixed melting point with 18,19-dihydro-19-corynantheone (m.p. 225–228 °C): 143–215 °C, confirming nonidentity with the latter.

Alkaline epimerization of 19-corynantheidone. A solution of 19-corynantheidone (19 mg) in 2 M methanolic sodium methoxide (3 ml) was refluxed for 3 h. After dilution with water and extraction with chloroform the extract was filtered through a small quantity of alumina and concentrated to give a crystallizing oil. Recrystallization from benzene–cyclohexane and methanol gave 18,19-dihydro-19-corynantheone (*8a*) (15 mg); m.p. 225–228 °C, $[\alpha]_D^{28}$ −59° (*c* 1.5, chloroform). Lit.[6] 225–228 °C, $[\alpha]_D$ −57°. Mixed melting point and IR proved the identity with an authentic sample of *8a*.

*Ajmaliciol (*9a, 19S*) and 19-isoajmaliciol (*9a, 19R*) from* 8a. A solution of 18,19-dihydro-19-corynantheone (243 mg) and a large excess of $NaBH_4$ in 80% aqueous methanol (20 ml) was kept at 0 °C for 4 h and then at 50 °C for 30 min. The mixture was acidified with dilute HCl, made basic again with NH_4OH and then kept at 0 °C for 4 h to give needles of crude ajmaliciol (132 mg). Extraction of the mother liquors with chloroform and concentration of the extract gave a residue that afforded more ajmaliciol (25 mg) from aqueous methanol. Removal of the solvents from these mother liquors and recrystallization of the residue from benzene gave 19-isoajmaliciol (100 mg).

19-Isoajmaliciol apparently forms solvated crystals both from benzene and methanol, m.p. *ca.* 110 and 118 °C, respectively; on further heating it resolidifies and melts at *ca.* 175° and 185 °C with transient intermediary crystallization. A sample was distilled in a tube at 170 °C and 0.1 mm pressure and the amorphous distillate was kept for 15 min at 150 °C *in vacuo* to give the high melting modification of 19-isoajmaliciol as tetrahedral prisms, m.p. 195–197 °C, $[\alpha]_D^{28}$ −101° (*c* 1.5, pyridine). Found: C 76.02, H 8.82, N 9.16. Calc. for $C_{19}H_{26}N_2O$: C 76.47, H 8.78, N 9.39. IR (Nujol): OH 3570 (w), NH 3310 (m).

Ajmaliciol (lit.[6] m.p. 200–201 °C, $[\alpha]_D$ −25° in pyridine), prepared as described previously,[6] was now found to have $[\alpha]_D^{28}$ −109° (*c* 1.5, pyridine), and to exhibit multiple melting points when crystallized from methanol (m.p. 110–120 °C, 135–145 °C, 176–179 °C and 198–200 °C. From benzene only the high-melting modification was obtained (m.p. 198–200 °C).

*Tetrahydroalstonine (*16a*) from akuammigine (*16b*).* When dehydrogenated by the palladium–maleic acid method as described previously,[5] akuammigine gave a tetradehydro compound, the perchlorate of which melted at 227–229 °C. However, an authentic sample of alstonine perchlorate (m.p. 241–246 °C d.) could be converted to a low-melting modification (m.p. 228–230 °C) by seeding its methanolic solution with the present dehydrogenation product; the two preparations gave no melting point depression.

Tetradehydroakuammigine perchlorate (10.8 mg) was hydrogenated in 0.05 M methanolic KOH (2 ml) in the presence of Raney nickel W-2 (20 mg). The hydrogenation slowed down after an uptake of 1.8 molar equivalents of H_2. The reaction mixture was filtered after acidification with acetic acid, diluted with water, made alkaline with NH_4OH and extracted with chloroform. Concentration of the extract and recrystallization from aqueous ethanol gave tetrahydroalstonine (4.5 mg), m.p. 227–230 °C, undepressed on admixture with an

authentic sample (m.p. 229 – 230 °C). IR confirmed the identity of the two preparations.

Acknowledgements. The authors are indebted to the U.S. Public Health Service for financial support of this work. This paper is submitted in honour of Professor Holger Erdtman on the occasion of his 80th birthday in appreciation of his contributions to organic chemistry.

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Studies on Pitch Problems Caused by Pulping and Bleaching of Tropical Woods. XIV.* Chemistry of the Aurone Derivatives at the Conventional Bleaching Stages

YOSHITO OHTANI and MASASHI SUMIMOTO

Faculty of Agriculture, Kyushu University, Higashi-ku Hakozaki, Fukuoka 812, Japan

Rengasin (*1*), which is responsible for the colored specks on a sheet of bleached sulfate pulp from the woods of rengas (Anacardiaceae), was converted into R_1 *7* having a novel carbon skeleton. A closely related analogue, S_1 *8*, prepared from sulfuretin (*2*) was used as a model to investigate further conversions under bleaching conditions. Chlorination of S_1 *8* produced an equilibrium mixture involving compound *9* as a dominant compound. The latter was transformed by the treatment with chlorine dioxide into an ortho-quinone (*16*) *via* two pathways containing *10* in one and *15* in another. The monomeric quinone (*16*) immediately dimerized in acidic media to give *20* under the same conditions as above, and the further oligomerization by the process similar to that in dimerization of *16* finally afforded a mixture of colored oligomers *26*.

Some characteristic phenomena as regards the formation of brown pitch specks on sheets of bleached sulfate pulps (BKP) from rengas wood (*Gluta* or *Melanorrhoea* sp., Anacardiaceae) grown in Sarawak and their exclusion from the pulps were mentioned in our previous papers.[1,2] Rengasin (*1*) responsible for the brown specks was chemically converted during the alkaline cooking and subsequent bleaching stages into the final colored specks, while the latter were effectively removed by application of a phase transfer catalyst to the alkaline extraction stage in the conventional multistage bleaching. This proves that all the conversion reactions of rengasin (*1*) proceeded in the interior of the neutral hydrophobic membrane formed with the components of wood extractives. As for the chemical conversion of rengasin (*1*) during the cooking stage, 4-methoxy-6-hydroxy-coumaranone (*3*), protocatechualdehyde (*5*), and an intermediate R_1 (*7*) were isolated from the pulp extractives but not from the wood extractives. This seems to indicate the presence of a thermal equilibrium[3] among these compounds which is slightly different from the scheme shown in Fig. 1. Sulfuretin (*2*) is not only a closely related derivative of rengasin (*1*) but also responsible for colored pitch specks on sheets of BKP from the wood of sepetir paya (*Pseudosindora palustris*).[4] Sulfuretin also affords intermediate S_1 *8*[5] as a component of an equilibrium system similar to that involving R_1 *7* formed from rengasin (*1*). In the present paper, therefore, intermediate S_1 *8* was adopted as one of the aurones to elucidate the mechanism for the formation of colored specks during the bleaching stages, since S_1 can be synthesized more readily than R_1. Although the colored specks can be produced by treatment with chlorine dioxide (ClO_2) alone, it has been suggested[1] that chlorination prior to the ClO_2 treatment plays an important role in making stable colored specks. The chlorination of S_1 will be discussed first under these considerations.

RESULTS AND DISCUSSION

Treatment of S_1 8 with chlorine. Although chlorination of *8* at 25 °C was found to be nearly completed within twenty minutes as confirmed by thin layer chromatography (TLC), the reaction was

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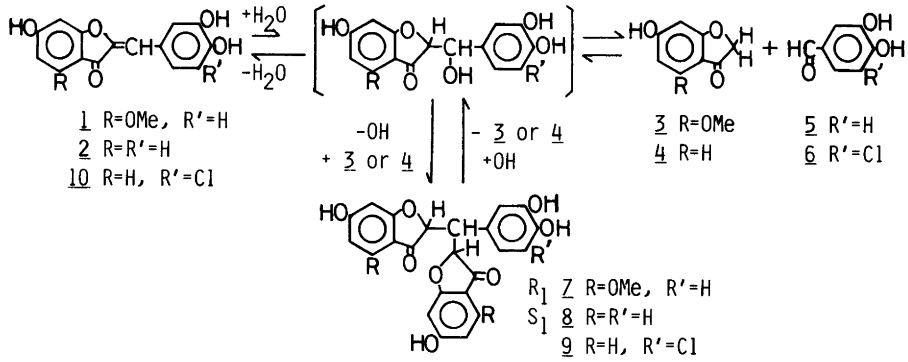


Fig. 1. Acid-catalyzed equilibria between aurones (*1*, *2* and *10*) and coumaranone adducts (*7*, *8* and *9*).

continued for a further twenty minutes. The resulting products were chromatographed on a silica-gel column to separate into seven fractions. The contributing yields and ratios [1] of the individual fractions to the total speck area are shown in Table 1. Fractions 2 and 3 were crystallized and identified as 6-hydroxy-coumaranone (*4*) and 5-chloro-protocatechualdehyde (*6*), respectively. In another experiment, the authentic sample of *6* for identification was synthesized by chlorination of protocatechualdehyde (*5*). Fraction 4, showing the highest yield as well as the highest contributing ratio, also gave crystalline compound *9* whose mass spedctrum and elemental analysis are consistent with molecular formula $C_{23}H_{15}O_8Cl$. Its mass fragmentation pattern was quite similar to that of S_1 *8* and R_1 *7* [3] and, therefore, suggested the presence of a chlorine atom in the B-ring of the same carbon skeleton as that of S_1 *8*. Spectral data obtained by ^{13}C nuclear magnetic resonance (CMR) of acetates *11*, *13*, *12* and *14*, all of which have been prepared by acetylation of *2*, *8*, 5'-chloro-sulfuretin (*10*), and *9*, respectively, are summarized in Table 2 and their numbering system is given in Fig. 2. Assignments of

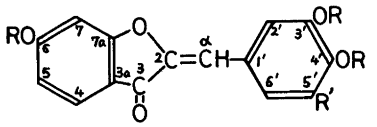
Table 1. Yields and contribution ratios of each fraction separated from the chlorinated mixture of *8* to the total speck areas.

Fraction number	Compound	A[a]	B
1		8.6	5.8
2	*4*	19.7	17.1
3	*6*	13.7	15.3
4	*9*	29.2	35.8
5		3.3	3.5
6		8.7	10.1
7		11.6	12.4

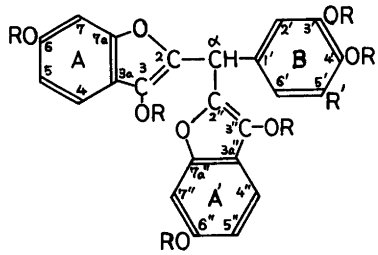
[a] A, Yield (weight percent to the total chlorinated mixture). B, Contribution ratio to the total speck areas (percent).

Table 2. Comparison of ^{13}C NMR spectral data (ppm from TMS) of the acetates *11*, *12*, *13*, and *14* in DMSO-d_6.

Carbon	*11*	*12*	*13*	*14*
2	146.80	147.77	151.67	151.71
3	182.18	182.18	143.20	143.19
3a	124.20	127.71	120.42	120.41
4	125.24	125.66	130.96	131.22
5	110.50	107.47	119.27	119.47
6	166.00	166.26	148.38	148.53
7	107.21	104.66	117.93	118.06
7a	167.88	166.96	148.40	148.54
α	118.35	118.00	106.05	106.09
1'	142.17	142.17	134.62	135.62
2'	125.85	124.38	123.90	122.05
3'	143.08	143.08	141.50	143.72
4'	157.40	157.78	141.99	139.61
5'	129.75	148.30	123.90	142.61
6'	130.41	130.46	112.76	127.34
2''	—	—	151.67	151.71
3''	—	—	143.20	143.19
3a''	—	—	120.42	120.41
4''	—	—	130.96	131.22
5''	—	—	119.27	119.47
6''	—	—	148.38	148.53
7''	—	—	117.93	118.06
7a''	—	—	148.40	148.54



11 R=$COCH_3$, R'=H
12 R=$COCH_3$, R'=Cl



13 R=$COCH_3$, R'=H
14 R=$COCH_3$, R'=Cl

Fig. 2. The acetates of the compounds *2*, *10*, *8* and *9*.

the signals were carefully made in reference to the CMR spectral data with those of aurone derivatives[6] and flavone derivatives.[7] All of the signals due to 23 carbons of *14* show δ-values almost identical with those of *13* but one due to 5'-carbon. The latter signal demonstrates a remarkable down-field shift compared to the corresponding signal for *13*, which is nearly in accordance with a difference in resonance field between the 5'-carbon signals of *11* and *12*. However, the unusually high down-field shift caused by the substituted chlorine atom is known as a characteristic feature of a tri-substituted B-ring.[8] The above results are, therefore, consistent with structure *14*. A further evidence for structure *9* was provided by the alkaline fission of the compound to afford 6-hydroxy-coumaranone (*4*) and 5'-chloro-sulfuretin (*10*). The fission mode is in good agreement with those of R_1 *7*[3] and S_1 *8*.[5] The authentic sample of 5'-chloro-sulfuretin (*10*) for identification was synthesized independently by condensation of 6-hydroxy-coumaranone (*4*) with 5-chloro-protocatechualdehyde (*6*) in the usual manner. In the light of the accumulated spectral and chemical evidence, structure *9* was assigned to the major chlorination product from S_1 *7*.

Since the chlorination mixture at 25 °C was confirmed to contain *4* and *6* together with *9*, an equilibrium shown in Fig. 1 must be established. In this case, however, 5'-chloro-sulfuretin (*10*) was not detected in spite of our expectation. As has been already mentioned,[3] a methanolic solution of *4* and *5*, which was adjusted to pH 2.5 by adding hydrochloric acid at room temperature, gave S_1 *8* in a 15% yield. When a methanolic solution of 6-hydroxy-coumaranone (*4*) and 5-chloro-protocatechualdehyde (*6*) was treated in the manner as mentioned above, it afforded *10* and *9* in 13.4 and 9.1% yields, respectively. These findings actually indicate the presence of an equilibrium during the chlorination as shown in Fig. 1, and allow one to propose that *10* produced in a low yield on chlorination of S_1 *8* immediately undergoes the addition of chlorine to its double bond, followed by an oxidative cleavage. As shown in Fig. 1, however, the equilibrium system in acidic media at the present low temperature must involve some other reaction pathway different from that in neutral media at 170 °C shown in our previous paper.[5] In any case, occurrence of the equilibrium may be primarily attributed to the somewhat sterically unstable nature of bonds around the tertiary carbon atoms placed at the center of the molecules, *8*, *7* and *9*.

Chlorine dioxide treatment of 9. Chlorine dioxide prepared as a bleaching agent in the laboratory as well as in the bleach plant generally contains a few percent of chlorine[9] which lowers the pH of bleaching liquor to about 4.0 – 4.5, and the reaction temperature is usually maintained at 60 °C.

When a methanolic solution of *9* (1.0 mmol) was adjusted by dilute hydrochloric acid to pH 4.0 and kept at 60 °C for ten to ninety minutes, the resulting mixture afforded *ca.* 0.5 mmol of *10*, 0.4 mmol of *9*, and 0.4 mmol of *4* regardless of duration of the reaction. The constant yields of the above compounds, therefore, prove that a certain equilibrium is achieved immediately at that temperature. However, the equilibrium thus attained is in favor of the formation of *10* relative to the case when S_1 *8* is chlorinated at such a low temperature and pH. As mentioned in the previous section, 5'-chloro-sulfuretin (*10*) cannot be obtained by direct chlorination of sulfuretin (*2*) but only by a splitting reaction from *9* at the stage of ClO_2 treatment.

When *9* was treated with ClO_2 in *t*-butanol – water for ten minutes at 60 °C, the reddish quinoid compound (*15*) was furnished in a yield of 18.2%. Its IR[10] and UV spectra[11] indicate an ortho-quinone structure. Reduction of *15* with sodium borohydride ($NaBH_4$) afforded *9* as a sole product. The quinoid compound is, therefore, formulated as *15*. When *15* was further treated at

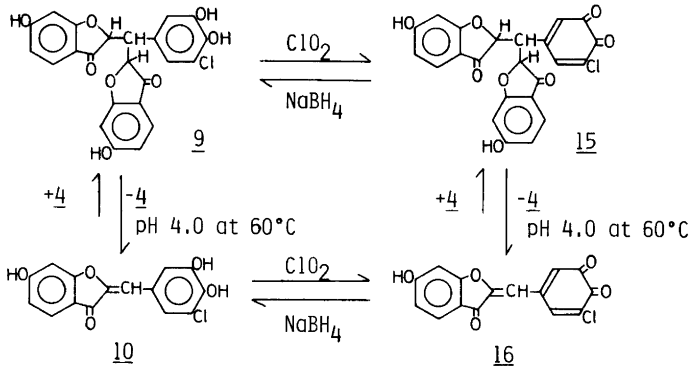


Fig. 3. Transformation of *9* to *16* in the reaction media with ClO_2.

60 °C in weakly-acidic media, it underwent a fission to give 6-hydroxy-coumaranone (*4*) along with the formation of another reddish product. The latter was also confirmed to be an ortho-quinone by spectral data and reduced with $NaBH_4$ to afford *10*. Accordingly, the second quinone must have structure *16*.

On the other hand, compound *16* was also obtained in a relatively high yield by oxidation of *10* with ClO_2. After all, a major fraction of *9*, the dominant product on chlorination of S_1, can be transformed into *16* by the treatment with ClO_2 *via* the two different pathways as shown in Fig. 3. In other words, as a result of the complete chemical conversion of *2* caused by cooking during the bleaching process, an important intermediate (*16*) was produced in a comparatively high yield. While *16* is stabilized by the chlorine substituent[12] at 5′-position, it can be subjected further to an acid-catalyzed oligomerization by treatment with ClO_2. However, the intermediate (*16*) used for the experiments in later sections was prepared by oxidation of *10* with sodium metaperiodate ($NaIO_4$),[13] because ClO_2 oxidation of *10* was accompanied by some undesirable reactions.

Reaction of 16 *in weakly-acidic media.* A further reaction of *16* in weakly-acidic media at 60 °C afforded four products as confirmed by TLC of the reaction mixture. Quantitative analysis of these products at various lengths of reaction time was performed by using a chromato-scanner. The products were designated as I–IV on the basis of the magnitude of R_F values on TLC in a decreasing order, IV being zero. The changes in yields of these components are shown in Fig. 4. While the starting material (*16*) rapidly decreases within five minutes, III appeared instantaneously and increased until the elapse of thirty minutes, and decreased gradually thereafter. In contrast, the reddish product (IV) increased slowly. In fact, the direct conversion of III to the final product (IV) was achieved under the same conditions as above and this proves that the former is one of the major precursors for the latter. The chemical structure for III is to be discussed in the next section. Component II, which appeared fifteen minutes after the initiation of reaction, was obtained but in a low yield and identified as *10*. This suggests the occurrence of some intermolecular oxidation-reduction, though the detail is still unknown.

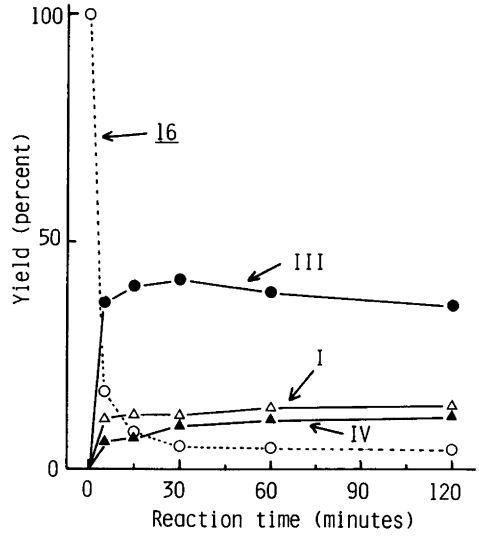


Fig. 4. Change in the yields of I, III and IV obtained from the reaction mixture of *16*.

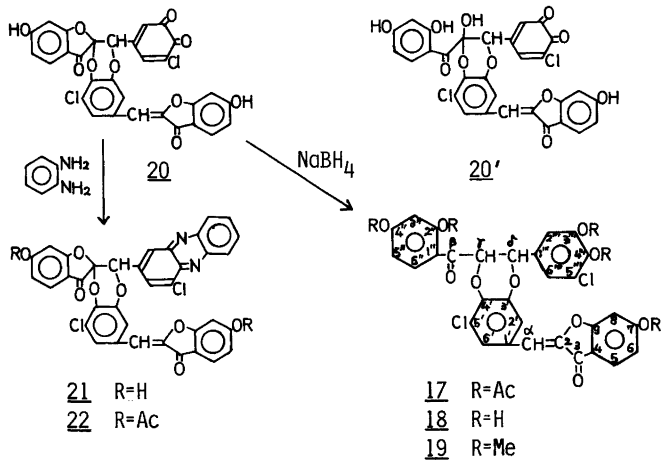


Fig. 5. The dimer III *20* and the derivatives.

The chemical structure for III. The reddish crystalline compound (III) obtained by reaction of *16* in weakly-acidic media, is taken as the direct precursor for the final product (IV). However, III is unfortunately too labile in the free form to be purified. The compound (III) was, therefore, reduced with $NaBH_4$, and then acetylated with acetic anhydride and pyridine to afford *17*. Its FD and CMR spectra indicated that the product has a carbon skeleton of 5'-chloro-sulfuretin (*10*). On the basis of its ^1H NMR spectrum (PMR), which revealed signals due to two vicinal methine protons, chemical structure *17* was assigned to the acetate. To confirm this, reduction product *18* from III was methylated with dimethyl sulfate and potassium carbonate in acetone, and the resulting methyl ether *19* (420 mg) was ozonized. This was followed by decomposition of the ozonide with hydrogen peroxide and acetic acid to afford 38 mg of 4-methoxy-salicylic acid (*23*), 23 mg of 2,4-dimethoxy-benzoic acid (*24*), and 135 mg of an unknown mixture. The latter mixture was, therefore, oxidized further with alkaline permanganate and yielded solely 10 mg of 5-chloro-veratric acid (*25*). As shown in Fig. 5, *23*, *24* and *25* must originate from rings A, A' and B' of *19*, respectively. The extent of yields and that of procurement of the acids under the experimental conditions may allow structure *19* to be satisfactorily assigned to the methyl ether, and hence, structure *18* to the reduction product from III. This leads to confine possible chemical structures for III, an ortho-quinonoid compound, to *20* and *20'*. It is conceivable that structure *20'* accounts better than *20* for the ready reduction to *17*, but it may not for the stable feature toward an oxidant, ClO_2. However, III afforded a phenylenediamine adduct *21* under mild conditions and the latter produced a stable crystalline acetate

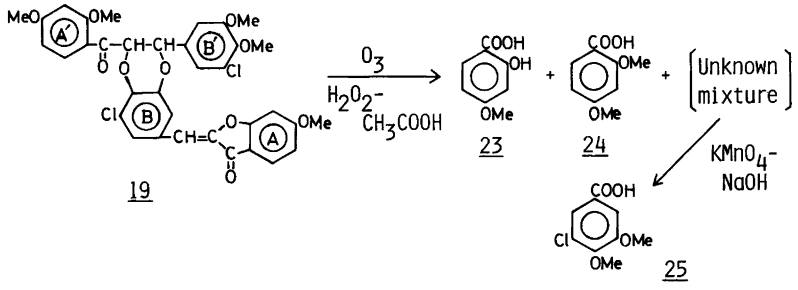


Fig. 6. Degradation of the methyl ether *19*.

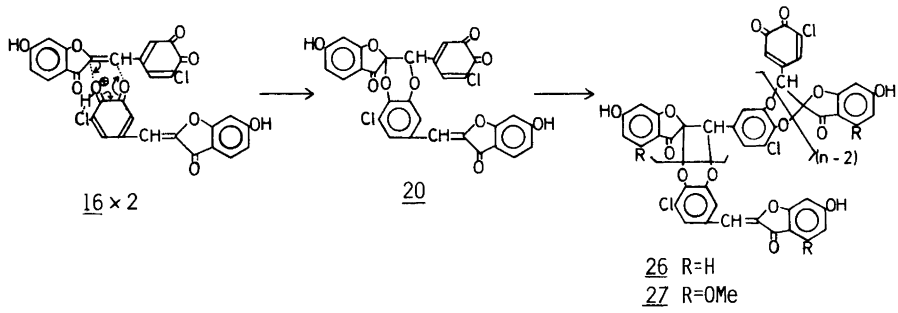


Fig. 7. Condensation of *16 via* the dimer *20* to the final oligomers *26*.

(*22*). The PMR spectrum of the crystalline acetate revealed proton signals due to two acetoxyl groups and one methine proton, as a singlet, fully corresponding to structure *22*. The elemental analysis was also consistent with *22*. Based on the findings mentioned above, structure *20* for III is preferred to *20'*.

It has been generally realized that the ortho-quinone undergoes two types of condensation reactions, one is the diene addition[13,14] between two quinone rings through the Diels-Alder process, and another is the formation of benzodioxene derivatives.[15-17] However, the third type of intermolecular condensation through acid-catalyzed oxidation-reduction between a quinone and a phenolic moiety has been reported very recently from our laboratory.[18] Therefore, the formation of the dimer (*20*) apparently belongs to the second type which is also an acid-catalyzed condensation as shown in Fig. 7. Nevertheless, the PMR spectrum of impure dimer III disclosed signals corresponding to those of methine protons,[14] which may be derived from the products furnished by the first type of reaction. Therefore, dimer III, having difficulty in purification, may be contaminated by a small amount of dimers produced by the Diels-Alder process.

The colored oligomers, IV. The elemental and chlorine analyses of the final product (IV) separated by column chromatography proved that the elemental composition was in good agreement with that of *16*. The UV and IR spectra of IV are quite similar to those of *20*. Reduction of IV with $NaBH_4$ followed by acetylation gave the product, whose CMR spectrum is almost identical with that of *17* and particularly indicates signals referred to β, γ, and δ carbons. The numbering system for the skeletal carbon atoms of *17* is shown in Fig. 5. The colored substance (IV) apparently consists of a mixture of oligomers of *16*. The oligomerization seems to proceed by the same process as dimerization of *16* to give *20*. This leads us to postulate structure *26* for IV as shown in Fig. 7. However, the gel-permeation chromatogram of *26* indicated the molecular weights of the predominant fractions to be about 1500 – 2000, corresponding to the pentamer or hexamer of *16*. Accordingly, the color of IV is developed only from the terminal ortho-quinone in *26*.

It must be emphasized again that the complete chemical conversions mentioned above proceed in the interior of the neutral hydrophobic membrane, through which chlorine and ClO_2 penetrate.[19] The final oligomers (*26*) are almost insoluble in many organic solvents, in a manner similar to that observed for the colored specks which appeared on sheets of BKP from the wood of rengas as well as sepetir paya.[4] The oligomers (*26*) and conceivable analogues *27* originated from rengasin *1* are, therefore, suggested to constitute one of the major parts of the colored speks in the pulps from sepetir paya and rengas, respectively.

CONCLUSION

The chemical conversions of S_1 *8* to the final colored oligomers (*26*) occurred through bleaching reactions were discussed. The dominant product (*9*) at the chlorination stage is transformed *via* two pathways into 5'-chloro-sulfuretin quinone (*16*) as the major product at the early stage of ClO_2 treatment. However, the monomeric quinone (*16*) readily undergoes condensation in acidic media to the dimer (*20*) and then gradually to the final colored oligomers (*26*) at the later stage of ClO_2 treatment.

The latter (*26*) and its plausible analogues *27* are suggested to be one of the major constituents of the colored specks in BKP's from the woods of sepetir paya and rengas, respectively.

EXPERIMENTAL

Synthesis of S_1 8. This was prepared by the method used for the synthesis of R_1 7.[3] The product obtained by the treatment of the compounds (*4*) and (*5*) with 0.5 N HCl-methanol (MeOH) was chromatographed on silica-gel column (benzene – ethyl acetate – formic acid = 10:4:1, designated as solvent A to afford the fraction, recrystallization of which from MeOH furnished S_1 *8*. (Yield 11.0%, m.p. (d.p.) 275 °C.

Chlorination of S_1 8. S_1 *8* (2.0 g) was dissolved in 200 ml of *t*-butanol (t-BuOH), to which 200 ml of aqueous chlorine solution (24 mmol) was added under stirring. After 40 min the solution was neutralized and evaporated to small volume under reduced pressure and the residual solution was extracted with ethyl acetate (EtOAc). The EtOAc solution was washed sufficiently with water and then evaporated to dryness. The residue (2.4 g) was separated on a silica-gel column (solvent A) into seven fractions. Each fraction was subjected to determination of the contributing ratio to the total speck area according to the previous method.[1] Among them Fraction 2 was recrystallized from MeOH to afford orange plates (260 mg), having m.p. 245 – 247 °C. This was identified as 6-hydroxy-coumaranone (*4*) by comparing the m.p. mixed m.p., and IR spectra with those of the authentic sample. Fraction 3 was recrystallized from MeOH – H_2O to afford white powder (150 mg), m.p. 228 – 230 °C. MS: 172 (M). Found: C 48.46; H 3.12. Calc. for $C_7H_5O_3Cl$: C 48.52; H 2.91. UV [abs. MeOH (log ε)]: 240 (3.18), 291 (3.12), 328 (2.88) nm. IR (KBr): 1640, 1590, 770 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.73 (1H, s, CHO), 7.37 (1H, d, *J* 2.0 Hz), 7.28 (1H, d, *J* 2.0 Hz). The results and direct comparison to the authentic sample confirmed the identity with 5-chloro-protocatechualdehyde (*6*).[20] The authentic sample (*6*) was prepared by direct chlorination of the compound (*5*) in *t*-BuOH – H_2O followed by chromatography on silica-gel column (solvent A) (yield 12%). Fraction 4 was recrystallized from MeOH to afford white powder *9* (550 mg), m.p. over 270 °C. MS: 454 (M), 304, 269, 168, 150, 121. Found: C 59.12; H 3.21. Calc. for $C_{23}H_{15}O_8Cl.1/2\ H_2O$: C 59.55; H 3.48. UV [abs. MeOH (log ε)]: 230 (4.27), 276 (4.15), 317 (3.95) nm. IR (KBr): 1680, 1590, 1420, 800 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.42 (1H, q, *J* 8 Hz, Hα), 5.08 (2H, d, *J* 8 Hz, $H_{2,2''}$), 6.30 (2H, d, *J* 2 Hz, $H_{7,7''}$), 6.46 (1H, d, *J* 2 Hz, $H_{2'}$), 6.52 (2H, dd, *J* 8 Hz, $H_{5,5''}$), 7.06 (1H, d, *J* 2 Hz, $H_{6'}$), 7.40 (2H, s, *J* 8 Hz, $H_{5,5''}$).

The compound (*9*) was acetylated with acetic anhydride and pyridine to produce the acetate, which was recrystallized from isopropyl ether to afford white crystalline powder *14*, m.p. 168 – 170 °C. MS: 706 (M). Found: C 54.23; H 3.98. Calc. for $C_{23}H_9O_2Cl(OCOCH_3)_6$: C 54.54; H 3.85. UV [abs. MeOH (log ε)]: 254 (4.15), 311 (4.12), 371 (3.94) nm. IR (KBr): 1760, 1610, 1480, 890, 810 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.06 (6H, s, OAc × 2), 2.31 (12H, s, OAc × 4), 6.34 (1H, s, Hα), 6.96 (2H, dd, *J* 8 Hz, $H_{5,5''}$), 7.18 (2H, d, *J* 2 Hz, $H_{7,7''}$), 7.24 (1H, d, *J* 2 Hz, $H_{6'}$), 7.30 (2H, d, *J* 8 Hz, $H_{4,4''}$), 7.35 (1H, d, *J* 2 Hz, $H_{2'}$). The chemical shifts of its ^{13}C NMR spectrum were shown in Table 2, and all ^{13}C NMR spectra were measured in DMSO-d_6 with tetramethylsilane as an internal standard.

The compound (*9*) (230 mg) was dissolved in 15% KOH-MeOH and refluxed for an hour. The solution was acidified and extracted with EtOAc. The extracts evaporated were chromatographed on silica-gel column (solvent A) to afford two compounds. The one (45 mg) was identified as 6-hydroxy-coumaranone (*4*) and the other was recrystallized from MeOH – H_2O to afford yellow powder (58 mg), m.p. over 270 °C. MS 305 (M), 270 (M – 35), 150. Found: C 54.36; H 3.59. Calc. for $C_{15}H_9O_5Cl{\cdot}3/2\ H_2O$: C 54.31; H 3.64. UV [abs. MeOH (log ε)]: 259 (3.82), 390 (4.01) nm. IR (KBr): 1680, 1580, 810 cm^{-1}. ^1H NMR (DMSO-d_6): δ 6.72 (1H, dd, *J* 8 Hz, H_5), 6.73 (1H, d, *J* 2 Hz, H_7), 6.90 (1H, d, *J* 2 Hz, $H_{6'}$), 6.92 (1H, s, Hα), 7.64 (1H, d, *J* 8 Hz, H_4), 7.80 (1H, d, *J* 2 Hz, $H_{2'}$). This was identified as 5′-chloro-sulfuretin (*10*) through direct comparison of spectral data with those of the authentic sample, which was obtained by condensation[21] of *4* with *6*.

The methanolic solution (100 ml) of 240 mg of *4* and 140 mg of *6* was adjusted with HCl to pH 2.5 and stood for 40 min under stirring. To the equilibrium mixture a large volume of water was added and this was extracted with EtOAc. After evaporation of the solvent the residue (374 mg) was chromatographed on a silica-gel column (solvent A) to afford 50 mg of *10* and 34 mg of *9*.

ClO_2 treatment of 9. Quantitative determination of the individual component in the reaction mixture was made by Iatron-chromatoscanner using the developing solvents (benzene – ethyl acetate – formic acid = 20:4:1).

The compound (*9*) (400 mg) was dissolved in 60 ml of *t*-BuOH, to which 50 ml of ClO_2 solution (4.4 mmol) was added under stirring. After standing for five minutes at 60 °C the reaction mixture was diluted with a large volume of water and this was extracted with ethyl ether (Et_2O). After evaporation of the solvent the residue (426 mg) was subjected to the preparative TLC (PLC: solvent A) for isolation

and further purification of the reddish compound (*15*). (Yield 22%). MS: 454 (M+2), 452 (M). Found: C 57.25; H 3.60. Calc. $C_{23}H_{13}O_8Cl\cdot 3/2H_2O$: C 57.56; H 3.36. UV [abs. MeOH (log ε)]: 260 (3.71), 373 (3.69), 415 (sh, 241) nm. IR (KBr): 1680, 1640, 1600 cm^{-1}. On reduction with $NaBH_4$ by the usual manner this afforded *9*.

The methanolic solution (20 ml) of *15* (80 mg) was adjusted with dilute HCl to pH 4.0 and stood for 10 min at 60 °C under stirring. After dilution with a large volume of water the resulting solution was extracted with Et_2O, and the solvent was evaporated to dryness. The residue was subjected to PLC (solvent A) and this yielded 15 mg of 6-hydroxy-coumaranone (*4*) and 28 mg of the reddish powder (*16*), which newly appeared, m.p. (d.p.) 250 °C. MS: 304 (M+2), 302 (M). Found: C 55.88; H 3.08; Cl 11.82. Calc. for $C_{15}H_7O_5Cl\cdot H_2O$: C 56.17; H 2.83; Cl 11.73. UV [abs. MeOH (log ε)]: 268 (3.32), 370 (3.67), 445 (sh, 2.90) nm. IR (KBr): 1685, 1660, 1590 cm^{-1}.

The solution of *16* (20 mg) in pyridine (1 ml) was mixed with a solution of *o*-phenylenediamine (15 mg) in the same solvent (0.2 ml). After standing for 1 h at room temperature a part of the solvent was removed under reduced pressure. On addition of water it gave a crystalline precipitate which was recrystallized from MeOH – H_2O. Red crystalline powder, m.p. over 270 °C. MS: 374 (M). Found: C 66.88; H 3.12; N 7.21. Clac. for $C_{21}H_{11}O_3N_2Cl$: C 67.29; H 2.96; N 7.47. UV [abs. MeOH (log ε)]: 256 (3.63), 352 (3.62), 415 (sh, 3.28) nm. IR (KBr): 1680, 1600, 840, 760 cm^{-1}. Reduction of *16* with $NaBH_4$ yielded *10*.

Reaction of 16 *in weakly-acidic solution at 60 °C.* The compound (*16*) (2.15 g), prepared by the treatment of *10* with $NaIO_4$,[13] was dissolved in 100 ml of MeOH and the methanolic solution was adjusted with dilute HCl to pH 4.0 and stood for 30 min at 60 °C. The solution was evaporated to small volume and extracted with Et_2O. The etherial solution was washed sufficiently with water and dried over Na_2SO_4 and then evaporated to dryness. The extracts (2.11 g) were chromatographed on silica-gel column (solvent A) to afford 133 mg of I, 80 mg of II, and 485 mg of III. Recrystallization of II from MeOH – H_2O gave yellow powder, m.p. over 270 °C. This was identified as 5'-chloro-sulfuretin (*10*) by comparing the mass and IR spectra with those of an authentic sample.

The product III (*20*) was purified by the repeated PLCs (solvent A). Found: C 57.44; H 2.85. Calc. for $C_{30}H_{14}O_{10}Cl_2\cdot H_2O$: C 57.79; H 2.59. UV [abs. MeOH (log ε)]: 271 (3.91), 348 (4.11), 410 (sh, 3.16) nm. IR (KBr): 1700, 1660, 1640, 1600, 1420 (dioxene C–O band[22]).

The solution of *20* (50 mg) in pyridine (3 ml) was mixed with the solution of o-phenylenediamine (30 mg) in the same solvent (0.5 ml). After standing for 1 h at room temperature the solvent was evaporated to dryness under reduced pressure. Recrystallization of the residue from MeOH gave reddish crystalline powder, m.p. over 270 °C. MS: 676 (M). Found: C 63.45; H 2.87; N 3.98. Calc. for $C_{36}H_{18}O_8N_2Cl_2$: C 63.82; H 2.68; N 4.13. This adduct (*21*) was acetylated with acetic anhydride and pyridine to afford the acetate (*22*), which was recrystallized from MeOH to yield brown powder, m.p. 167–170 °C. MS: 718 (M). Found: C 62.76; H 3.18; N 3.50. Calc. for $C_{40}H_{22}O_{10}N_2Cl_2$: C 63.08; H 2.91; N 3.68. ^1H NMR ($CDCl_3$): δ 2.31 (6H, s, OAx × 2), 5.81 (1H, s, H_δ), 6.70–7.10 (6H, m), 7.10–7.38 (4H, m), 7.42–8.20 (6H, m).

Reduction of III (420 mg) gave the yellow compound *18*, which was acetylated with acetic anhydride and pyridine to yield the acetate. The latter was recrystallized from MeOH to afford yellow crystalline powder *17* (310 mg), m.p. 163–165 °C. FD-Mass: 820 (M). Found: C 58.54; H 3.18. Calc. for $C_{30}H_{13}O_5Cl_2(OCOCH_3)_5$: C 58.61; H 3.44. UV [abs. MeOH (log ε)]: 266 (4.20), 330 (4.07), 365 (4.00), 381 (4.03) nm. IR (KBr): 1780, 1735, 1710, 1650, 1440, 1416 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.30 (15H, s, OAc × 5), 5.88 (1H, d, *J* 8 Hz, H_γ), 5.93 (1H, d, *J* 8 Hz, H_δ), 6.79 (1H, d, *J* 2 Hz, $H_{3''}$), 6.90 (1H, d, *J* 2 Hz, $H_{2'''}$), 6.96 (1H, dd, *J* 8 Hz, H_6), 7.20 (1H, d, *J* 2 Hz, H_8), 7.24 (1H, s, Hα), 7.26 (1H, d, *J* 2 Hz, $H_{2'}$), 7.54 (1H, dd, *J* 8 Hz, $H_{5''}$), 7.59 (1H, d, *J* 8 Hz, $H_{6''}$), 7.80 (1H, d, *J* 8 Hz, H_5), 7.81 (1H, d, *J* 2 Hz, $H_{6'''}$), 8.12 (1H, d, *J* 2 Hz, $H_{6'}$). ^{13}C NMR (carbon number): δ 71.55 (γ), 97.87 (δ), 104.25 (3''), 107.12 (7), 110.27 (5), 115.48 (6''), 118.06 (2'''), 118.58 (α), 118.62 (6'''), 119.87 (1'''), 121.39 (5''), 124.78 (3a), 125.20 (4), 125.43 (2'), 126.25 (1''), 127.54 (5'''), 128.24 (5'), 128.65 (6'), 137.83 (3'''), 140.99 (1'), 141.52 (3'), 147.77 (2), 157.61 (4'), 159.56 (4'''), 159.83 (2''), 166.09 (6), 166.61 (4''), 166.79 (7a), 170.01 (β), 182.06 (3).

The compound (*18*) (400 mg) was refluxed with dimethyl sulfate and potassium carbonate in acetone. The solution of the resulting methyl ether (*19*) (420 mg) in dichloromethane was cooled in ice and subjected to the treatment with ozonized oxygen (40 mmol of O_3). The resulting mixture was decomposed by addition of 10% H_2O_2 – CH_3COOH. After addition of water the solution was extracted with EtOAc. The extracts (353 mg) were chromatographed on silica-gel column (solvent A) to yield three products, 38, 23, and 135 mg. Each of the former two was recrystallized from MeOH – H_2O to yield colorless needles, m.p. 158–159 °C and m.p. 107–108 °C, respectively. The former was identified as 4-methoxy-salicylic acid (*23*) (lit. m.p. 159 °C[23]), the latter as 2,4-dimethoxy-benzoic acid (*24*) (lit. m.p. 107–108 °C[24]) by comparing the mixed m.p. IR with those of authentic samples, respectively.

The third product (135 mg) dissolved in aqueous sodium hydroxide (100 ml) was mixed with 200 ml of potassium permanganate solution (5%) under stirring and stood overnight at room temperature. The solution was then acidified and extracted with Et_2O. The solvent was evaporated to dryness to afford a single compound (24 mg), which was recrystallized from MeOH to yield colorless powder, m.p. 189 – 190 °C. This was identified as 5-chloro-veratric acid [25] by comparing the mixed m.p. and IR with those of an authentic sample.

Analyses of IV, 26. After elution of I – III the residual column was washed well with solvent A to remove impurities. Subsequently IV was eluted with EtOAc – HCOOH (1:1) and the solvents were evaporated to dryness. The resulting compound (270 mg) was purified repeatedly by precipitation with $CHCl_3$ to yield 174 mg of *26*. Found: C 58.77; H 2.62; Cl 12.01. Calc. for $(C_{15}H_7O_5Cl)_5 \cdot H_2O$: C 58.81; H 2.44; Cl 11.59. UV [abs. MeOH (log ε)]: 272 (3.10), 325 (sh, 3.00), 410 (sh. 2.62) nm. IR (KBr): 1700, 1655, 1640, 1600, 1450, 1440 cm^{-1}. IV was subjected to gel-permeation chromatography (Sephadex LH-20) using MeOH – $CHCl_3$ (1:1).

IV *26* was reduced and subsequently acetylated by the same manner as mentioned in the preparation of *17* from III, and the resulting acetate was subjected to the ^{13}C NMR spectroscopy. ^{13}C NMR (carbon number): δ 71.11 (γ), 97.31 (δ), 169.92 (β).

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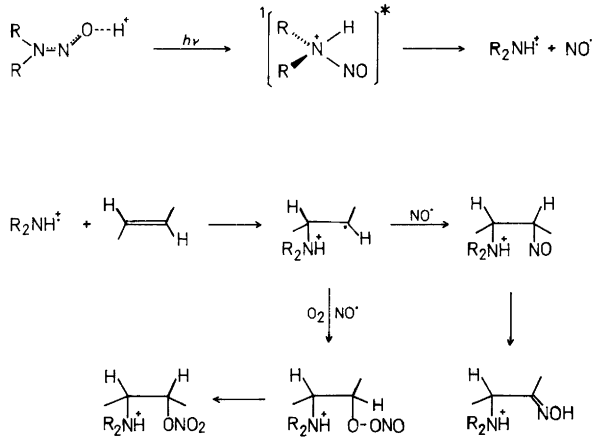
Nitrosamine Photolysis as a Synthetic Method: The Addition of Aminium Radicals to Unsaturated Carbon–Carbon Bonds

YUAN L. CHOW,* CARLOS J. COLON, DAVID W. L. CHANG, K. SOMASEKHAREN PILLAY, ROBERT L. LOCKHART and TAKAHIRO TEZUKA**

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Acid complexed nitrosamines decompose from their lowest singlet excited state to give aminium radicals and nitric oxide radical transients. Aminium radicals initiate addition to various unsaturated groups to give 1-amino-2-nitroso compounds under an inert atmosphere, or 1-amino-2-nitrates under oxygen. In this report, photoaddition of nitrosamines to olefins, acetylenes and fused aromatic hydrocarbons, and the subsequent transformations of the intermediates are described. Aminium radical initiated intramolecular cyclization to prepare tetracyclic aza compounds is also described. While photoaddition of nitrosamines to 4-propenylanisole or 3-butenol was efficient, that to 3-butenyl benzoates under oxidative conditions was only fair, obviously due to the presence of a benzene ring. The oxidative photoaddition to 3-butenyl halide was followed by spontaneous cyclization to an azaspiro compound. The photoaddition to phenyl-substituted acetylenes gave β-nitroso enamines which hydrolyzed to diketomonoximes under neutral conditions but decomposed extensively under acidic conditions. Certain fused aromatic hydrocarbons acted as singlet sensitizers as well as substrates to induce similar addition giving amino nitroso adducts. These adducts took different courses of conversion dependent on reaction conditions, and on steric and electronic factors.

Some years ago[1-3] we discovered that *N*-nitrosodialkylamines add efficiently across a carbon–carbon double bond under photolysis in acidic solution but not in neutral solution. That is, these nitrosames are photolabile only in acidic solution.[1]



Scheme 1.

* Address enquiries to this author.
** On study leave from Tohoku University, Sendai. Present address: Department of Chemistry, Tsukuba University, Japan.

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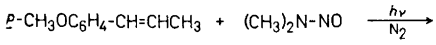
In the ensuing investigation, we established that the aminium radicals are the reactive intermediates involved in this photoaddition [4,5] and that they are generated from the lowest singlet excited state of acid-complexed nitrosamines,[6] and that the photoaddition occurs by a stepwise radical addition.[4,5] The reaction pattern is summarized in Scheme 1. Photoaddition carried out under nitrogen gives α-amino oximes *via* the nitroso intermediate.[4,5] However, under oxygen the photoaddition is cleanly diverted to the formation of α-amino nitrates presumably *via* the peroxynitrite intermediates.[7-10] In a series of publications, the efficiency and applicability of the oxidative and non-oxidative photoaddition to olefins in preparations of various oximes, aminonitrates and amino alcohols have been described.[4] We now report the photoaddition of *N*-nitrosodimethylamine (NND) and *N*-nitrosopiperidine (NNP) to various unsaturated compounds, and certain accompanying complications.

RESULTS AND DISCUSSION

(1) Photoaddition to olefins. In analogy to previously reported photoadditions of nitrosamines to olefins,[1-3] photoaddition of NND to 4-propenylanisole (*1*) in acidic solution under nitrogen gave *syn*-1-(*p*-methoxyphenyl)-2-*N*,*N*-dimethylaminopropan-1-one oxime (*2s*), the corresponding *anti*-isomer (*2a*) and 1-(*p*-methoxyphenyl)-1-*N*,*N*-dimethylaminopropan-2-one oxime (*3*) in 68, 11 and 3% isolated yields, respectively. The structures of these oximes were determined from their elemental analysis as well as spectroscopic data as given in the Experimental section. The *syn-anti* configuration of *2s* and *2a* were decided by the NMR quartets for the methine protons at 3.55 and 3.27 ppm according to the correlation of α-proton signals of oximes proposed previously.[11] The minor oxime *3* exhibited a methyl singlet at 1.38 ppm because of a methyl ketoxime structure. In agreement with the aminium radical initiated addition to the conjugated double bond [12] the attack occurs predominantly at the β-carbon of *1* to yield the more stabilized benzyl radical intermediate leading to oximes *2s* and *2a*. Regioselectivity is not complete, however, yielding a small amount of *3*.

The oxidative photoaddition of NNP was demonstrated using 3-butenol and 3-butenyl esters as substrates. The photolysis was carried out in the presence of one of the 3-butenyl derivatives *4a*–*4h* under constant oxygen purging. These photoadditions yielded the amino nitrates *5* and the amino alcohols *6* in nearly a 1:1 ratio. Only in the case of the addition to 3-butenol, *5a* and *6a* were separated by taking advantage of their solubility difference. In the photoaddition to *4b*–*4g*, the crude products were reduced with lithium aluminum hydride to *6a* or by catalytic hydrogenation to give the corresponding amino alcohols *6*. The presence of the nitrates *5* and alcohols *6* in each crude product could be recognized from the NMR quintet signal for the methine protons and pertinent IR absorptions at 1630, 1280 and 865 cm^{-1} for a nitrate group [7,8] and 3440 and 1045 cm^{-1} for a hydroxyl group in addition to the typical absorptions for other functionalities. The ratios of *5*:*6* could be estimated from the NMR triplet of the C-4 protons. In contrast to the good yields of the photoadducts from *4a* and *4b*, the yields from benzoates *4c*, *4d* and *4e* were low and that from tosylate *4f* was nil. The cause of the yield variation merits further investigation.

The percent yields of amino alcohols *6* were surprisingly high in comparison to the cases of the



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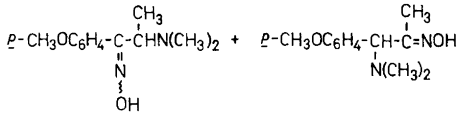
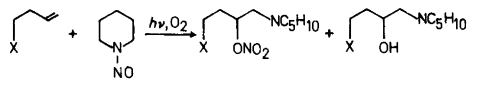
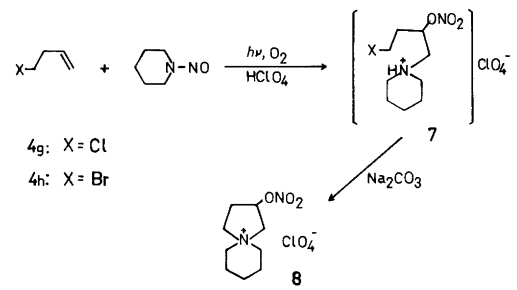


2s, 2a 3



4 5 6

	X	%yields of 5 and 6
4a	OH	72
4b	$OCOCH_3$	80
4c	$OCOC_6H_4CH_3(p)$	26
4d	$OCOC_6H_4OCH_3(p)$	32
4e	$OCOC_6H_4CN(p)$	33
4f	$OSO_2C_6H_4CH_3(p)$	0



4g: X = Cl
4h: X = Br

7 8

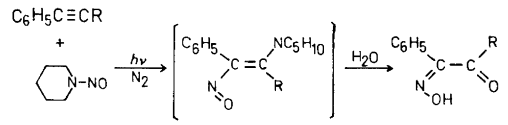
oxidative photoaddition to simple olefins where amino nitrates were always obtained as the major product.[7,8] Undoubtedly, the ester groups at the C-4 position intervene in an intramolecular displacement, probably at the peroxynitric stage, to give solvolysis products.

Oxidative photoaddition of NNP to 3-butenyl chloride and bromide in the presence of perchloric acid proceeded smoothly to give the perchlorate of 2-nitrato-5-azoniaspiro[4,5]decane (*8*) in 38 and 46%, respectively, which was isolated by continuous extraction with methylene chloride. No doubt the azaspiro compound *8* was derived from the expected nitrate ester 7, the primary photoaddition product, by cyclization. The hydroxy compound corresponding to *8* was also formed but could not be isolated. The structure *8* was confirmed by analysis and NMR decoupling experiments which unraveled the coupling patterns of the C-1, C-2 and C-4 protons; the details of the decoupling results are described in the thesis presented by Pillay.[13]

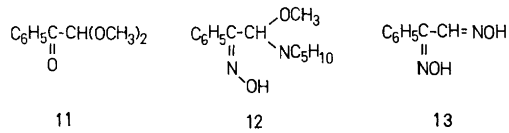
(2) Photoaddition to acetylenes. Photolysis of NNP in the presence of diphenylacetylene[14] under nitrogen also proceeded rapidly to give benzil monoxime (*9*) in 61% yield. Similar photolysis in the presence of phenylacetylene gave phenylglyoxal ketoxime *10* in 65% yield only when the photolysis was carried out at dry ice–methanol temperature and the photolysate was neutralized with sodium carbonate immediately after the photolysis. Similar photolysis without these precautions yielded ketoxime *10* in 27% yield in addition to 2,2'-dimethoxyacetophenone (*11*, 22%), 2-methoxy-2-piperidinoacetophenone oxime (*12*, 24%) and phenylglyoxal dioxime (*13*, 9%). These phenylglyoxal derivatives were most likely formed by acid-catalyzed addition of methanol, substitution and/or transoximation from the primary photoadduct *14*, a *β*-nitroso enamine.

Both ketoximes *9* and *10* were derived from hydrolysis of the primary photoadduct, the *β*-nitroso enamine *14*, that occurred readily under neutral or slightly basic conditions. As the intermediate *14* contained a *β*-nitroso enamine, a chromophore which would certainly absorb in the 300–350 nm region, a light filter with cutoff at 320–340 nm was used in order to minimize the secondary photodecomposition of *14*. Indeed, when a Pyrex filter was used, the yields of ketoximes *9* and *10* were much lower and many minor products were obtained.

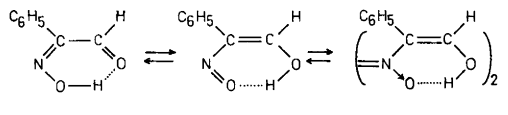
Compound 10 exhibited interesting tautomerization behavior between phenylglyoxal ketoxime *10* and the dimer of 1-nitroso-2-hydroxystyrene (*10a*). A chloroform solution of the compound exhibited a strong carbonyl absorption at 1700 cm^{-1} indicating the existence of ketoxime form *10*. Evaporation of



14 9: R = C_6H_5; 10: R = H



11 12 13



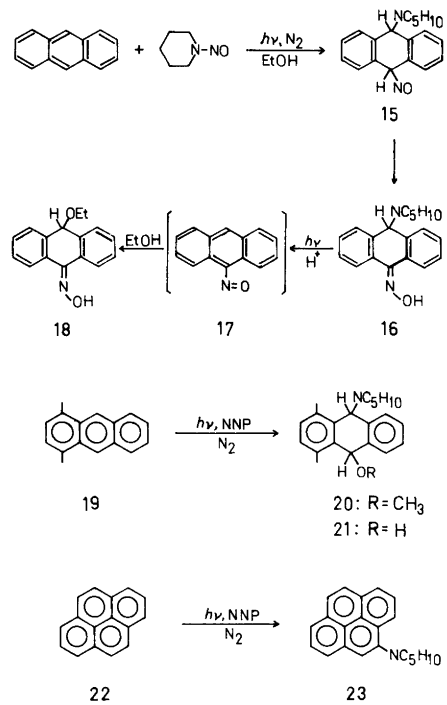
10 (m.p. 48.5–50°) 10a (m.p. 113–115°)

chloroform left an oil which crystallized to give *10*, m.p. 48.5 – 50.0, IR absorption in Nujol at 1705 cm^{-1}. The crystals gradually transformed at room temperature to another crystal, m.p. 113 – 115 °C, showing IR absorption at 1210 (s) and 1590 (m) cm^{-1} indicating the presence of the *trans*-dimeric structure *10a*. Dissolution of *10a* in various organic solvents caused taumerization to give *10* as shown by its NMR and IR spectra. Sublimination of *10a* gave *10* and slow crystallization of *10* from chloroform gave *10a*.

Other alkylacetylenes, such as 1-hexyne, were also used as substrate in the photoaddition but gave no products that could be extracted with a variety of solvents. While water soluble products were formed, attempts to isolate them have not been successful.

(3) Sensitized photoaddition to aromatic hydrocarbons. Photolysis of NNP in the presence of benzene, toluene, anisole and benzonitrile in acidic solution causes a rapid decomposition of the nitrosamine but gives no addition product to the benzene derivatives. Careful analysis shows that piperidine is the only isolable product. The reason for the lack of addition may be that the attack of the aminium radical on the π-system of the benzene rings requires a higher activation energy than those required in other processes, *e.g.*, the hydrogen abstraction from methanol. Fused aromatic hydrocarbons would provide sites of a less delocalized double bond and higher electron densities; such double bonds could be attacked by aminium radicals to cause photoaddition. Fused aromatic hydrocarbons generally absorb light strongly in the 300 – 400 nm region [15,16] and it is impractical to design a photoreaction in which nitrosamines (absorption maximum at 345 nm) are excited in the primary photoexcitation. It also occurred to us that since most of these aromatic hydrocarbons possess substantial fluorescence quantum yields [15] and reasonably long singlet lifetimes,[16] it might be possible to use certain aromatic hydrocarbons as singlet sensitizers as well as substrates to carry out the photoaddition. NND (and other nitrosamines) has the lowest excited singlet state at about 75 kcal/mol and the lowest triplet state energy of 59 kcal/mol;[6] a classical sensitization mechanism by energy transfer from the singlet state of these hydrocarbons is thus possible.

Our conclusion that nitrosamines photolytically dissociate from their lowest singlet state was further supported by the failure of benzophenone [16] ($\tau_{s\_} < 10^{-12}$ s, $\phi_F = 0$, $\phi_{ISC} = 1.00$), an excellent triplet sensitizer, to sensitize the photodecomposition of NNP in acid solution. Anthracene sensitized photolysis of NNP in acidic ethanol under helium gave 9-piperidinoanthrone oxime [17] (*16*) in a 70% yield and a small amount of 9-ethoxyanthrone oxime (*18*) in addition to trace amounts of unidentified compounds. The photolysis solution contained a low concentration of NNP (0.014 M) so that more than 95% of the incident light was absorbed by anthracene. It was obvious that the oxime *16* was a tautomeric product of the primary photoadduct *15*. The oxime *18* was a secondary photolysis product since under similar photolysis conditions oxime *16* was slowly converted to *18*. The photoconversion could be explained by the elimination of piperidinium ion followed by the addition of ethanol to 9-nitrosoanthracene (*17*). The assigned structures for *16* and *18* were supported by analysis and spectroscopic data. When a similar sensitized photoreaction was repeated in the presence of cyclohexene or *cis*-4-methyl-2-pentene, only trace amounts of the addition products to olefins [1,2] were obtained but *16* was isolated as the major product. Nitrosamines are known to form loose collision complexes with aromatic π-electron systems in solution.[18] Such complexes conceivably



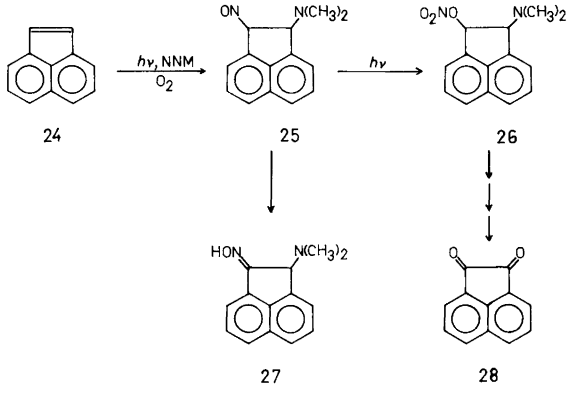
facilitate the sensitization process as well as the addition to anthracene. The process may be represented by the mechanism (1), where *A* is anthracene having[16] $E_s = 76.3$ kcal/mol, $\phi_f = 0.27$ and $\tau_s = 5$ ns.

$$A + C_5H_{10}NNO \xrightarrow[H^+]{h\nu} {}^1A^* + C_5H_{10}NNO\cdots H^+ \rightarrow$$
$$A + {}^1[C_5H_{10}NNO\cdots H^+]^* \rightarrow$$
$$A + C_5H_{10}NH^{\cdot +} + NO^{\cdot} \rightarrow \textit{15} \qquad (1)$$

Sensitized photoaddition of NNP to 1,4-dimethylanthracene (*19*) in acidic methanol gave 1,4-dimethyl-9-piperidino-10-methoxy-9,10-dihydroanthracene (*20*, 48%) and a trace of 2,4-dimethyl-9-piperidino-10-hydroxy-9,10-dihydroanthracene (*21*). The stereochemistry of these two compounds was not determined. It is simplest to assume that these products arise from the nucleophilic substitution of the nitroso group in the primary photoadduct (*i.e.* the 1,4-dimethyl analogue of *15*) by methanol or water. The intermediate *C*-nitroso compound would be difficult to tautomerize to the corresponding oxime because of the steric crowding from the methyl group at the *peri*-position. The steric hindrance of the dimethyl group is also reflected by the low yields of the products. In these photoreactions, the dimers[19] of the anthracenes were also formed. The photoaddition of nitrosamines to 1,3-dimethylanthracene and 1,2-benzanthracene also gave the corresponding products in low yields; these results and experiments are described in the thesis presented by C. J. Colon.[20]

Sensitized photoaddition of NNP to pyrene (*22*), $E_s = 79$ kcal/mol, $\tau_s = 450$ ns, $\phi_f = 0.58$[16] in acidic methanol solution proceeded rapidly to give blackish solution and could not be carried to completion. It gave 4-piperidinopyrene *23* in 52% yield together with some recovered pyrene from the neutral extract. While the spectroscopic data could not indicate the complete structure, the substitution at C-4 was deduced on the basis that the π bond at C-4,5 is localized more than others for ozone addition and is probably just as susceptible to an electrophilic radical attack. Obviously *23* was formed from the C-nitroso intermediate, the primary adduct corresponding to *15*, by elimination of HNO. Mechanistically the elimination could occur either by acid catalysis or sensitized photolysis. The driving force for the elimination may be ascribed to the tendency to achieve extended conjugation. Interestingly, sensitized photoaddition of NNP to phenanthrene gave only a trace amount of a piperidinophenathrene and was not pursued further.

Acenaphthene is not exactly a fused aromatic but it structurally resembles these compounds. As it possesses no absorption >320 nm, irradiation of a mixture with nitrosamines is more likely to cause direct excitation of the nitrosamines. Photoaddition of NND to acenaphthene (*24*) in acidic methanol under nitrogen gave 2-dimethylamino-acenaphth-1-one oxime (*27*, 51%) in addition to large amounts of the dimer of acenapthene[22] and acenapthoquinone (*28*). The oxime fraction consisted of *anti* and *syn* isomers in a 7:3 ratio and only *anti-25* was isolated in the pure state. The stereochemistry was deduced from the chemical shifts of the methine and methyl protons in the NMR spectra.[11] The *anti*-oxime exhibited the singlets at 5.22 and 2.14 ppm while the *syn*-oxime showed the corresponding signals at 5.43 and 2.42 ppm. The copious yield of acenaphtho-

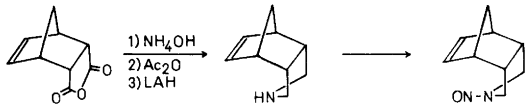


24 25 26 27 28

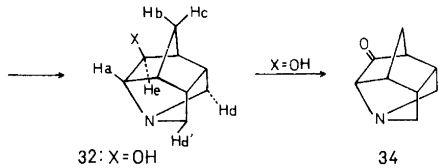
quinone *28* was surprising and indicated that oxidizing species were formed during photolysis.

(4) Intramolecular photoaddition. We have demonstrated that a $\Delta^{4,5}$-alkenylaminium radical generated from the corresponding nitrosamine efficiently cyclizes exclusively to form a five-membered pyrrolidine intermediate, that is, the aminium radical center intramoleculary attacks the C-4 of the olefin exclusively.[23-25] It was also shown that such a C-radical intermediate can be scavenged by oxygen to form a nitrate ester and by bromotrichloromethane to form a bromo compound as the final product.[25] Applications of this process for synthesis of azapolycyclic oximes, nitrates and bromides have been reported.[24,25] The synthesis of a tetracyclic aza compound is described here.

The tricyclic nitrosamine *31* was prepared from readily available *endo*-norbornene-*cis*-5,6-dicarboxylic anhydride (*29*) by a four-step operation as described in the Experimental section. Photolysis of *31* in acidic methanol under oxygen was expected to give, by the oxidative intramolecular aminium radical addition, the nitrate ester which was reduced by LAH to give a good yield of tetracyclic amino alcohol *32*; this compound was oxidized to the corresponding ketone *34*. When a similar photolysis of nitrosamine *31* was carried out in the presence of bromotrichloromethane, the tetracyclic amine bromide *33* was isolated in 46% yield. The structures of these tetracyclic aza compounds were decided from analysis and spectral data. The configuration of the OH and Br groups in *32* and *33* was determined from the NMR coupling pattern of the geminal proton, H_e, which was shown not to be, or only weakly, coupled to the adjacent proton H_a and to be coupled by a long range spin–spin interaction with H_c; the information was gained by extensive decoupling experiments with the aid of an Europium shift reagent. The details and spectra were described in the thesis written by R. L. Lockhart.[26]



29 30 31



32: X = OH
33: X = Br

34

CONCLUSION

The examples described above demonstrate that photoexcited nitrosamines are a good source of aminium radicals and react readily with various unsaturated systems to give 1-amino-2-nitroso compounds or 2-aminonitrates depending on the presence or absence of oxygen. These *C*-nitroso compounds exhibit some interesting chemistry and may reveal even more unexpected behavior when the nitroso group is in conjugation with other functional groups. The photoaddition is a valuable general method for synthesis of C-nitroso compounds; their preparation as well as their chemistry have not been investigated extensively. Unfortunately, *nitrosamines are carcinogens*[27] and, thus, utmost precaution is required for their handling.

Aminium radicals can be generated by thermal or photolytic decomposition of chloramines in highly acidic media, *e.g.*, 2 M H_2SO_4 or higher in acetic acid.[28] Nitrosamine photolysis is a much milder method for aminium radical generation and a more amenable method for the investigation of aminium radical reactivity. Because of propensity to attack a π-bond over hydrogen abstraction from alkyl or allyl groups, aminium radicals are potentially useful reactive intermediates in synthesis.[29]

EXPERIMENTAL

General conditions. Unless specified otherwise the following conditions were used. Infrared spectra were recorded with a Perkin-Elmer 457 spectrophotometer using liquid films or Nujol mulls. Ultraviolet spectra were taken with either a Cary 14 or a Unicam SP8000 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian A-56/60 or an XL-100 in CCl_4 or $CDCl_3$ with Me_4Si as the internal standard. The chemical shifts of NMR were reported in δ-value (ppm) from Me_4Si and coupling constants in Hertz (Hz). The decoupling experiments were performed with the XL-100 spectrometer. Mass spectra were recorded with a Hitachi Perkin-Elmer RMU-6E mass spectrometer. High resolution mass spectra were

performed at the University of British Columbia, Mass Spectrometric Services. Elemental analyses were carried out by Mr. M. K. Yang using a Perkin-Elmer 240 microanalyzer. Gas chromatographic analyses were performed on a Varian 1200 flame analytical machine using a Varian Aerograph Model 20 recorder equipped with a Model 224 disc chart integrator.

Photoaddition of nitrosamies to olefins. 1. 4-Propenylanisole (1). A solution of NND (1.24 g, 17 mmol), *1* (3.05 g, 21 mmol) and concentrated hydrochloric acid (1.5 ml, 18 mmol) in methanol (350 ml) was irradiated in an ice bath with a Rayonet lamp (350 nm) for 5 h and 40 min. The photolysate was evaporated to dryness. Water (50 ml) was added and the aqueous solution was extracted with ether (3 × 25 ml). The combined ethereal solution was dried ($MgSO_4$) and evaporated to give the recovered *1* (0.63 g). The aqueous solution was adjusted with a saturated solution of sodium carbonate to pH 10, and extracted with methylene chloride (3 × 25 ml). The combined methylene chloride solution was evaporated to give a solid (2.97 g) which was taken up in benzene and chromatographed on neutral alumina to give the following compounds in the order of elution.

(i) A solid (2.55 g, 68 %) which was recrystallized from a benzene–cyclohexane mixture and was sublimed at 85 °C/0.1 mmHg to give *syn*-1-(*p*-methoxyphenyl)-2-*N*,*N*-dimethylaminopropan-1-one-oxime (*2s*): m.p. 93–94.5 °C; IR 1605, 1520, 1030, 954, 926 and 855 cm^{-1}; NMR δ 1.42 (d, $J=7$ Hz, 3H), 2.37 (s, 6H), 2.55 (q, $J=7$ Hz, 1H), 3.78 (s, 2H) and 7.17 (*AB* q, $\Delta\nu=44$, $J=9$ Hz, 4H); *m/e* (%) 222 (M^+, 25), 205(20), 189(9), 176(6), 163(6), 147(71), 133(71), 115(16), 103(40), 90(36), 78(27) and 72(100); Calc. for $C_{12}H_{18}N_2O_2$: C, 65.84; H, 8.16; N, 12.60. Found: C, 65.06; H, 8.27; N, 12.46.

(ii) A solid (0.42 g, 11 %) which was recrystallized from a benzene–cyclohexane mixture and sublimed at 90 °C/0.1 mmHg to give *anti*-1-(*p*-methoxyphenyl)-2-*N*,*N*-dimethylaminopropan-1-one-oxime (*2a*): m.p. 112.5–114 °C; IR 3300, 1620, 1525, 1260, 1182, 1038, 942 and 830 cm^{-1}; NMR δ 1.18 (d, $J=7$ Hz, 3H), 2.24 (s, 6H), 3.37 (q, $J=7$ Hz, 1H), 3.77 (s, 3H)) and 7.15 (*AB* q, $\Delta\nu=35$, $J=9$ Hz, 4H); *m/e* (%) 222 (M^+, 4), 205(100), 189(14), 179(14), 176(24), 163(11), 147(8), 133(72), 118(10), 103(27), 90(30), 78(14) and 72(50).

(iii) An oil (100 mg) which was distilled at 85 °C/0.1 mm to give a colorless oil which was tentatively assigned as 1-(*p*-methoxyphenyl)-1-*N*,*N*-dimethylaminopropan-2-one oxime (*3*): IR 2940, 2870, 2830, 2780, 1675, 1600, 1510, 1455, 1260, 1235, 1170, 1100, 1030, 930 and 845 cm^{-1}; NMR δ 1.38 (s, 3H), 2.28 (s, 6H), 3.70 (s, 3H) and 7.15 (*AB* q, 4H); *m/e* (%) 222 (M^+, <1), 205(3), 176(2), 151(60), 148(100), 121(5), 107(5), 92(8), 77(15) and 72(10): Calc. for $C_{12}H_{18}N_2O_2$: C, 64.84; H, 8.16; 12.60; Found: C, 64.95; H, 8.08; N, 12.67.

2. 3-Butenol. A solution of NNP (2.736 g, 0.024 mol), 3-butenol (*4a*, 1.44 g, 0.02 mol) and perchloric acid (70 %, 4 ml) in methanol (320 ml) was irradiated (200 watt Hanovia lamp) through a Nonex filter under oxygen at 0 °C. After 1.5 h, the absorption at 350 nm disappeared completely and a colourless solution was obtained. The photolysate was concentrated under reduced pressure at 10 °C and was diluted with water to *ca.* 100 ml and extracted with ether (4 × 50 ml) to give an oil (66 mg) which contained unreacted 3-butenol as shown by IR and NMR analysis. The aqueous acidic solution was cooled to 5 °C, basified with saturated sodium carbonate solution (pH 9.5) and extracted with methylene chloride (5 × 50 ml). The methylene chlroide extract was worked up in the usual manner to afford 3-nitrato-4-piperidinobutanol (*5a*) as an oil (2.209 g, 51 %): IR 3370 (m), 2800 (m), 1625 (s), 1225 (s), 1055 (m), 865 (m) and 855 (m) cm^{-1}; NMR δ 5.22 (qi, $J=6.0$ Hz, 1H), 5.15 (bs, D_2O exch., 1H), 3.71 (t, $J=5.5$ Hz, 2H), 2.58 (d, $J=6.0$ Hz, 2H), 2.45 (m, 4H), 1.97 (q, $J=6.0$ Hz, 2H) and 1.5 (m, 6H).

Continuous extraction of the basic solution with methylene chloride gave 4-piperidino-1,3-butanediol (*6a*) as an oil (710 mg, 21 %): IR 3370 (s), 2800 (m), 1975 (s), 1050 (s) and 1040 (s) cm^{-1}; NMR δ 4.62 (s, D_2O exch., 2H), 3.87 (qi, $J=6.5$ Hz, 1H), 3.72 (t, $J=6.0$ Hz, 2H), 2.47 (m, 4H), 2.40 (d, $J=6.5$ Hz, 2H), 1.68 (q, $J=6.0$ Hz, 2H) and 1.53 (m, 6H); m.s. *m/e* (%) 174 (M^+, 7), 128(30), 99(37), 98(100), 84(27), 55(28), 42(25) and 41(31).

Reduction of the aminonitrate *5a* (2.2 g) with LAH (1.9 g, 0.05 mol) in ether (150 ml) for 24 h followed by the usual work-up afforded diol *6a* (1.824 g). Treatment of diol *6a* (173 mg) with *p*-nitrobenzoyl chloride (186 mg) in dry THF (2 ml) gave a solid which was recrystallized from 2-propanol to give the hydrochloride of 3-hydroxy-4-piperidinobutyl-*p*-nitrobenzoate (216 mg, 60 %): m.p. 192–193 °C (d); IR 3260 (s), 1720 (s), 1525 (s), 1350 (s), 1340 (s), 1285 (s) and 720 (s) cm^{-1}; NMR δ 1.95 (m, 8H), 3.9–2.7 (m, 6H), 4.3 (m, 1H), 4.57 (t, $J=6.5$ Hz, 2H) and 8.3 (m, 4H): Calc. for $C_{16}H_{23}N_2O_5Cl$: C, 53.56; H, 6.46; N, 7.82. Found: C, 53.86; H, 6.28; N, 7.92.

3. 3-Butenyl esters 4b–4f. The butenyl esters *4b–4f* were prepared from 3-butenol with appropriate acyl halides. They were purified and characterized as described in the thesis.[13]

Similar oxidative photoaddition of NNP to the butenyl esters *4b–4f* were carried out and worked up as described above. The neutral fraction contained the starting butenyl esters as shown by GC co-injection and IR spectra. The basic fraction was examined by IR and NMR spectroscopy to estimate the ratio of *5* to *6*. They are described below.

(i) *4b:* The crude basic fraction (80%) contained 3-nitrato-4-piperidinobutyl acetate (*5b*) and 3-hydroxy-4-piperidinobutyl acetate (*6b*) in the approximate ratio 1:1 as shown by the intensities of the triplets at 4.23 and 4.18 ppm: IR 3440(m), 1740(s), 1630(s), 1280(s), 1240(s), 1045(s), 895(s), 865(s) and 855(s) cm^{-1}; NMR δ 5.27 (qi, $J=6.0$ Hz), 4.77 (bs, D_2O exch.), 4.23 (t, $J=6.5$ Hz), 4.18 (t, $J=6.5$ Hz), 4.18 (t, $J=6.5$ Hz), 3.72 (qi, $J=5.5$ Hz), 2.47(m), 2.04(s) and 1.52(m). Reduction of the crude fraction (5.6 g) with LAH in ether gave diol *6a* (3.938 g) in an overall yield of 80%.

(ii) *4c:* The basic fraction (26%) contained 3-nitrato-4-piperidinobutyl-*p*-methylbenzoate *5c* and 3-hydroxy-4-piperidinobutyl-*p*-methylbenzoate *6c* in a 1:1 ratio as shown by the intensities of the NMR triplets at 4.49 and 4.43 ppm: IR 3420(m), 1720(s), 1630(s), 1278(s), 1180(s), 1108(s), 860(s) and 758(s) cm^{-1}; NMR δ 1.51(m), 2.42(s), 2.5(m), 5.37 (qi, $J=6.5$ Hz), 4.49 (t, $J=6.5$ Hz) 4.43 (t, $J=6.0$ Hz), 3.67 (s, D_2O exch.), 3.8(m), 7.24 and 7.92 (A_2B_2, $J=8.0$ Hz, $\Delta\nu=41$ Hz).

This mixture was hydrogenated in methanol (50 ml) in the presence of platinum oxide (125 mg) to afford *6c* as an oil (934 mg); IR 3400(s), 1715(s), 1280(s), 1180(m), 1105(s) and 755(s) cm^{-1}; NMR δ 1.53(m), 1.85 (q, $J=6.5$ Hz, 2H), 2.42 (s, 3H), 2.40 (m, 6H), 3.90 (qi, $J=6.5$ Hz, 1H), 4.50 (t, $J=6.5$ Hz, 2H), 4.95 (m, D_2O exch., 1H), 7.23 and 7.93 (A_2B_2, $J=8.5$ Hz, $\Delta\nu=42$ Hz, 4H). The hydrochloride of *6c* was recrystallized from 2-propanal and was analyzed; m.p. 184–185 °C.

(iii) *4d:* The basic fraction (32%) contained 3-nitrato-4-piperidinobutyl-*p*-methoxybenzoate (*5d*) and 3-hydroxy-4-piperidinobutyl-*p*-methoxybenzoate (*6d*) in the approximate ratio 1:1 as indicated by the intensities of the NMR signals at 4.47 and 4.42 ppm: IR 3420(m), 1703(s), 1630(s), 1608(s), 1280(s), 1260(s), 1173(s), 1100(s), 1033(s), 853(s) and 733(s) cm^{-1}; NMR δ 1.5(m), 2.46(m), 3.86(s), 4.42 (t $J=6.0$ Hz), 4.47 (t, $J=6.5$), 5.37 (qi, $J=6.5$ Hz), 4.12 (s, D_2O exch.), 3.76(m), 6.92 and 7.99 (A_2B_2, $J=9.0$ Hz). This mixture was hydrogenated in methanol (50 ml) in the presence of platinum oxide (125 mg) to give *6d* as an oil (1.12 g): IR 3400(s), 1710(s), 1605(s), 1280(s), 1260(s), 1170(s), 1100(s), 1030(m) and 770(m) cm^{-1}; NMR δ 1.54(m), 1.84 (q, $J=6.5$ Hz, 2H), 2.4(m), 3.87 (s, 3H), 3.79 (m, 1H), 4.48 (t, $J=6.5$ Hz, 2H), 4.66 (m, 1H), 6.92 and 8.00 (A_2B_2, $J=9.0$ Hz, 4H). The hydrochloride of *6d* (m.p. 159–160 °C) was recrystallized from 2-propanol and was analyzed.

(iv) *4e:* The basic fraction (33%) consisted of 3-nitrato-4-piperidinobutyl-*p*-cyanobenzoate (*5e*) and 3-hydroxy-4-piperidinobutyl-*p*-cyanobenzoate (*6e*) in a 1:1 ratio as judged from the intensities of the NMR signals at 4.55 and 4.51 ppm: IR 3400(m), 2230(m), 1725(s), 1628(s), 1275(s), 1105(s), 1120(s), 860(s), 768(s) and 690(s) cm^{-1}; NMR δ 8.13 and 7.75 (A_2B_2, $J=8.0$ Hz, $\Delta\nu=23$ Hz), 5.35 (qi, $J=6.0$ Hz), 4.55 (t, $J=6.5$ Hz), 4.51 (t, $J=6.0$ Hz), 3.97(m), 3.98 (bs, D_2O exch.), 2.45(m) and 1.5(m). Part of the crude basic mixture was reduced with LAH in ether to give diol *6a*.

(v) *4f:* The neutral fraction contained the unreacted *4f* (95%) and the basic fraction gave some piperidine and dipiperidylmethane.

*4. 3-Butenyl chloride (*4g*) and bromide (*4h*).* A methanol solution (320 ml) of NNP (2.74 g, 0.024 mol), 3-butenyl chloride (1.81 g, 0.02 mol) and perchloric acid (70%, 3.5 ml) was photolyzed under oxygen as described before. After irradiation (1.5 h), the colourless photolysate was worked up in the usual manner to give a neutral (62 mg) and basic (520 mg) fraction; the latter contained predominantly piperidine and dipiperidylmethane. The aqueous solution was re-extracted continuously with methylene chloride for several days to give a solid (2.263 g, 38%) which was recrystallized from metanol to give the perchlorate of 2-nitrato-5-azoniaspiro[4,5]decane (*8*): m.p. 134.5–135.5 °C; IR 3040(w), 1643(s), 1303(s), 1293(s), 1275(s), 1095(s), 870(s), 860(s) and 625(s) cm^{-1}; NMR (acetone-d_6) δ 5.96 (m, H-2), 4.23 (2H, m, H-1, $J=14.5$ and 3.5 Hz), 3.98 (2H, m, H-4, $J=15$, 7 and 4 Hz), 3.71 (4H, m), 2.82 (2H, m, H-3, $J=16$, 7 and 4 Hz) and 2.3–1.6 (6H, m). Calc. for $C_9H_{17}N_2O_7Cl$: C, 35.95; H, 5.70; N, 9.31. Found: C, 36.01, H, 5.84; N, 9.31. A similar photolysis of NNP in the presence of 3-butenyl bromide gave 46% of *8*.

Photoaddition of NNP to Acetylenes. 1. Diphenylacetylene. A solution of NNP (7.72 g, 68 mmol), diphenylacetylene (4.66 g, 26 mmol) and concentrated hydrochloric acid (5.8 ml, 69 mmol) in methanol (200 ml) was irradiated in an ice bath with a Hanovia lamp (450 W) through a filter solurion (cut off at 350 nm) for 2 h. The photolysate was evaporated to dryness. Water (50 ml) was added and the aqueous solution was extracted with ether (3 × 25 ml). The ethereal solution was evaporated to give an oil (4.37 g) which contained *N*-nitrosopiperidine (0.22 g) and a solid (4.15 g, 61%). The solid was recrystallized from benzene to give benzil monoxime (*9*): m.p. 136–138 °C; (lit. 137 °C);[30] IR 3340, 3060, 1640, 1600, 1375, 1310, 1215, 1010, 930, 875 and 695 cm^{-1}; *m/e* (%) 225 (M^+, 7), 122(18), 105(100), 103(56) and 77(56).

2. Phenylacetylene. A solution of NNP (7.87 g, 69 mmol), phenylacetylene (8.37 g, 82 mmol) and concentrated hydrochloric acid (6 ml, 72 mmol) in methanol (300 ml) was irradiated in a dry ice–methanol bath with a Hanovia lamp (450 W) through a Uranium glass filter for 15 h. While the absorption at 340 nm decreased gradually, new peaks at 305, 280 and 276 nm increased. The photolysate was rendered basic with solid sodium

carbonate (8 g) immediately on termination of the irradiation. The precipitate was filtered off and the filtrate was evaporated to dryness. An aqueous solution of the residue was worked up in the usual manner to give a basic and a residue fraction. The basic fraction was chromatographed on a silicic acid column to give NNP (2.58 g), a yellow liquid (49 mg), the oily major product (4.53 g) and an unknown solid mixture (300 mg). The major oil was distilled at 95 °C/0.1 mmHg to give crystalline phenylglyoxal ketoxime (*10*): m.p. 48.5 – 50 °C; IR ($CHCl_3$) 3240, 3060, 3030, 2840, 1700, 1590, 1440, 1370, 1280, 1050, 990, 960 and 685 cm^{-1}; NMR δ 7.47 (s, 5H) and 9.80 (s, 1H); *m/e* (%) 149.0520 (calc. for $C_8H_7NO_2$: 149.0478, 20), 119(53), 103(45), 91(24) and 77(100); Calc. for $C_8H_7NO_2$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.29; H, 4.77; N, 9.05. The *bis*-phenylhydrazone was recystallized once from dilute ethanol solution, and twice from a mixture of benzene and light petroleum to give a crystalline solid: m.p. 147 – 149 °C (lit. 152 °C);[30] IR 3300, 3180, 1590, 1540, 1490, 1370, 1265, 1245, 1160, 1070, 1010, 950, 750 and 685 cm^{-1}; *m/e* (%) 314 (M^+, 100), 222(75), 209(36), 195(12), 116(12), 104(24), 83(80) and 77(50).

The neat oil resulting from chromatography crystallized on standing overnight. The crystals partially dissolved on heating in chloroform, leaving a solid nitroso dimer of 1-nitroso-2-hydroxystyrene (*10a*) which was filtered: m.p. 113 – 115 °C; IR 3230, 3060, 1590, 1310, 1280, 1210, 1130, 1055, 990, 960 and 700 cm^{-1}; NMR (DMSO-d_6) δ 7.47 (s, 5H), and 9.80 (s, 1H); *m/e* (%) 149 ($1/2M^+$, 46), 119(98), 103(37), 91(15) and 77(100); Calc. for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.07; H, 4.92; N, 9.12. The dimer *10a* was dissolved in chloroform on prolonged heating and the chloroform solution was evaporated to give *10* as shown by the IR absorption at 1700 cm^{-1} and the NMR signal at 9.80 ppm. The dimer *10a* was sublimed at 110 °C/0.1 mmHg to give an oil *10* which crystallized (m.p. 48 – 50 °C) and showed the typical IR and NMR spectra.

Separately, a similar photoreaction was carried out with an ice bath, and the acidic photolysate was evaporated. The residue was worked up to give acidic and basic fractions. From the acidic fraction (2.81 g), NNP (390 mg), the acetophenone *11* (1.40 g, 22%), ketoxime *10* (1.2 g mg, 27%) and dioxime *13* (540 mg, 9%) were obtained by chromatography on a silicic acid column. Chromatography of the basic fraction gave oxime *12* (2.0 g, 24%) and four unknown minor compounds. The detail of chromatography and characterization of these compounds are described in the thesis presented by D. W. L. Chang.[31]

Photoaddition of Nitrosamines to Fused Aromatic Compounds. 1. Anthracene. NNP (2.3 g, 0.02 mol), concentrated hydrochloric acid (2 ml, 0.024 mol) in ethanol (1.4.1) exhibited an optical density of 1.40 at 350 nm. Anthracene (5.4 g, 0,03 mol) was added and vigorously stirred for 1/2 h. The supernatant liquid exhibited optical density of 0.34 at 350 nm with 1/100 dilution. The heterogeneous solution was irradiated for 14 h with a Rayonet RPR 3500 Å lamp. The photolysate was concentrated to 60 ml and diluted with water. The precipitated anthracene and its dimer[19] (2.8 g) were filtered. The acidic aqueous layer was extracted with CH_2Cl_2 (50 ml × 3). The CH_2Cl_2 extract was washed with water, dried ($MgSO_4$) and evaporated to give a resin (1.5 g) which was chromatographed on silicic acid (40 g) to give anthracene (76 mg), NNP (500 mg) and a solid (360 mg). The solid was sublimed to give 9-ethoxyanthrone oxime (*18*, 360 mg): m.p. 155 – 158 °C; IR 3400, 1680, 1320, 1295, 1000, 980, 950, 800, 780, 715, 693 cm^{-1}; NMR δ 8.5 (m, 1H), 7.95 (m, 1H), 7.47 (m, 6H), 5.40 (s, 1H), 3.56 (q, 2H, $J = 7$ Hz), 1.24 (t, 3H, $J = 7$ Hz); m.s. (15 eV) *m/e* (%) 253(89), 208(100).

The aqueous layer was made basic with a sodium carbonate solution to give a white precipitate (3.3 g) which was recrystallized from 2-propanol to give *16*: m.p. 182 – 184 °C (decomposition with gas evolution); IR 3300, 2400, 1500, 1325, 1193, 1165, 1144, 1118, 1098, 1072, 1060, 1038, 1000, 978, 962, 953, 940, 930, 894, 878, 850, 782, 758, 740, 719, 660 cm^{-1}; NMR (pyridine-d_5) δ 9.1 (m, 1H), 8.2 (m, 1H), 7.5 (m, 6H), 4.82 (s, 1H), 2.46 (m, 4H), 1.25 (m, 6H); Calc. for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.90; n, 9.58. Found: C, 77.73; H, 7.00; N, 9.49.

The oxime *16* (100 mg), concentrated hydrochloric acid (0.2 ml) and anthracene (200 mg) in ethanol (100 ml) were irradicated under nitrogen as above. The crude neutral fraction, after removal of anthracene and its dimer, was chromatographed to give oxime *18* (6 mg).

2. 1,4-Dimethylanthracene. NNP (3.05 g, 0.03 mol) concentrated hydrochloric acid (17.5 ml) and 1,4-dimethylanthracene (1,4-DMA, 3.24 g, 0.014 mol) were dissolved in methanol (400 ml). The solution was irradiated under nitrogen with a 200 watt Hanovia lamp for 6 h. The deposited crystals were filtered to give the 1,4-DMA dimer (335 mg): m.p. 242 – 250 °C; IR 1160, 1030, 938, 807, 760, 750, and 660 cm^{-1}; NMR δ 6.84 (m, 8H), 6.44 (s, 4H), 4.73 (s, 4H), 2.26 (s, 12H). The filtrate was worked up in the usual manner to give the syrupy neutral (5.3 g) and the basic fraction (930 mg). This crude neutral resin (1.0 g) was chromatographed on silicic acid (50 g). Elution with $CHCl_3$ gave NNP (100 mg). Elution with $CHCl_3$ containing up to 5% methanol gave the hydrochloride of *20* (400 mg); IR 3450, 2490, 1600, 1500, 1250, 900, 820, 750 cm^{-1}; NMR δ 8.25 (m, 2H), 7.48 (m, 4H), 6.63 (s, 1H), 5.50 (s, 1H), 3.56 (s, 3H), 3.44 (b, 4H), 2.69 (s, 3H), 2.52 (s, 3H), 1.70 (m, 6H). One of these fractions (153 mg) was treated with a saturated

K_2CO_3 solution to give *20*.

The basic syrup was treated with 2-propanol to give crystals (155 mg) which were recrystallized 4 times and sublimed (115 °C, 0.2 mm Hg) to afford an analytical sample of 1,4-dimethyl-9-pipedidino-10-methoxy-9,10-dihydroanthracene (*20*); m.p. 145 – 146 °C; IR 1612, 1585, 1078, 935, 825, 810, 760, 740 cm^{-1}; NMR δ 7.40 (s, 4H), 7.13 (s, 2H), 5.23 (s, 1H), 4.30 (s, 1H), 3.48 (s, 3H), 3 – 2 (m, 4H), 2.52 (s, 3H), 2.48 (s, 3H), 1.4 (m, 6H); Calc. for $C_{22}H_{27}NO$; C, 82.84; H, 8.41; N, 4.36. Found C, 82.16; H, 8.34; N, 4.51. The mother liquour was evaporated to dryness (384 mg) and chromatographed on alumina (40 g) to give *20* (90 mg) and a solid (30 mg). The solid was crystallized from methanol to give 1,4-dimethyl-9-piperidino-10-hydroxy-9,10-dihydroanthracene (*21*): m.p. 206 – 207 °C; IR 3300, 1600, 1500, 1108, 1070, 1039, 980, 970, 950, 828, 820, 750 cm^{-1}; NMR δ 7.24 (m, 4H), 7.00 (s, 2H), 6.62 (s, 1H), 4.37 (s, 1H), 2.50 (s, 3H), 2.40 (s, 3H), 2.5 (m, 4H), 1.4 (m, 6H); m.s. (1,7 kV) *m/e* (%) 307(81), 224(100), 204(80); M^+) Calc. for $C_{21}H_{25}NO$; 307.1936. Found 307.1932.

3. Pyrene. A heterogeneous solution of NNP (2.3 g, 0.02 mol), concentrated hydrochloric acid (2 ml, 0.024 mol) and pyrene (2.1 g, 10.01 mol) in methanol (300 ml) was irradiated under nitrogen with a 450 W Hanovia lamp for 3 h. At this time the solution had turned black. The photolysate was evaporated to 100 ml and filtered to give crystals of pyrene (502 mg): m.p. 110 – 118 °C. The photolysate was worked up in the usual manner to give neutral (2.53 g) and the basic (58 mg) fractions. The neutral oil (1.5 g) was chromatographed on alumina (45 g) to give a solid (700 mg), NNP (100 mg) and unidentified mixtures (200 mg). The solid was recrystallized 3 times from benzene-2-propanol to give 4-piperidinopyrene *23*: m.p. 90 – 91 °C; IR 1600, 1590, 1513, 1226, 840 cm^{-1}; NMR δ 8.00 (m, 9H); 3.1 (m, 4H), 1.75 (m, 6H); Calc. for $C_{21}H_{19}N$: C, 88.38; H, 6.71; N, 4.91. Found: C, 88.00; H, 6.82; N, 5.00. Compound *23* dissolved in $CHCl_3$ turned a brown-purple color after some days.

4. Acenaphthene. A solution of the NND (3.70 g, 0.05 mol) concentrated hydrochloric acid (7.5 ml, 0.09 mol) and acenaphthene (9.10 g, 0.06 mol) in methanol (400 ml) was irradiated with a 200 watt Hanovia lamp for 8 h. The solution was then filtered to give crystals of acenaphthene dimer (603 mg): m.p. 227 – 228 °C, lit.[22] 234 °C. The solution was evaporated to 40 ml and cooled to give crystals (800 mg, m.p. 230 – 235 °C dec) which were crystallized from 2-propanol three times to give acenaphthoquinone (*28*): m.p. 252 – 255 °C, lit.[22] 261 °C; IR 1720, 1260 cm^{-1}.

The concentrated photolysate was worked up in the usual manner to give a neutral (3.7 g) and a basic (5.9 g) fraction. The neutral fraction contained acenaphthene and NND by IR and NMR spectral analysis. The basic fraction was treated with benzene to afford crystals (1.05 g) which were recrystallized five times from benzene to afford an analytical sample of *anti*-2-dimethylaminoacenaphth-1-one oxime (*27*); m.p. 123 – 125 °C; IR 3100, 970, 930, 858, 850, 800, 785 cm^{-1}; NMR δ 11.0 (b, 1H, D_2O exch.), 8.4 (m, 1H), 7.7 (m, 5H), 5.22 (s, 1H), 2.14 (s, 6H).

The presence of *syn*-2-dimethylaminoacenaphth-1-one oxime (*27*) was indicated by the singlet signals at 5.43 and 2.42 ppm (the ratio of 1:6) in addition to the signals indicated above in the recovered material. The ratio of the *syn* to *anti* isomers was 3:7.

Preparation of N-*nitroso-4-azatricyclo*[*5.2.1.0*^{2,6}]-*dec-8-ene* (*31*). *Endo*-Norbornene-*cis*-5,6-dicarboxylic anhydride (*29*) (50 g) was treated with concentrated NH_4OH (150 ml) to give the ammonium salt as a solid (45 g): m.p. > 250 °C; IR 3460(s), 3200(s), 1710(m), 1660(s), 1628(s), 1412(s), 1290, 1270, 1232(s) and 730(s) cm^{-1}. This solid (23 g) was refluxed in acetic anhydride (12 ml) for 2 h to give *endo*-norbonene-*cis*-5,6-dicarboximide (19.75 g): m.p. 186 – 187 °C (lit. 185 – 186.5 °C):[32] IR 3155, 3060(s), 1750, 1700(s), 1295(s), 1230(m), 1190, 1120, 992(s), 840, 738 and 640(s) cm^{-1}; NMR δ 8.50 (m, D_2O exch. 1H), 6.20 (m, 2H), 3.33 (m, 4H), 1.78 and 1.52 (AB q, $J = 9$ Hz, 2H).

The imide (16 g, 0.1 mol) was reduced with LAH (16 g, 0.41 mol) in dry THF (700 ml) to yield a semisolid (15.5 g). Recrystallization from ethyl acetate – light petroleum (30 – 60 °C) or vacuum sublimation (25 °C/0.05 mmHg) gave 4-azatricyclo-[5,2,1,0^{2,6}]dec-8-ene (*30*): m.p. 59 – 60 °C; IR 3350 (s, br), 3050(w), 2720(w), 2460(w), 1625(m), 1512(s), 1255, 1230(m), 820, 752 and 720(m) cm^{-1}; NMR 6.22 (m, $W_{1/2} = 4$ Hz, 2H), 5.00 (s, D_2O exch., 2H), 2.89 (m, 8H), 1.55 and 1.40 (AB q, $J = 8.5$ Hz); m.s. *m/e* (%) 135(41), 134(10), 94(53), 69(41) and 68(100): Found: C, 48.8; H, 6.39; N, 6.02.

A crude amine *30* was treated with $NOBF_4$ to yield *N*-nitrosamine *31*. An analytical sample was prepared by repeated sublimation (25 °C/0.5 mm): m.p. 73 – 73.5 °C; IR 3060(w), 1452(s), 1412(s), 1312(s), 1220, 842, 802, 770, 742(m) and 720(m) cm^{-1}; NMR δ 6.18 (m, 2H), 4.10 (m, H-5, 2H), 3.40 (m, H-3, 2H), 3.02 (m, 5H), 1.65 and 1.47 (AB q, $J = 9$ Hz); m.s. *m/e* (%) 164 (M^+, 62), 134(29), 105(54), 79(71), 68(91), 67(54) and 66(100); UV (methanol) 348 nm (ε, 87): Calc. for $C_9H_{12}N_2O$: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.80; H, 7.22; N, 17.11.

Photolysis of nitrosamine 31. *1. Under oxygen.* A solution of nitrosamine *31* (2 g, 0.012 mol) and concentrated HCl (1.1 ml, 0.066 N) in methanol (200 ml) was photolyzed under oxygen for 2.75 h. The basic fraction was extracted into ether (4 × 50 ml) which was immediately dried and stirred overnight with LAH (2 g). The usual work-up gave a clear oil

(2.63 g) which showed one major spot at R_f 0.12 on TLC (silica gel, 10% methanol – CH_2Cl_2) and the NMR spectrum corresponded essentially to that of alcohol *32* with weak signals of unidentified products. The oil (100 mg) was purified by preparative TLC to give a semisolid (36 mg) which was recrystallized several times from ether to give tetracyclic amino alcohol *32*: m.p. 150–153 °C; IR 3200 (s, br), 1270(m), 1140, 1092(m), 1040(s), 1005(s), 960, 940, 902, 815 and 778(m) cm^{-1}; NMR δ 5.08 (s, D_2O exch.), 4.30 (m, $W_{1/2}=4$ Hz, H_e), 3.59 (m, H_a), 3.25 (dd, $J=12$ and 2 Hz, H_d), 3.02 (m, 1H), 2.8–2.2 (unresolved, 7H), 1.65 and 1.60 (AB q, $\Delta\nu=38$, $J=10.5$ Hz, H_b and H_c) and 1.60 (m, 1H); m.s. *m/e* (%) 151 (M^+, 45), 134(33), 122(55), 85(55), 80(50), 79(50), 68(44) and 57(100).

The picrate of *32* was recrystallized three times from ethanol – light petroleum: m.p. 245–255 °C (decomp): Calc. for $C_{15}H_{16}N_4O_8$: C, 47.37; H, 4.24; N, 14.73. Found: C, 47.70; H, 4.47; N, 14.53.

Amino alcohol *32* (30 mg) in acetone (3 ml) was treated with $CrO_3-H_2SO_4$ at 0 °C. The green solution was stirred for 10 min at which time the acetone was evaporated and the residue was basified with aqueous Na_2CO_3. Extraction with CH_2Cl_2 gave crystalline ketone *34* (18 mg, 62%): m.p. 115–117 °C; IR 1750(s), 1490(w), 1282, 1162, 990, 958(m), 820, 770 and 732(m) cm^{-1}; NMR δ 3.2–2.5 (unresolved, 8H) and 1.55 (AB q, $\Delta\nu=24$, $J=11.5$ Hz, 2H); m.s. *m/e* (%) 149 (M^+, 11), 121(100), 120(60), 100(54), 93(60), 80(48), 79(54) and 77(55).

2. In bromotrichloromethane. A solution of nitrosamine *31* (1 g, 0.006 mol) and concentrated HCl (0.52 ml) in methanol – $CBrCl_3$ (1:4, 120 ml) was photolyzed under nitrogen for 1.5 h. The blue solvent mixture was distilled under vacuum and the usual work-up gave a neutral extract (260 mg) and a basic extract (750 mg). The basic fraction (500 mg) was chromatographed on silica gel where elution with ethyl acetate gave bromoamine *33* (335 mg): IR 1505(w), 1300, 1280, 1260, 1228(m), 1180, 1130, 1055, 1008, 928(m), 880(s), 802, 770, 745(s), 730 and 678(m) cm^{-1}; NMR 4.70 (m, $W_{1/2}=3.5$ Hz, H_e), 3.40 (m, $W_{1/2}=9$ Hz, H_a), 3.21 (dd, $J=12.5$ and 2.5 Hz, H_d), 2.7–2.2 (unresolved, 5H), 2.10 (m, 1H) and 1.60 (d, $J=10.5$ Hz, H_c); m.s. *m/e* (%) 215(1.5), 213 (M^+, 1.5) and 134(100).

The picrate of *33* was recrystallized four times from ethanol to give yellow needles: m.p. 224–235 °C with slow decomposition: Calc. for $C_{15}H_{15}N_4O_7Br$: C, 40.65; H, 3.41; N, 12.64. Found: C, 40.85; H, 3.31; N, 12.84.

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Crystal and Molecular Structure of Dihydrodigoxigenin Hydrate

ARVID MOSTAD

Department of Chemistry, University of Oslo, Oslo 3, Norway

The crystal and molecular structure of the 20,22-dihydro derivative of digoxigenin has been determined by X-ray crystallographic methods using 1501 reflections recorded on a SYNTEX $P\bar{1}$ diffractometer. The crystals are orthorhombic, space group $P2_12_12_1$ with unit cell dimensions $a=8.027(2)$ Å, $b=14.801(5)$ Å, $c=18.376(7)$ Å. The structure was refined to a conventional R-factor of 0.05.

Estimated standard deviations are 7×10^{-3} Å and 0.4° in the interatomic distances and angles when hydrogen atoms are not involved. The asymmetric center created at C20 by the hydrogenation is shown to be S in the 3β,12β,14β trihydrozy derivative.

Structure-activity studies of cardiac glycosides have disclosed the importance of the unsaturated lactone ring and that a hydrogenation of the C20–C22 double bond in this ring abolishes activity.[1] The C20–C22 bond saturation, which seems to be included in the metabolic pathway of such compounds, will change the conformation of the lactone ring as well as introduce a center of asymmetry at C20.[2] Catalytic hydrogenation of dioxygenin has been shown to yield two components which are separable in a "major" and a "minor" fraction,[2] and the present paper presents the crystal and molecular structure of the "minor" component of the hydrogenation process.

EXPERIMENTAL

A sample of about 1.9 mg of dihydrodigoxigenin hydrate was supplied by Dr. Richard H. Reuning, College of Pharmacy, The Ohio State University. The sample was recrystallized from methanol, giving a cluster of prismatic crystals. Only one crystal in the sample was suitable for X-ray crystallographic work and was used throughout the investigation. The experimental conditions are described in Table 1.

Cell parameters were determined by a least squares fit to the diffractometer settings for 15 general reflections. The standard deviations in the

Table 1. Experimental conditions.

Instrument	SYNTEX $P\bar{1}$
Radiation	Graphite crystal monochromated Mo$K\alpha$, $\lambda=0.71069$ Å
Crystal dimensions/mm	$0.2\times0.2\times0.2$
Scanning mode	$\theta/2\theta$
Scan speed/° min^{-1}	3.0 ($2\theta<45.0°$)
	Variable depending on intensity for $2\theta>45.0°$
Scan range	$2\theta\alpha_1-0.7$ to $2\theta\alpha_2+0.9$
Background counts	For 0.35 of scan time at scan limits
Temperature/K	121
2θ range	$2.0<2\theta<60.0$
Number of reflections meas.	2027
Number of reflections $I>2.5\sigma(I)$	1501
Number of standard reflections	3
Number of reflections between standard reflections	57

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measured intensities were calculated as $\delta(I)=|C_T+(0.02\ C_N)^2|^{\frac{1}{2}}$, 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. The variation in the intensities of the test reflections was less than 1 % and no corrections were made on this basis. Scattering factors used were those of Doyle and Turner[3] for O and C and of Stewart, Davidson and Simpson[4] for H.

CRYSTAL DATA

Dihydrodigoxigenin hydrate, $C_{23}O_5H_{36}\cdot H_2O$, orthorhombic, $a=8.027(2)$ Å $b=14.801(5)$ Å, $c=18.376(7)$ Å, $V=2183.2$ Å^3, $M=410.5$, $Z=4$, $F(000)=896$, space group $P2_12_12_1$.

STRUCTURE DETERMINATION

The structure was solved by direct methods using the program assembly MULTAN.[5] Successive Fourier syntheses indicated the positions of all the non-hydrogen atoms. The positions of the hydrogen atoms were introduced from considerations of the molecular geometry and of the hydrogen bond system. All positional parameters, anisotropic temperature factors for the non non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms were refined in successive least squares calculations. The final R-factor was 0.049 and the goodness of fit: $S=[\Sigma w\Delta^2/(m-n)]^{\frac{1}{2}}=1.22$. The corresponding parameters are given in Tables 2 and 3. Tables of observed and calculated structure factors are available from the author.

Table 2. Fractional atomic coordinates and thermal parameters multiplied by 10^4. The anisotropic temperature factor is given by $\exp -2\pi^2(u_{11}a^*h^2+\cdots+2u_{12}a^*b^*hk+\cdots)$. Estimated standard deviations in parentheses.

Atom	9793(4)	4540(2)	3637(2)	255(22)	329(22)	259(20)	27(20)	18(19)	145(17)
O12	7634(4)	1299(2)	6927(2)	284(22)	145(18)	246(19)	12(18)	10(19)	11(14)
O14	7266(4)	4358(2)	7920(2)	257(22)	250(19)	160(17)	46(19)	30(18)	−93(16)
Ow	0408(5)	4762(2)	8357(2)	319(22)	351(23)	327(21)	−104(21)	97(21)	55(19)
O21	4038(5)	2023(3)	9709(2)	383(26)	527(27)	252(22)	−98(24)	77(22)	71(20)
O23	5836(5)	1092(3)	10262(2)	461(28)	711(31)	326(25)	−140(28)	41(23)	228(24)
C1	10345(6)	3306(3)	4865(2)	151(27)	269(29)	148(25)	15(26)	32(25)	135(24)
C2	8820(6)	3313(3)	4359(3)	198(3)	231(28)	157(26)	16(28)	19(24)	−14(22)
C3	8429(6)	4250(3)	4102(3)	186(31)	244(31)	241(31)	−14(26)	6(27)	49(26)
C4	8221(6)	4888(3)	4741(3)	169(28)	187(28)	299(31)	−26(25)	5(27)	40(26)
C5	9658(7)	4886(3)	5284(3)	184(27)	139(26)	224(28)	0(24)	61(27)	46(24)
C6	9322(7)	5503(3)	5935(3)	257(33)	139(26)	276(30)	9(26)	0(29)	−22(24)
C7	8024(6)	5124(3)	6454(3)	248(32)	211(28)	236(28)	48(27)	62(26)	−100(24)
C8	8461(6)	4170(3)	6715(3)	178(29)	197(29)	129(26)	−15(24)	22(24)	25(23)
C9	8716(6)	3543(3)	6054(3)	185(3)	196(28)	165(26)	21(25)	58(24)	−21(23)
C10	10098(6)	3914(3)	5544(2)	168(29)	185(27)	173(25)	−21(25)	14(23)	−27(23)
C11	8951(6)	2554(3)	6296(3)	195(28)	195(27)	140(25)	55(26)	14(24)	44(23)
C12	7520(7)	2249(3)	6766(3)	218(3)	121(25)	213(27)	89(25)	5(28)	−14(22)
C13	7369(7)	2801(3)	7486(3)	160(27)	202(27)	133(24)	24(25)	0(25)	25(22)
C14	7172(6)	3815(3)	7272(3)	217(32)	204(28)	150(26)	56(27)	8(23)	−35(22)
C15	5341(6)	3886(3)	7025(2)	148(25)	212(28)	187(26)	32(24)	73(25)	−57(24)
C16	4412(6)	3285(3)	7571(3)	155(28)	282(29)	270(28)	−13(28)	25(27)	−12(26)
C17	5670(6)	2558(4)	7862(3)	192(3)	258(29)	180(26)	−54(27)	33(26)	−25(25)
C18	8935(7)	2647(3)	7952(3)	235(3)	274(32)	180(28)	7(28)	2(26)	28(25)
C19	11807(6)	3947(3)	5938(3)	165(29)	264(30)	242(29)	33(26)	16(25)	37(26)
C20	5659(6)	2538(3)	8697(3)	207(3)	197(27)	219(27)	−17(26)	24(26)	−50(24)
C21	3894(7)	2468(4)	9005(3)	335(35)	337(32)	241(30)	9(33)	66(29)	34(28)
C22	6480(7)	1695(4)	9051(3)	263(32)	329(32)	235(28)	−17(30)	11(28)	62(27)
C23	5481(8)	1546(4)	9739(3)	345(38)	506(43)	247(33)	−159(37)	109(34)	23(31)

Table 3. Fractional atomic coordinates and isotropic temperature factors for the hydrogen atoms.

Atom	*X*	*Y*	*Z*	*B*
HO3	0.941	0.484	0.334	1.8
HO12	0.682	0.101	0.678	6.2
HO14	0.821	0.446	0.801	11.2
H104	0.083	0.466	0.875	2.0
H204	0.087	0.531	0.823	9.0
H1C1	1.131	0.355	0.460	2.6
H2C1	1.064	0.270	0.507	2.3
H1C2	0.901	0.289	0.394	0.8
H2C2	0.784	0.307	0.466	0.9
HC3	0.734	0.424	0.381	0.2
H1C4	0.806	0.552	0.457	0.5
H2C4	0.710	0.473	0.498	1.3
HC5	1.052	0.511	0.503	0.3
H1C6	1.037	0.560	0.623	1.0
H2C6	0.902	0.614	0.577	0.7
H1C7	0.790	0.552	0.691	0.4
H2C7	0.691	0.510	0.620	1.5
HC8	0.951	0.415	0.698	3.4
HC9	0.766	0.363	0.578	−0.1
H111	0.905	0.218	0.587	2.0
H211	1.011	0.246	0.657	3.2
HC12	0.646	0.237	0.650	−0.3
H115	0.494	0.455	0.706	−0.0
H215	0.523	0.367	0.650	1.8
H116	0.401	0.370	0.800	2.4
H216	0.343	0.299	0.738	1.3
HC17	0.533	0.196	0.768	1.1
H118	0.881	0.289	0.838	2.8
H218	0.918	0.197	0.802	1.0
H318	0.992	0.286	0.770	0.3
H119	1.176	0.429	0.641	4.7
H219	1.223	0.333	0.610	0.3
H319	1.270	0.417	0.562	2.7
HC20	0.620	0.308	0.889	−0.4
H121	0.333	0.307	0.908	1.5
H221	0.313	0.209	0.868	5.1
H122	0.766	0.181	0.922	2.1
H222	0.628	0.116	0.872	3.4

Table 4. Bond lengths and angles. Estimated standard deviations are 7×10^{-3} Å in the bond lengths and 0.4° in the angles.

Bond lengths (Å)		Bond angles (°)	
C1–C2	1.537	C1–C2–C3	111.3
C2–C3	1.498	C2–C3–C4	110.8
C3–O3	1.454	C3–C4–C5	114.7
C3–C4	1.516	C4–C5–C6	112.2
C4–C5	1.525	C4–C5–C10	112.0
C5–C6	1.529	C5–C10–C1	108.5
C6–C7	1.521	C10–C1–C2	112.4
C7–C8	1.532	C2–C3–O3	107.5
C8–C9	1.543	O3–C3–C4	110.8
C9–C10	1.552	C5–C10–C19	108.4
C10–C1	1.552	C5–C10–C9	110.5
C10–C5	1.557	C1–C10–C19	106.3
C10–C19	1.551	C9–C10–C19	111.2
C9–C11	1.542	C1–C10–C9	111.8
C11–C12	1.507	C5–C6–C7	113.1
C12–O12	1.440	C6–C7–C8	112.3
C12–C13	1.560	C7–C8–C9	109.8
C13–C14	1.560	C8–C9–C10	110.9
C14–O14	1.438	C8–C9–C11	111.1
C14–C8	1.548	C7–C8–C14	111.5
C14–C15	1.542	C9–C11–C12	110.9
C15–C16	1.535	C11–C12–O12	111.2
C16–C17	1.569	C11–C12–C13	112.8
C17–C13	1.570	O12–C12–C13	110.1
C13–C18	1.539	C12–C13–C14	107.4
C17–C20	1.535	C12–C13–C18	109.3
C20–C21	1.529	C12–C13–C17	108.7
C20–C22	1.554	C8–C14–O14	108.9
C22–C23	1.513	C8–C14–C13	115.2
C21–O21	1.457	C14–C13–C18	111.5
C21–C23	1.358	C18–C13–C17	115.5
C23–O23	1.207	C13–C14–C15	103.7
		C13–C14–O14	108.9
		C14–C15–C16	103.4
		C15–C16–C17	107.9
$\overline{C-H}$	1.03	C16–C17–C13	104.6
		C16–C17–C20	110.5
$\overline{O-H}$	0.80	C13–C17–C20	116.7
		C17–C20–C21	112.1
		C17–C20–C22	115.5
		C21–C20–C22	100.6
		C20–C22–C23	104.0
		C20–C21–O21	106.6
		C21–O21–C23	109.8
		O21–C23–C22	110.0
		O21–C23–O23	121.5
		C22–C23–O23	128.4

DESCRIPTION AND DISCUSSION

The labelling of the atoms is indicated in Fig. 1 which also illustrates the molecular packing as well as the hydrogen bond system. Bond lengths and angles are given in Table 4 and the values are found to be in agreement with those reported for similar structures.[6-11] The atomic coordinates in Table 2 describe the steroid nucleus in the well-known configuration of a 3β,12β,14β-trihydroxy derivative, the six-membered rings being in chair conformation and the five-membered D-ring existing in a 14β envelope form. An analysis of the ring conformations is given in Table 5. A least squares plane through the six atoms: C5, C6, C8, C9, C12, C13 shows

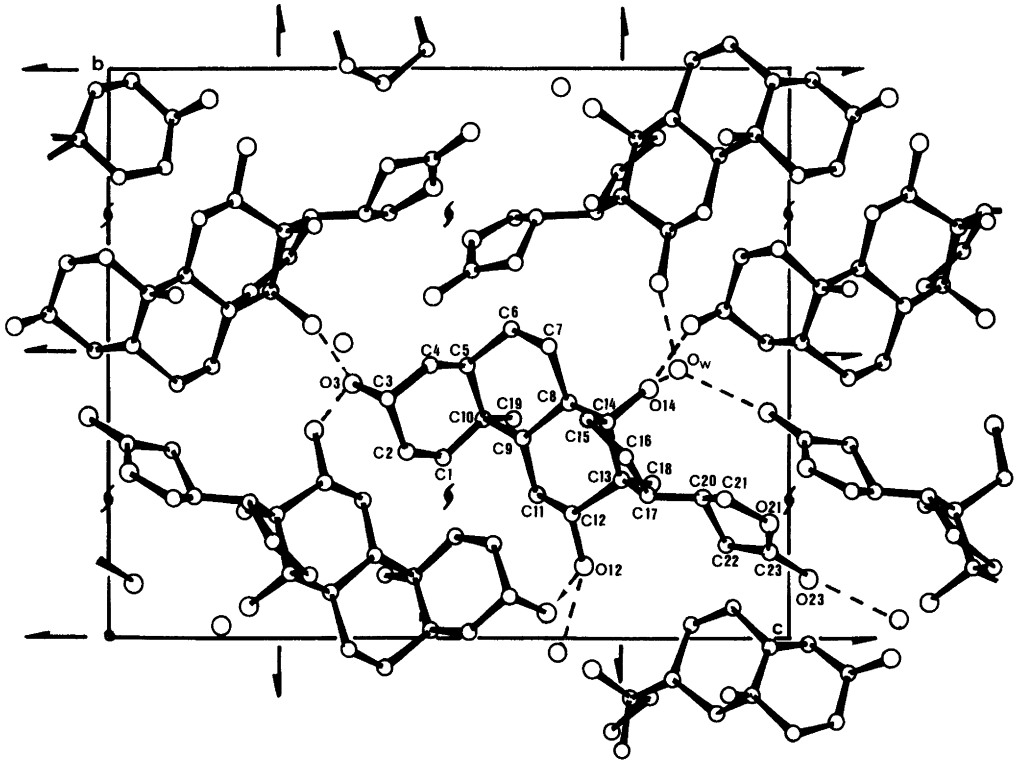

Fig. 1. Packing of dihydrodigoxigenin molecules in the crystal as seen along the *a*-axis.

Table 5. Distances from least squares planes in the ring system. The atoms defining the respective planes are all less than 0.016 Å from these planes; the largest deviations found in the C- and D-rings.

A-ring	B-ring	C-ring	D-ring	Lactone ring
Least squares planes defined by				
C2,C3,C5,C10	C5,C10,C7,C8	C9,C11,C13,C14	C13,C15,C16,C17	O23,O21,C23,C22
Distance (Å)				
C1: 0.68	C6: 0.63	C12: −0.69	C14:0.61	C21: −0.08
C4: −0.60	C9: −0.69	C8: 0.56		C20: 0.41

Table 6. Distances and angles concerning the hydrogen bond system.

D	A	Ekv. pos	D−A	D−H	H···A	∠D−H···A
O3	O14	$1.5-x, 1-y, z-\frac{1}{2}$	2.670	0.77	1.95	155.6
O12	O3	$x-\frac{1}{2}, \frac{1}{2}-y, 1-z$	2.795	0.83	1.97	172.0
O14	O4	$1+x, y, z$	2.714	0.79	1.93	172.4
O4	O12	$1-x, \frac{1}{2}+y, 1.5-z$	2.813	0.92	1.93	164.5
O4	O23	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	2.854	0.82	2.12	149.4

deviations of ±0.08 Å indicating a twist between the B and C ring. The angle between the planes through C5, C6, C9 and C8, C12, C13 is found to be 7.7°.

The lactone ring in the present structure is saturated and hence not planar and the conformation of the ring may be described as a C20-envelope form. The conformation about the C17–C20 bond is staggered with HC17 and HC20 in *trans* position. The torsion angle C13–C17–C20–C22 is −76° and C13–C17–C20–C21 is 169°. Thus in the present structure neither C21 nor C22 but HC20 is in a synclinal position relative to C13 and C16 and in this respect the conformation about the C17–C20 is different from those earlier reported for similar structures.[8] The absolute configuration at C20 is *S* in the "minor" component of the 3β,12β,14β derivative and this is actually equivalent with the *R* configuration in (20*R*)-3β-hydroxy-22-methylene-5β-card-14-enolide.[8]

An extensive hydrogen bond system binds the molecules together in the crystal. Each of the three hydroxy groups is involved in two hydrogen bonds, both as donor and acceptor, whereas the carbonyl oxygen is engaged as acceptor in one hydrogen bond. The O3–O12 bond connects molecules in a helix about one of the screw axes and the O3–O14 bond connects molecules in a chain along the *c*-axis. The rest of the hydrogen bonds involve water molecules each of which is engaged in three such bonds, two as a donor and one as an acceptor. The geometry of the hydrogen bond system is described in Table 6.

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Intramolecular Cyclisation of Iminoxyl Radicals

SHIRAVANATE ATMARAM, ALEXANDER R. FORRESTER, MELVYN GILL, RUSSELL J. NAPIER and RONALD H. THOMSON

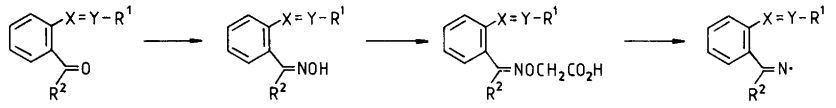
Department of Chemistry, University of Aberdeen, Meston Walk, Old Aberdeen AB9 2UE, Scotland

Iminyl radicals, generated by oxidation of the corresponding *O*-carboxymethoximes with persulphate, do not cyclise onto an adjacent azo group but the structurally related iminoxyls cyclise to give indazoles. ESR evidence is presented for the intramolecular addition of iminoxyl radicals to carbon–carbon double bonds and to aryl rings.

Iminyls cyclise onto an adjacent aromatic ring[1] or carbon–carbon double bond[2] given favourable geometry. In principle, cyclisation onto other unsaturated groups is also possible. Accordingly, we have extended our previous investigations[2] to iminyls of type *4*.

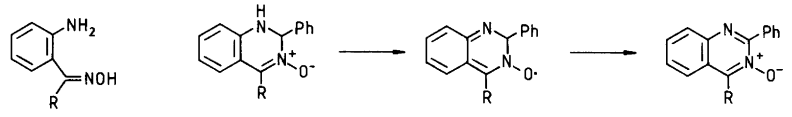
The iminyl precursors[3] most suitable for chemical studies are methylene-imino-oxyacetic acids *3* and their derivatives. These are usually prepared from the corresponding ketones *1 via* the oximes *2* thus providing the initial synthetic objectives of the work.

Precursors of o-*azo-*, o-*azoxy- and* o-*imino-diphenyliminyls.* Although the ketones *1a*, *1b*, *1e*[4] were readily prepared, numerous attempted condensations with hydroxylamine, methoxyamine, and amino-oxyacetic acid, under forcing conditions, failed to yield the desired products. The oximes *2a* and *2b* were eventually prepared[5] from the *o*-aminoketoximes *5a* and *5b* by condensation with nitrosobenzene and converted into *3a* and *3b* in the usual way.[3] However, oxidation with aqueous persulphate gave no major product, and the many minor coloured products were not investigated. Condensation of the amino-ketoximes *5a* and *5b* with benzaldehyde failed to give the desired *o*-imino-ketoximes *2c* and *2d*. Instead, the dihydroquinazoline *N*-oxides *6a* and *6b* were formed as earlier reported. Confirmation of structure *6b* was achieved by ^{13}C NMR spectroscopy. In particular the signal at δ 79.55 (doublet) is attributed to C–2. The imino carbon of *2c* would be expected to resonate at lower field. Oxidation of the dihydroquinazoline *N*-oxides *6a* and *6b* to *8a* and *8b* apparently proceeds *via* the corresponding nitroxides *7a* and *7b*. Thus, treatment of *6a* and *6b* with lead tetra-acetate in the cavity of an ESR



1 2 3 4

Scheme 1. *1a–4a*, X=Y = N=N, $R^1=R^2$=Ph; *1b–4b*, X=Y = N=N, R^1=Ph, R^2=Me; *1c–4c*, X=Y = N=CH, $R^1=R^2$=Ph; *1d–4d*, X=Y = N=CH, R^1=Ph, R^2=Me; *1e*, X=Y = N=$\overset{+}{N}(O^-)$, R^1=Me, R^2=Ph.



5 6 7 8

Scheme 2. *5a–8a*, R=Ph; *5b–8b*, R=Me.

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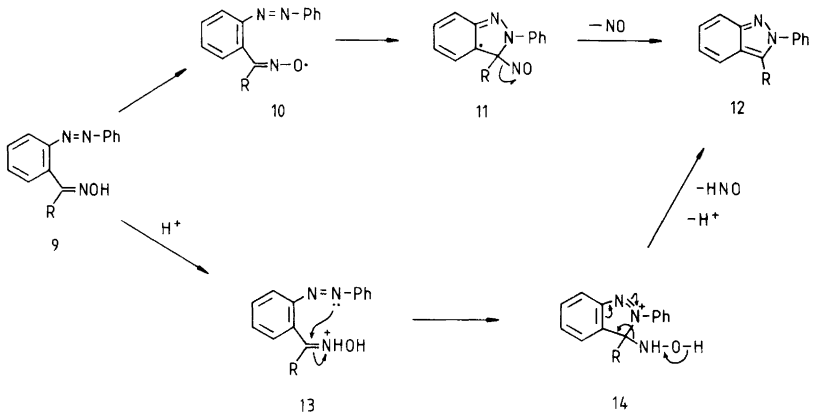


Scheme 3. *9a*–*14a*, R = Ph; *9b*–*14b*, R = Me.

spectrometer led to the detection of the nitroxides *7b*, $a_{N-3}=9.25$ and $a_{N-1}=2.5$ G, and *7a*, $a_{N-3}=7.25$, $a_{N-1}=3.55$, $a_H=1.0$ G. The broad lines of the former spectrum (~1.25 G) prevented resolution of the proton splitting. The differences in the a_{N-1} values of the two spectra is surprising and at present unexplained.

Following these failures to observe intramolecular addition of iminyls to azo and imino groups attention was turned to the possibility of adding the corresponding iminoxyl radicals to adjacent multiple bonds. Oximes are potential precursors of both iminyls and iminoxyls and several suitable examples with adjacent, azo, alkenyl and aryl groups were available from this and earlier work.[1,2]

o-*(Phenylazo)phenylmethyleneiminoxyls.* Treatment of *o*-phenylazobenzophenone oxime *9a* with lead tetra-acetate at −60 °C in the cavity of an ESR spectrometer gave a weak spectrum of the corresponding iminoxyl *10a*, $a_N=31$ G. The spectrum

Table 1. ESR data on radicals derived from oximes *15*.

Oxime	Method[a]	Temp. °C	a_N (G)		Radical type
15a	a	20	12.5		Dialkyl nitroxide *20*
	a	−60	19.75	5.4(1H) (Ha); 1.2(1H) (Hb)	Bicyclic nitroxide *18a*
	b	−40	19.75	5.25(1H) (Ha); 1.25(1H) (Hb)	Bicyclic nitroxide *18a*
15b	a	−60	20.1	5.25(1H) (Ha); 1.2(1H) (Hb)	Bicyclic nitroxide *18b*
	a	−60	11.5		Dialkyl nitroxide *20*
	b[+]	−30	20.0	5.25(1H) (Ha); 1.25(1H) (Hb)	Bicyclic nitroxide *18b*
15c	a	−60	11		Dialkyl nitroxide *20*
	b	−30 to −10	30.5		Iminoxyl *16c*
			32.0		
	b	−10 to +10	19.75	5.25(1H) (Ha); 0.9(4H) (Hb,Me)	Bicyclic nitroxide *18c*
15d	a	−70	30.75		Iminoxyl *16d*
	a	−10	11.8		Dialkyl nitroxide *20*
	b	0	30.75		Iminoxyl *16d*
15e	a	−50	31.0		Iminoxyl *16e*
	a	10	12.0		Dialkyl nitroxide *20*
	b	−50	31.0		Iminoxyl *16e*

[a] Method of oxidation. a, treatment with lead tetra-acetate; b, photolysis in di-*t*-butyl peroxide; +, $g=2.0069(3)$.

faded with increasing temperature and at room temperature the solution was shown to contain the diphenylindazole *12a*. More efficient conversion into the indazole *12a* was achieved by treatment of the oxime with lead tetra-acetate or di-t-butyl peroxalate (source of *t*-butoxyl) at or above room temperature when evolution of nitrogen dioxide ($NO+O_2$) was obvious. *o*-Phenylazoacetophenone oxime *9b* behaved similarly. Product formation is rationalised by the sequence *10*→*11*→*12* which includes a favourable 5-exo trigonal cyclisation.[7] Cyclisation of the oximes *9a* and *9b* to the indazoles *12a* and *12b* was also achieved by reaction with acid. Clearly, iminoxyls *10* do not mediate in this cyclisation and an alternative sequence *9*→*13*→*14*→*12* involving final elimination of nitroxyl (HNO) is postulated.

o-Alkenylphenylmethyleneiminoxyls. Continuous photolysis of a solution of the oxime *15a* in di-*t*-butyl peroxide in an ESR spectrometer did not give the spectrum of the corresponding iminoxyl (a_N 30 G). Instead, at −40 °C a triplet of double doublets [$a_N=19.75$, $a_H=5.4$(1H), $a_H=1.2$ G(1H), $g=2.0069$] was detected. A nearly identical spectrum was obtained from the phenyl analogue *15b*. Even more interesting was the result with *15c*. From −30 to −10 °C overlapping spectra of two iminoxyls (a_N 30.0 and 32 G) were present. From −10 to +10 °C these signals were gradually replaced by another spectrum which had $a_N=19.75$, $a_H=5.25$(1H), $a_H=$ 0.9 G (4H). Above 20 °C only the latter spectrum persisted. The other two oximes listed in Table 1 only gave spectra of the corresponding iminoxyls.

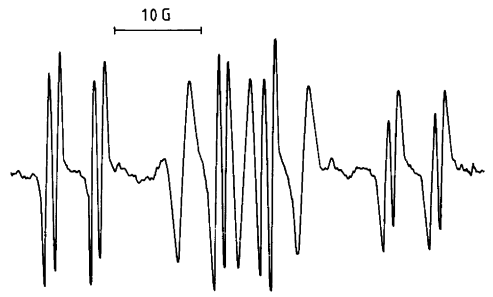
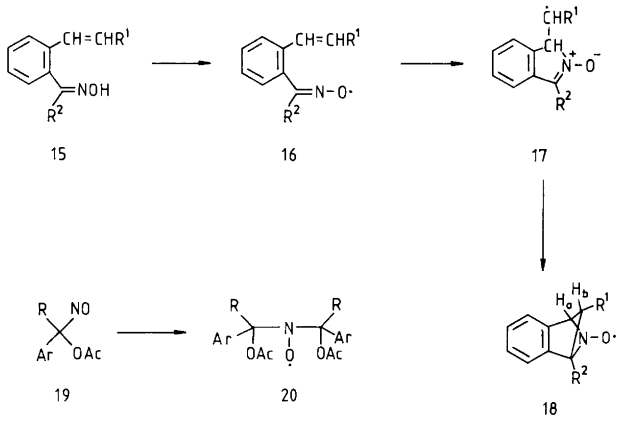


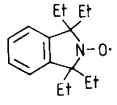
Fig. 1. Spectrum obtained by oxidation of oxime *15a* with lead tetra-acetate at −60 °C.

When lead tetra-acetate was used as oxidant the spectrum of either the iminoxyl ($a_N\sim30$ G) or that of the radical with $a_N\sim20$ G was detected but not both. These spectra were usually accompanied by a second spectrum with $a_N\sim11-12$ G which usually intensified with increasing temperature as the iminoxyl decayed.

The iminoxyl spectra were similar to that observed from *o*-ethylphenyl phenylmethyleneiminoxyl and are not remarkable. Also, nitroxides with $a_N=11-12$ G are often detected when oximes are treated with lead tetraacetate and are attributed[8] to di-*t*-alkyl nitroxides *20* formed from the corresponding nitroso-acetates *10*. The spectra with $a_N\sim20$ G are more interesting and probably arise from the corresponding iminoxyls since in all but one case they are not detected when spectra of the iminoxyl appear and *vice versa*. Exceptionally, with



Scheme 4. *15a*−*18a*, $R^1=Ph$, $R^2=Bu^t$; *15b*−*18b*, $R^1=R^2=Ph$; *15c*−*18c*, $R^1=Me$, $R^2=Ph$; *15d*, $R^1=Et$, $R^2=Bu^t$; *15e*, $R^1=Pr^i$, $R^2=Bu^t$.



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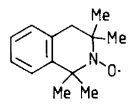

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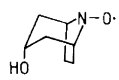
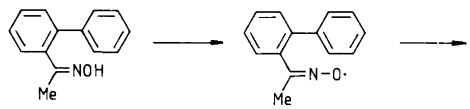


23

15c when both were detected that with $a_N \sim 20$ G intensified as the iminoxyl spectrum faded. Since no spectra with $a_N \sim 20$ G were detected when aryl methylene oximes with saturated *ortho*-alkyl substituents were oxidised we consider that such spectra arise from radicals formed by cycloaddition of the iminoxyl onto the adjacent double bond. However, 5- and 6-membered cyclic dialkyl nitroxides[9] such as *21* and *22* have a_N values (14 – 16 G) significantly smaller than 20 G. Indeed, only bicyclic nitroxides have a_N values in this range,[9] *e.g.* nitroxide *23*[10] has $a_N = 19.8$, $a_H = 5.6$(2H) and $a_H = 1.4$ G (4H). Hence, we suggest that the iminoxyls undergo 5-exo-trigonal cyclisation followed by intramolecular spin trapping of the ensuing alkyl radicals *17* by the nitrone group to give the bicyclic nitroxides *18* whose coupling constants are very similar to those of *23*. It is known[11] that exo-intramolecular addition is kinetically more favourable than endo addition for such cyclisations and that spin trapping of alkyl radicals by nitrones is fast.[12] However, we cannot satisfactorily account for our failure to detect bicyclic nitroxides from *15d* and *15e*. Preliminary product studies using lead tetra-acetate as oxidant and the oxime *15a* showed (TLC) that many products are formed but none is major.



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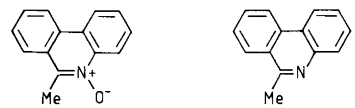

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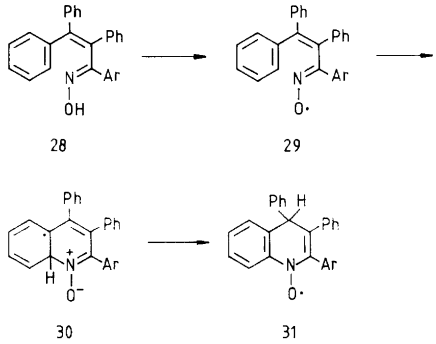
Methyl-o-phenylphenylmethyleneiminoxyl. Oxidation of the oxime *24* with lead tetra-acetate gave 6-methylphenanthridine *27* and its *N*-oxide *26* in low yield. The latter is the expected product of cyclisation of the iminoxyl *25* but *27* is not derived from *26* by reaction with lead tetra-acetate. Previously,[2] we produced *27* by cyclisation of the corresponding iminyl but we would not expect iminyls to mediate in the above oxidation. The ESR spectrum of the iminoxyl *25* [$a_N = 32$, $a_H = 1.5$ G (1H)] was detected on reaction of the oxime *24* with lead tetra-acetate at room temperature in the ESR spectrometer. However, this rapidly faded leaving a strong signal due to a di-*t*-alkyl nitroxide ($a_N = 11.75$ G) presumably due to a radical of type *20*.

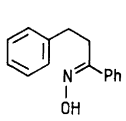
Aryl vinylmethylene iminoxyls. Since we had already shown[2] that iminyls derived from oximes such as *28a* and *28b* (Scheme 5) readily cyclised to



28 29 30 31

Scheme 5. 28a – 31a, Ar = Ph; *28b – 31b*, Ar = *o*-MeC_6H_4.

give quinolines we reasoned that iminoxyls might behave similarly. Hence, photolysis of the oxime *28b* in the ESR cavity gave an intense spectrum of a radical with $a_N = 7.15$, $a_H = 2.75$ (3H) and $a_H = 0.90$ G (2H). A very similar spectrum was obtained when the oxime in dichloromethane was treated with lead tetra-acetate at −60 °C but this faded as the temperature was increased. The related oxime *28a* behaved similarly on photolysis in di-*t*-butyl peroxide but with lead tetra-acetate mixed spectra were produced which changed with temperature and so were difficult to interpret. Iminoxyls were not detected under any of the sets of conditions used although with oximes *32* and *33* iminoxyl spectra (a_N 30 G) were detected without difficulty. Hence, it appears that the iminoxyls *29* are undergoing a reaction not available to iminoxyls derived from *32* and *33*. In view of our previous findings[2] that iminyls derived from oximes *28a* and *28b* cyclised to give quinolines but those from *32* and *33* did not, we believe that iminoxyls *28a* and *28b* are behaving similarly. The product radicals detected are most



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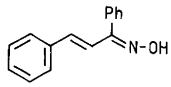

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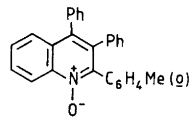

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likely to be the nitroxides *31a* and *31b* ($a_{4-H}=a_{6-H}=a_{8-H}$) indicated. However, when oxime *28b* was oxidised with nickel peroxide or with lead tetra-acetate on a preparative scale the quinoline *N*-oxide *34* was not detected in the complex product mixtures.

Iminoxyls are known[13] to dimerise and couple with other radicals but addition to multiple bonds and aryl rings has not previously been observed.

EXPERIMENTAL

IR spectra were measured as KBr discs and NMR spectra in deuteriochloroform unless stated otherwise. Merck GF_{254} silica was used for chromatographic separations.

o-*(Phenylazo)benzophenone* 9a. Aniline (0.31 g, 3.5 mmol) was added dropwise to a solution of *o*-nitrosobenzophenone[14] (0.74 g, 3.5 mmol) in acetic acid (10 ml) and the mixture was stirred for 18 h. Ether was added and the etherial solution was washed with 2 M sodium hydroxide, water, and dried. Removal of solvent left the crude product as a red oil which was purified by PLC using dichloromethane as eluant to give *o*-phenylazobenzophenone (57 %), m.p. 56–57 °C (from hexane) [I.R. 1670 cm^{-1}. Anal. $C_{19}H_{14}N_2O$]. *o*-Phenylazobenzophenone did not react with (a) hydroxylamine hydrochloride in aqueous alcohol under reflux for 4 h; (b) amino-oxyacetic acid hemihydrochloride in aqueous ethanol under reflux for 3 h nor in a sealed vessel at 90 °C for 9 h; (c) methoxyamine hydrochloride in aqueous alcohol under reflux for 3 h nor in a sealed vessel at 90 °C for 5 h.

o-*Methylazoxybenzophenone 1e* was prepared by the literature method.[4] It did not react with hydroxylamine hydrochloride in aqueous alcohol under reflux for 5 h.

o-*Aminoacetophenone oxime*[15] *5a* and *o*-aminobenzophenone oxime,[16] were obtained from the corresponding aminoketones by reaction with hydroxylamine in the usual way.

o-*Phenylazoketoximes* 9. A solution of the *o*-aminoketoxime (0.015 mol) and nitrosobenzene (0.015 mol) in acetic acid (50 ml) was stirred at room temperature for 17 h. Ether was added and the solution was washed with 2 M sodium hydroxide, water, and dried. Removal of solvent left the crude product o-*(phenylazo)acetophenone oxime 9a* (64 %) which gave orange needles, m.p. 134.5–136.5 °C (from ether–hexane) [^{1}H NMR: 2.33 (3H, s), *ca.* 7.55 (9H, s). IR 3220 cm^{-1}. Anal. $C_{14}H_{13}N_3O$]. o-*(Phenylazo)benzophenone oxime* was purified by chromatography (column) using ether as eluant. It formed fine orange needles, m.p. 147–150 °C (from ether–hexane) [IR 3220 cm^{-1}. Anal. $C_{19}H_{15}N_3O$].

*Methyl(*o*-phenylazo)phenylmethyleneamino-oxyacetic acids* 3. Bromoacetic acid (2.1 g, 0.015 mol) and sodium hydroxide (1.24 g, 0.03 mol) were added to a solution of the *o*-(phenylazo)ketoxime (0.01 mol) in water (50 ml) and ethanol (25 ml). The mixture was heated under reflux for 21 h. Ethanol was removed *in vacuo* and the aqueous residue was acidified with hydrochloric acid. The acid solution was extracted with ether and the ether was extracted with aqueous bicarbonate solution. After acidification this was extracted with chloroform. Evaporation of the dried extracts and crystallisation of the residue gave the product. o-*(Phenylazo)acetophenone oxime* gave *methyl(*o*-phenylazo)phenylmethyleneamino-oxyacetic acid* (51 %), as red needles from ether–hexane, m.p. 140–141.5 °C [^{1}H NMR: 2.4 (3H, s), 4.47 and 4.75 (2 H, both s), 7.38–7.95 (9H, m). IR 3440–2570, 1720, 1705 cm^{-1}. Anal. $C_{16}H_{15}N_3O_3$]. o-*(Phenylazo)benzophenone oxime* gave *phenyl(*o*-phenylazo)phenylmethyleneamino-oxyacetic acid* (42 %) as orange needles from chloroform–hexane, m.p. 163–165 °C [^{1}H NMR: 4.6 and 4.8 (2H, both s), 7.1–7.6 (14H, m). IR 3410, 1730, 1700 cm^{-1}. Anal. $C_{21}H_{17}N_3O_3$].

Oxidation of these two acids with persulfate in aqueous solution, as previously described,[1-3] gave complex product mixtures in which the indazoles *12a* and *12b* were the only identifiable products.

Reactions of o-*(phenylazo)acetophenone oxime* 9b. (a) *With acid.* To a solution of the oxime in chloroform a few drops of acetic acid or 2 M hydrochloric were added and the solution was left for 15 min when the colour faded. The solution was washed with aqueous bicarbonate solution, dried, and evaporated. Crystallisation of the residue from hexane gave 3-methyl-2-phenyl-2*H*-indazole *12b*.

(b) *With di-t-butyl peroxalate.* The peroxalate (23 mg) was added to a solution of the oxime (48 mg) in benzene. After 10 min the colourless solution was evaporated and the residue crystallised as before to give the indazole *12b* quantitatively. A similar

result was obtained when crystals of lead tetra-acetate (free from acetic acid) were added to a solution of the oxime *9b* in benzene.

Reactions of o-(phenylazo)benzophenone oxime 9a. This oxime reacted with lead tetra-acetate and di-*t*-butyl peroxalate as described for *9b* to give 2,3-diphenyl-2*H*-indazole.[5]

Oxidation of 1,2-dihydro-4-methyl-2-phenylquinazoline 3-oxide. 6b. Lead tetra-acetate (0.96 g) (free from acetic acid) was added to a solution of the quinazoline 3-oxide (0.48 g) in benzene (30 ml) and the mixture was stirred at room temperature for 3 h. The yellow brown solid remaining after removal of solvent was chromatographed (PLC) using dichloromethane ethyl acetate (9:1) as eluant to give pale yellow needles of 4-methyl-2-phenylquinazoline 3-oxide (60%) *8b*.

Similar treatment of 1,2-dihydro-2,4-diphenylquinazoline 3-oxide *6a* gave 2,4-diphenylquinazoline 3-oxide *8a*.

Bromo-alkenes. The following new bromoalkenes were prepared by a previously described method.[1] *o-(1'-Butenyl)bromobenzene* was an oil (b.p. 82–84 °C/0.4 kPa. [MS Mol. wt., obs. 210.0042, calc. for $C_{10}H_{11}{}^{79}Br$ 210.0045. ^1H NMR: 1.05 (3H, t), 2.2 (2H, q *J* 2Hz), 6.15 (1H, dt, *J* 5 Hz and 2 Hz), 6.67 (1H, d, *J* 5 Hz), 6.98 (1H, t, *J* 2 Hz), 7.18 (1H, t, *J* 2 Hz), 7.45 (2H, dd, *J* 2 Hz)]. *o-(3'-Methyl-1'-butenyl)bromobenzene* was a thick oil [MS Mol. wt. obs. 224.0200, calc. for $C_{11}H_{13}{}^{79}Br$ 224.0201. ^1H NMR: 1.08 (6H, d, *J* 2 Hz), 2.5 (1H, oct. *J* 2 Hz), 6.12 (1H, dd, *J* 5 Hz and 2 Hz), 6.68 (1H, d, *J* 5 Hz), 7.04 (1H, t, *J* 2 Hz), 7.23 (1H, t, *J* 2 Hz), 7.5 (2H, dd, *J* 2 Hz)].

Preparation of o-vinylketoximes 15. *General.* 1.6M Butyl-lithium (20 m, 0,032 mol) was added to a solution of the bromoalkene (0.013 mol) in ether under nitrogen, and the mixture was stirred at room temperature for 5 h. A solution of pivalonitrile (2.49 g, 0.03 mol) in ether was added dropwise and the mixture was then heated under reflux for 15 min. Ethanol (37.5 ml), acetic acid (1.5 ml) and hydroxylamine hydrochloride (1.75 g, 0.025 mol) were added and the mixture was heated under reflux for 5 h. After cooling, water (65 ml) was added and the ethanol was removed *in vacuo*. The aqueous solution was extracted with ether and the extracts were washed with water and dried. Removal of solvent left the crude oxime. t-*Butyl o-styrylphenyl ketoxime 15a* formed rhombs, m.p. 166–169 °C (from chloroform) [^1H NMR: 1.17 (9H, s), 6.94–7.55 (11H, m). IR: 3240 cm^{-1}. Anal. $C_{19}H_{21}NO$]. *t*-Butyl-*o*-(1'-butenyl)phenyl ketoxime *15d* gave plates, m.p. 122–124 °C (from hexane) [^1H NMR 1.02 (3H, t), 1.12 (9H, s), 2.17 (m, CH_2), 6.23 (2H, s), 6.95–7.6 (4H, m). IR: 3410 cm^{-1}. Anal. $C_{15}H_{21}NO$]. t-*Butyl o-(3'-methyl-1'-butenyl)-phenyl ketoxime 15e* formed plates, m.p. 106–109 °C (from chloroform–hexane) [^1H NMR: 1.08 (6H, d, *J* 7 Hz), 1.15 (9H, s), *ca.* 2.4 (1H, m), 6.19 and 6.27 (2H, both s), 7.05–7.75 (4H, m). IR: 3280 cm^{-1}. Anal. $C_{16}H_{23}NO$].

The oximes *15b* and *15c*, *28a*, *28b*, *24*, *32* and *33* were prepared by the literature methods.[1,2,18]

Oxidation of o-phenylacetophenone oxime. Lead tetra-acetate (0.98 g, 2.2 mmol) (free of acetic acid) was added to a solution of *o*-phenylacetophenone oxime (0.42 g, 2.0 mmol) in benzene and the mixture was stirred at room temperature for 2 h. The solid was collected and the filtrate was evaporated to dryness. The residue was chromatographed (PLC) using ethyl acetate–dichloromethane (1:19) as eluant giving 6-methylphenanthridine[18] (12%) and 6-methylphenanthridine *N*-oxide[19] (20%).

ESR measurements. ESR spectra were recorded on an E104A Varian spectrometer. The samples were prepared as follows.

(a) A solution of the oxime in dichloromethane was degassed and then cooled to −90 °C in the cavity of the spectrometer. One crystal of lead tetra-acetate, which had previously been freed from acetic acid by washing with dichloromethane, was then added. Spectra were recorded at regular intervals between −90 °C and room temperature.

(b) A degassed solution of the oxime in di-*t*-butyl peroxide was photolysed (1 kW Hg-vapour lamp) in the cavity of the spectrometer. Spectra were recorded between −60 °C and room temperature.

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Short Communications

Effects of Phospholipase C on Gastric Vesicle Membranes Containing H^+,K^+-ATPase *

HANS OLAISSON, SVEN MÅRDH and GÖSTA ARVIDSON

Institute of Medical and Physiological Chemistry, Biomedical Center, University of Uppsala, Box 575, S-75123 Uppsala, Sweden

Vesicular membranes of the gastric mucosa contain the H^+,K^+-ATPase which is part of the HCl secreting machinery of the stomach.[1] When ATP is added to vesicles containing KCl the ATP is hydrolyzed by the enzyme and hydrogen ions are transported into the vesicles in exchange for intravesicular K^+ which results in the accumulation of hydrochloric acid in the vesicles. The vesicular membranes are able to maintain a concentration gradient of hydrogen ions of more than 10^6 *in vivo*.[2] On the external (cytoplasmic) side of the membrane the H^+,K^+-ATPase is able to hydrolyze *p*-nitrophenylphosphate in the presence of extravesicular KCl.[3] The present experiments were conducted in order to gain some information on the organization and function of the lipids in the membranes of intact gastric vesicles. Such vesicles were treated with phospholipase C and the effects on the phospholipid content and composition, hydrogen ion transport and the *p*-nitrophenylphosphatase activity of the H^+,K^+-ATPase were recorded.

Experimental. Phospholipase C (4000 U/mg) from *Bacillus cereus* was obtained from Boehringer, Mannheim. Silica Gel H was a product of Merck, Darmstadt. ATP and *p*-nitrophenylphosphate (pNPP) were from Sigma. Ficoll 70 was from Pharmacia Fine Chemicals, Uppsala. Vesicular membranes were prepared from a homogenate of pig gastric mucosa by differential centrifugations in order to obtain a microsomal fraction.[2,3] This fraction was then layered on top of a step gradient, from the bottom consisting of 40 ml of 37% sucrose and 25 ml of 7.5% Ficoll in 0.25 M sucrose. After centrifugation at 75 000 × g for 2 h in a 6 × 100 ml MSE angle rotor, a vesicular fraction was collected from the top of the sucrose-Ficoll layer. Gastric vesicles at a protein concentration of 1 mg/ml were incubated with phospholipase C in 10 mM Hepes-Tris buffer, pH 7.5, containing 0.25 mM $CaCl_2$, 2 mM $MgCl_2$, 150 mM KCl at 30 °C. The phospholipase was added at a concentration of 3 units per mg of vesicular protein. Phospholipids were separated on thin layers of silica gel impregnated with magnesium acetate.[4] The plates were developed in two dimensions, first in chloroform–methanol–25% ammonia–water, 65:35:5:2 (v/v) and then in chloroform–methanol–acetic acid–water, 60:30:8:5 (v/v). The phospholipids were visualized and quantitated by phosphorus determination as previously described.[5]

Hydrogen ion transport in vesicles which had been equilibrated with 150 mM KCl was assayed at 21 °C in 2 mM $MgCl_2$, 150 mM choline-Cl, 10 μM acridine orange in 10 mM Hepes-Tris buffer, pH 7.0. The change of the absorbance at 493 nm upon the addition of 100 μM ATP as hydrogen ions were accumulated inside the vesicles was registered by means of a standard spectrophotometer equipped with a recorder.[6] The *p*-nitrophenylphosphatase activity of the H^+,K^+-ATPase was assayed by incubation of vesicles at 21 °C in 3 mM pNPP, 3 mM $MgCl_2$ and 10 mM KCl in 10 mM Hepes-Tris buffer, pH 7.5. The absorbance at 410 nm was measured.[7] Protein was measured according to the method of Lowry *et al.*[8]

Results and discussion. Approximately 50% of the membrane phospholipids were rapidly degraded by phospholipase C (Fig. 1). Concomitantly, marked changes occurred in the composition of the phospholipids (Fig. 2). These results indicate that sphingomyelin and phosphatidylinositol were left intact while the major part (80% of the original amounts) of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine seemed to be immediately accessible to hydrolysis by phospholipase C. This might reflect a preferential localization of these glycerophospholipids to the outer half, *i.e.* the cytoplasmic half of the lipid bilayer in the vesicles. Such a conclusion would be in general

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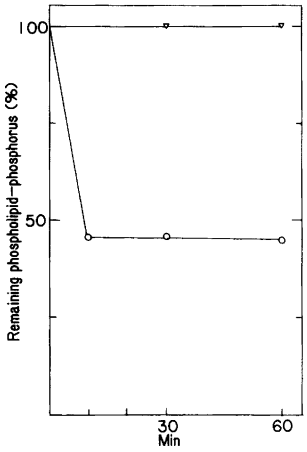

Fig. 1. Time dependent hydrolysis of phospholipids in gastric vesicles upon incubation with phospholipase C; ○, 3 units of phospholipase C per mg of vesicular protein were added at zero time; ▽, control, no phospholipase was added.

agreement with the findings of Saccomani *et al.*[9] who studied the effects of phospholipase A_2 on gastric vesicles. The *p*-nitrophenylphosphatase activity of the H^+,K^+-ATPase was inactivated in parallel with the hydrolysis of total phospholipids while the accumulation of hydrogen ions was more sensitive and decreased much more rapidly (Fig. 3).

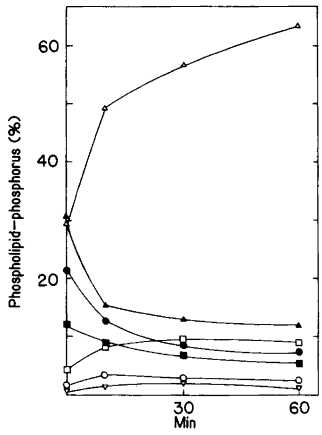

Fig. 2. Time dependent change of the phospholipid composition in gastric vesicles upon incubation with phospholipase C. ▲, phosphatidylethanolamine; ●, phosphatidylcholine; ■, phosphatidylserine; △, sphingomyelin; □, phosphatidylinositol; ○, lysophosphatidylethanolamine; ▽, lysophosphatidylcholine.

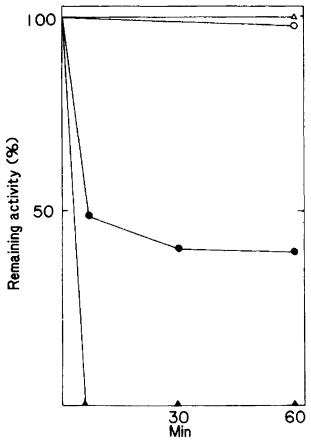

Fig. 3. Activities of p-nitrophenylphosphatase and hydrogen ion transport after incubation of gastric vesicles with phospholipase C. ○, ●, p-nitrophenylphosphatase; △, ▲, hydrogen ion transport. Open symbols represent control experiments in the absence of phospholipase C.

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Oxygen Supply to Immobilized Biocatalysts. A Model Study*

PATRICK ADLERCREUTZ and
BO MATTIASSON

Department of Pure and Applied Biochemistry, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund, Sweden

Enzymes and whole cells can carry out a wide variety of chemical reactions. Technical processes have been designed for large scale production of different substances with catalysts of biological origin (enzymes or whole cells). The production media used are aqueous solutions. When reactions are to be performed, which demand oxygen, problems arise due to the poor solubility of oxygen in water (the concentration of oxygen in water saturated with air is about 0.25 mM). The problems are especially serious when the catalyst is immobilized and used in packed bed reactors.

One obvious way of increasing the oxygen supply is to modify the medium so that it contains more oxygen. This report presents the use of the enzyme thermistor to monitor such modifications of the medium in a quick and reliable way. The oxidation of glucose by glucose oxidase (E.C. 1.1.3.4.) and catalase (E.C. 1.11.1.6.) was used as an indicator reaction. The enzyme preparation was quite stable and was used for several months without replacing the enzymes.

Substances added to increase the oxygen supply must, of course, be biocompatible. Two different potential oxygen carriers were tested. One is of natural origin, hemoglobin, and one is synthetic, the perfluorochemical FC-75.

Experimental. Materials. Hemoglobin was prepared from pig erythrocytes. FC-75 was obtained from 3M and Pluronic F-68 from Montoil AB. All other chemicals were of analytical grade.

Preparation of FC-75-emulsions. The buffer solution (water phase), the perfluorochemical (organic phase) and Pluronic F-68 (emulgator) were mixed in appropriate proportions. 10 ml of the mixture (in a 25 ml beaker) was cooled with crushed ice. The mixture was sonicated for about 30 sec (a probe was used on a A 350G from Ultrasonics Ltd., England).

Enzyme immobilization. Glucose oxidase (1,000 U) and catalase (300,000 U) were immobilized on 1 ml activated glass beads as described earlier.[1]

Experimental set-up. The enzyme thermistor has been used for analyses of many different substances.[2] With this technique, a suitable enzyme is immobilized on a solid support and the preparation is packed in a small column. When a sample containing a substrate of the enzyme is pumped through the column, heat is evolved. The increase in temperature is measured with a sensitive thermistor.

Several models of the enzyme thermistor have been used.[2] In the present investigation a standard enzyme thermistor was modified for oxygen analyses (Fig. 1). It was equipped with tubing of stainless steel to prevent leakage of oxygen. The buffer solution (saturated with nitrogen) was kept in a pressure vessel connected to a nitrogen bomb *via* a reduction valve. A pressure of 1.5 atm in the pressure vessel gave a suitable flow rate (1 ml/min) through the system. To assure that the flow rate remained constant, a peristaltic pump was connected to the effluent of the system. With these precautions the flow rate remained at 1.0 ml/min throughout the experiments. A sample loop of stainless steel (vol. 0.5 ml) was used.

The immobilized enzymes used were glucose oxidase and catalase. Glucose was added to the samples. The oxygen of the samples oxidized some of the glucose added in the reaction catalyzed by glucose oxidase. In this reaction hydrogen peroxide

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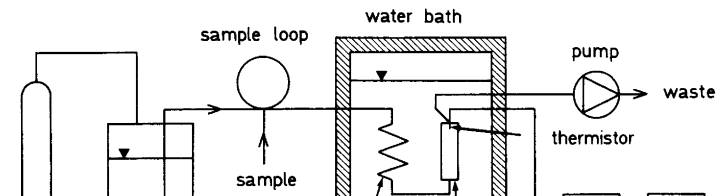

Fig. 1. Enzyme thermistor used for oxygen analyses.

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is formed. Catalase catalyzes the decomposition of hydrogen peroxide to oxygen and water. The reactions are:

$$\text{Glucose} + O_2 \rightarrow \text{Gluconolactone} + H_2O_2$$
$$H_2O_2 \rightarrow H_2O + 0.5\ O_2$$

The oxygen formed in the second reaction is used to oxidize more glucose. The net reaction is:

$$\text{Glucose} + 0.5\ O_2 \rightarrow \text{Gluconolactone} + H_2O$$

The thermistor was connected to a Wheatstone bridge unit, which in turn was connected to a recording devise. The response of the thermistor varied with the amount of glucose oxidized. When glucose was present in excess of oxygen, the thermistor response was proportional to the oxygen content of the sample. The enzyme thermistor was chosen for the oxygen analyses because it measures the oxygen *content* of a sample and not the oxygen *pressure* like, *e.g.*, a polarographic oxygen electrode.

Results and discussion. To calibrate the enzyme thermistor, buffer solutions with varying concentrations of dissolved oxygen were analyzed. The same solutions were also analyzed with a polarographic oxygen electrode. The response of the enzyme thermistor was plotted against the concentration of oxygen measured with the oxygen electrode (Fig. 2). When glucose was present in excess of oxygen (10 mM glucose) a straight line was obtained. When lower concentrations of glucose were used the curves levelled off and reached constant values. These plateau-values depended on the glucose concentration.

The effect of oxygen carriers in the medium was then tested. When hemoglobin supplied oxygen to the enzymes in the thermistor, the thermistor response varied with the concentration of hemoglobin in the solution (Fig. 3). Some levelling off was observed at higher concentrations of hemoglobin. This was probably due to incompleteness of the enzyme catalyzed reactions, caused by the short residence time in the column with immobilized enzymes.

The solubility of oxygen from air in the solution without hemoglobin was approximately 0.25 mM. Assuming that the addition of hemoglobin does not

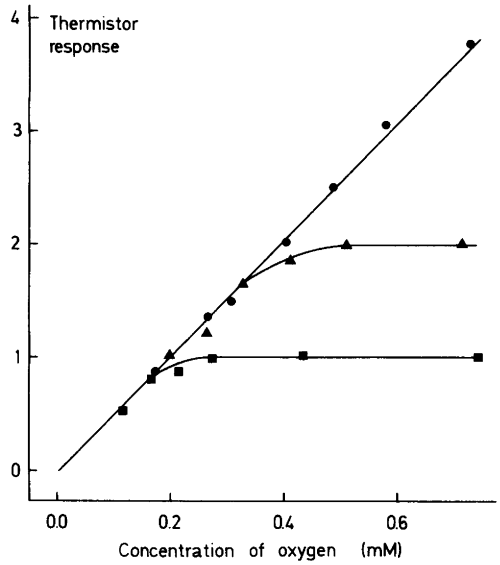

Fig. 2. Correlation between oxygen measurements with a polarographic oxygen electrode (Beckman) and with the enzyme thermistor. The samples were buffer solutions (0.1 M K-phosphate, pH 7.0) with varying concentrations of glucose and oxygen: 10 mM glucose (●), 1.0 mM glucose (▲) and 0.5 mM glucose (■).

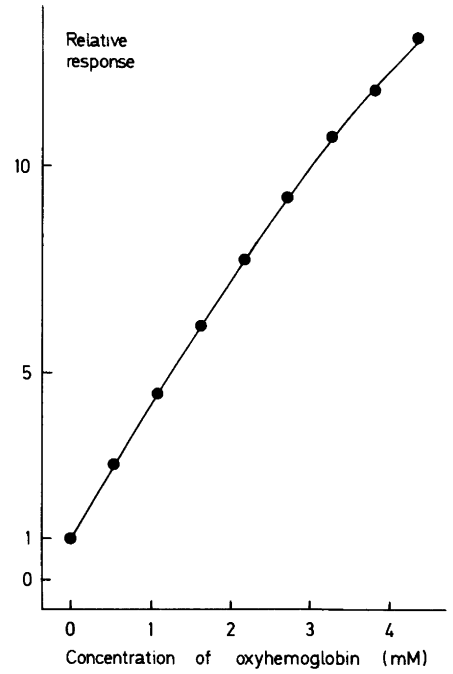

Fig. 3. Effect of hemoglobin concentration on the response of the glucose oxidase–catalase thermistor. Samples containing 0.1 M glucose and varying amounts of oxy-hemoglobin (expressed as concentration of heme-groups) were analyzed. The samples were saturated with air (750 mm Hg, 22 °C). The flow rate through the thermistor unit was 1.0 ml/min. The peak height of the sample without hemoglobin was set to 1.0.

alter the solubility of oxygen very much, the maximal concentration of oxygen in the 2.16 mM oxyhemoglobin solution (2.16 mM with respect to heme-groups) was 2.41 mM. The thermistor response for this solution was 7.8 times that of the solution without added hemoglobin. Assuming that the oxygen in this control was completely used up, the utilized concentration of oxygen in the 2.16 mM hemoglobin solution can be calculated to be 1.95 mM, corresponding to an efficiency of 81%. This efficiency can probably be raised by decreasing the flow rate and thereby increasing the residence time of the sample in the column. However, this would increase the time required for the analyses. The time routinely required per analysis was about 8 min with the conditions reported.

Hemoglobin proved to be a good oxygen carrier in the model system, however, in a potential technical process the oxygen carrier must be rather stable so that it can be used for a long time. Hemoglobin is a rather complex and sensitive substance, so perhaps it is not the ideal oxygen carrier for technical purposes. Other possible substances are perfluorochemicals.

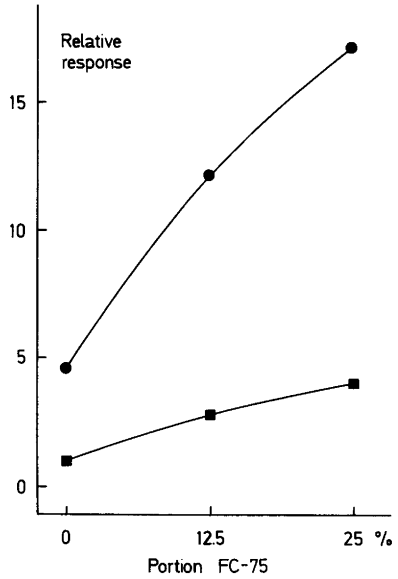

Fig. 4. Response of the glucose oxidase–catalase thermistor as a function of the percentage (v/v) of perfluorochemical in the emulsion analyzed. The perfluorochemical phase of the emulsion consisted of FC-75, while the water phase contained 40 mM glucose in 0.1 M K-phosphate buffer (pH 7.0). The emulgator used was Pluronic F-68 (0.21 g/ml FC-75). The samples were saturated with air (■) or oxygen (●). The atmospheric pressure was 760 mm Hg and the temperature of the samples was 22 °C. The response of the sample without perfluorochemical saturated with air was set to 1.0.

Perfluorochemicals are organic compounds in which all hydrogen atoms have been replaced by fluorine atoms. These compounds are very nonpolar, heat-stable and very chemically inert. Many gases, for example oxygen and carbon dioxide, have a high solubility in perfluorochemicals. These properties make perfluorochemicals suitable as blood substitutes. In this application, pure perfluorochemicals cannot be used, but emulsions of these have succesfully been used in "blood-transfusions" to several different animals [3] and recently also to humans.[4,5]

The ability of perfluorochemical emulsions to transport oxygen was tested with the enzyme thermistor. The oxygen-transporting capacity of an FC-75 emulsion increased with the amount of perfluorochemical added (Fig. 4). Since the solubility of oxygen in perfluorochemicals is proportional to the partial pressure of oxygen in the gas phase, the oxygen content of an emulsion can be increased by a factor of approximately 5 if it is saturated with pure oxygen instead of air. The response of the thermistor was 4–4.5 times greater for the emulsion saturated with oxygen compared to that saturated with air. The highest response (25% emulsion of FC-75 saturated with oxygen) was 17 times that of the buffer solution saturated with air.

The solubility of oxygen from air in FC-75 is reportedly 4.6 mM.[6] A calculation similar to that shown for hemoglobin reveals that the efficiency of oxygen transfer from the 25% emulsion saturated with air to the enzymes in the thermistor unit was 82%.

Conclusions. The enzyme thermistor proved to be a quick and reliable method for determining the oxygen content of a solution, even when an oxygen carrier was present.

Both hemoglobin and FC-75 could carry oxygen to the enzymes in the thermistor unit. The oxygen content of the media was increased by a factor of approximately 15.

Perfluorochemicals like FC-75 do not seem to be deliterious to enzymes. The immobilized enzyme preparation in the thermistor unit was stable for several months at room temperature.

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[2_6]Paracyclophanes – Large Ring Compounds with Extended π-Systems

ILONA RASTON and OLOF WENNERSTRÖM*

Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

Two [2_6]paracyclophanes with unsaturated bridges, containing perimeters with 36 π-electrons, have been prepared by fourfold Wittig reactions. Attempts to prepare cyclophanes with even larger π-systems are reported. The reversible two-electron reduction of the [2_6]paracyclophanes to the corresponding dianions is compared to that of [2_4]paracyclophanetetraene and correlated with the energies of the lowest unoccupied molecular orbitals (LUMOs) in the cyclophanes which have been calculated by simple Hückel theory.

Large carbocyclic compounds with conjugated π-systems around the ring have been extensively studied. The synthetic problems encountered in attempted preparation of such compounds are manifold, however, and have prevented more complete investigations of the many aspects of this type of compound which are of such interest for the development of theoretical models applicable to conjugated π-systems. Another major problem encountered in the annulenes is their flexibility, which increases with ring size, and the large number of rapidly interconverting isomers and conformers.[1] A relatively rigid and planar molecule is essential for π-electron delocalization which is a phenomenon of major interest in the annulenes.[1] These problems can be overcome by, for example, the preparation of rigid annulenes, as accomplished by the groups of Boekelheide[2] and Vogel.[3a]

The introduction of benzene rings into the annulenes changes their properties. This prevents bond-isomerization and endows a more rigid structure on the π-systems but, unfortunately, also diminishes the properties connected with π-electron delocalization over the molecule in the ground state.[3b] Large ring compounds consisting of aromatics linked by vinylene bridges behave essentially as normal open chain compounds.[4] We have recently found that certain cyclophanes can be reduced to dianions in a reversible two-electron process.[5] One very interesting condition for this is that the cyclophanes must contain a perimeter of $4n$ π-electrons in the neutral molecule. The rapid rotation of the benzene rings on the NMR time scale in the cyclophanes becomes hindered in the dianions, resulting in nonequivalence of inner and outer protons. A large difference in chemical shift for these two types of proton is observed, due to strong diamagnetic ring currents in the dianions.[6] It thus seems as if large paracyclophanes with unsaturated bridges, and their dianions, might be interesting model compounds for theoretical investigations of the conditions and effects of conjugation in large cyclic π-systems. In this paper, the synthesis and reduction of two cyclophanes of this type are described.

RESULTS AND DISCUSSION

Synthesis. Fourfold Wittig reactions between aromatic dialdehydes and bisphosphonium salts from bis(halomethyl)arenes have proved to be a facile method of preparing large cyclophanes with vinylene linkages between the aromatics.[4] Although the yields may be low, which is certainly expected for reactions in which four *cis* double bonds are formed in a one-pot reaction sequence, the simplicity of the approach outweighs the low yields and makes large cyclophanes readily available.

*To whom correspondence should be addressed.

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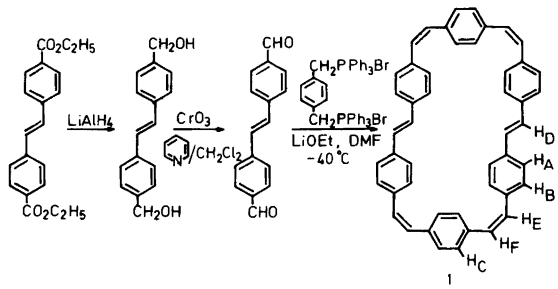
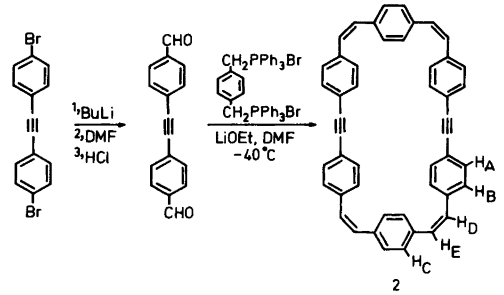


Scheme 1.

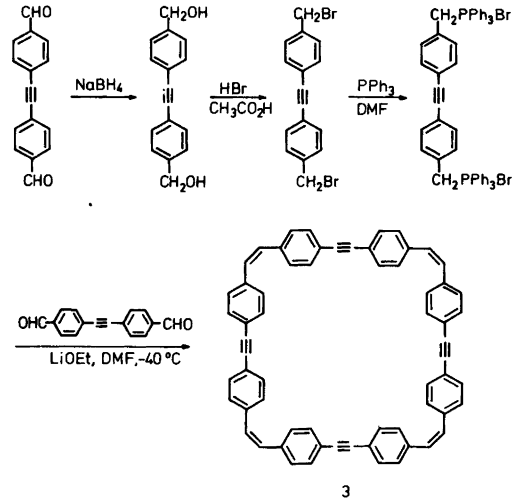
trans-Stilbene-4-4′-dicarbaldehyde reacts with the bisphosphonium salt from 1,4-bis(bromomethyl)benzene and base at −30 °C in DMF to give *cis,cis,trans,cis,cis,trans*-[2$_6$]paracyclophanehexaene, *1*, in 5 % yield (Scheme 1). The structure of the cyclophane follows from its mass and ^{1}H NMR spectrum. The latter shows the simple pattern expected for the cyclophane assuming rapid rotation of the benzene rings and the *trans*-double bond bridge, *i.e.* a singlet and an AA′XX′-pattern for the aromatic protons and a singlet and an AB-pattern for the olefinic *trans* and *cis* protons, respectively. The temperature-dependence of the NMR spectrum of *1* was checked. No significant broadening of the signals was observed down to 200 K in CD_2Cl_2, implying rapid rotation of the benzene rings and the *trans*-vinylene groups on the NMR time scale.

Tolan-4,4′-dicarbaldehyde reacts under similar conditions with the bisphosphonium salt from 1,4-bis(bromomethyl)benzene to give all-*cis*-[2$_6$]paracyclophanetetraenediyne, *2*, in 12 % yield (Scheme 2). The mass spectrum shows the molecular ion and the doubly charged molecular ion. The high symmetry of the cyclophane is revealed by its ^{1}H NMR spectrum which consists of a singlet and an AA′XX′-pattern for the aromatic protons and an AB-pattern for the olefinic protons.

In an attempt to prepare an even larger cyclophane with eight benzene rings, four *cis* double bonds, and four triple bonds, we reacted tolan-4,4′-dicarbaldehyde with the bistriphenylphosphonium salt from 4,4′-bis(bromomethyl)tolan (Scheme 3) under the standard conditions.[4] The dialdehyde and the bisphosphonium salt were prepared by standard procedures from bibenzyl, which was treated with bromine to give α,α′,4,4′-tetrabromobibenzyl. The product was refluxed with base to give 4,4′-dibromotolane, which, upon treatment with butyllithium and dimethylformamide followed by hydrolysis, gave tolan-4,4′-dicarbaldehyde. A portion of the dialdehyde was reduced with sodium-borohydride to the dialcohol which, upon reaction with hydrobromic acid, gave 4,4′-bis(bromomethyl)-



Scheme 2.



Scheme 3.

tolan. The bisphosphonium salt was generated in DMF by addition of triphenylphosphine (see also Scheme 3). The Wittig reaction between the dialdehyde and the bisphosphonium salt in the presence of base gave only trace amounts of products isolated after careful chromatography of the ethereal extract of the reaction product. The mass spectra of two of the products showed the molecular ion at *m/e* 808 consistent with a [2_8]paracyclophanetetraenetetrayne. The ^{1}H NMR spectra revealed the products to be the all-*cis* isomer and the *cis,trans,cis,trans*-isomer. The latter was not present in the reaction mixture but formed slowly from the all-*cis* isomer, probably by a light-induced *cis-trans* isomerization. The all-*cis* isomer shows an unusually simple ^{1}H NMR spectrum consisting of an AA'XX'-pattern for the aromatic protons and a singlet for the olefinic protons. The *cis,trans,cis,trans*-isomer shows two AA'XX'-patterns for the aromatic protons and two singlets for the olefinic protons, consistent with the assumption of rapid rotation of the benzene rings as well as the *trans*-double bonds. In one of the Wittig reactions, a product with a mass spectrum similar to those above but with a molecular ion of *m/e* 606 was observed and isolated. The ^{1}H NMR spectrum was very simple, consisting of just one AA'XX'-pattern in the aromatic region and a singlet in the olefinic region. The compound is most likely the [2_6]paracyclophanetrienetriyne. Its formation can be rationalized either by assuming impurities of a monophosphonium salt monoaldehyde in the Wittig reaction, originating from incomplete reduction of the dialdehyde to the dialcohol, or by assuming air oxidation of a small proportion of the intermediate ylid groups to aldehyde groups, which ultimately could lead to cyclophanes with three double bonds instead of the even number expected.

Electrochemistry and Hückel calculations. As mentioned above, several cyclophanes formally with a perimeter of 4n π-electrons undergo a completely reversible two-electron reduction to their dianions at a mercury electrode in DMF.[5] The reduction potential has been found to be proportional to the energy of the LUMO orbital obtained from simple Hückel calculations, even for cyclophanes that are far from planar as neutral species.[7] Within the Hückel approximation, cyclophanes *1* and *2* are similar. The energy of their LUMO orbitals is the same as that of [2_4]paracyclophanetetraene, *6*, (0.254 β). In fact, the HOMO and LUMO orbitals in these three cyclophanes can be regarded as arising from the degenerate nonbonding orbitals in the (4n) annulenes which have been split by the bridging benzene rings, to give a bonding and an antibonding orbital in the cyclophanes. The magnitude of the splitting does not depend on the size of the cyclophane, but only on the relative proportion of double bonds and benzene rings, in the series of unsaturated [2_n]paracyclophanes (n is even).

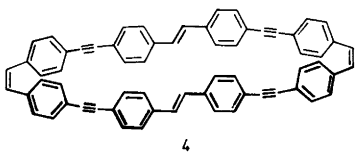
Cyclic voltammetry of *1* and *2* at a mercury drop in dry DMF containing tetraethylammonium perchlorate as supporting electrolyte and with [2_4]paracyclophanetetraene as a standard gave similar results. Both cyclophanes are reduced at -1.70 V *vs.* SCE, which is exactly the same reduction potential as that of [2_4]paracyclophanetetraene.[5] However, the latter undergoes a completely reversible two-electron reduction whereas the cyclophanes *1* and *2* undergo the same type of two-electron process, but less reversibly. Apparently, the dianions from *1* and *2* are more prone to abstract protons from the residual water in the solvent or from the solvent itself.

The dianion of [2_4]paracyclophanetetraene is planar enough to sustain a diamagnetic ring current when subjected to a strong magnetic field.[6] By analogy, the cyclophanes *1* and *2* should behave similarly. According to Haddon,[8] ring current should increase with the size of a cyclic conjugated π-system, whereas the resonance energy should decrease with ring size. Thus the dianions of cyclophanes *1* and *2*, if conformationally rigid, could well display large diamagnetic ring current effects. It is interesting to note the differences in the UV spectra of the cyclophanes *1*, *2* and *4* (Table 1). The photoexcitation process is too fast to allow for conformational changes whereas the electrochemical reduction allows for relaxation to the equilibrium geometries of the cyclophanes and their dianions. The simple Hückel calculations used here should, of course, better represent the latter situation. It seems reasonable to conclude that considerable geometrical changes occur in the cyclophanes on reduction to the dianions.

Most recent theories of electronic effects in large conjugated systems, and particularly in the annulenes, result in similar predictions of the resonance stabilization in charged and neutral systems with the same number of π-electrons.[8,9a] The total effect is small in annulenes with more than 18–22 π-electrons, and should be much smaller in cyclophanes with conjugated perimeters. In a recent paper, however, Bates *et al.*[9b] give consider-

Table 1. Comparison of calculated LUMO energies, reduction potentials (*vs. SCE*) and absorption maxima for cyclophanes *6*, *2* and *1*.

Cyclophane	Calculated energy of LUMO (β)	ΔE_{red} (V) to the dianions	λ_{max} (nm)	ε
6	−0.254	−1.70	304	57 500
2	−0.254	−1.70	324	86 000
1	−0.254	−1.70	344	116 000



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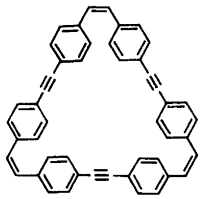

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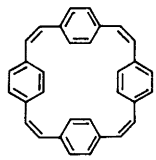

6

ably larger values for the resonance energy in annulene-dianions. The effect of Hückel's rule should be negligible for π-systems of the size and type discussed here. Nevertheless, an alternating behaviour has been observed for the reduction potential of a series of closely related cyclophanes with increasing number of π-electrons around the perimeter.[4] Further, the reversible transfer of two electrons to the cyclophanes with $4n$-π-electron perimeters is difficult to rationalize without assuming some extra stabilization of the dianions, relative to the radical anions, to compensate for the inter-electronic repulsion. For many aromatic hydrocarbons this repulsion results in a separation of the potentials for the reduction to the radical anions and the dianions of *ca.* 500 mV.[9c]

The Hückel calculations have shown the importance of the HOMO and LUMO orbitals for comparison with experimental reuction potentials. It might be adequate to restrict the discussion of these large conjugated systems to include only the frontier orbitals rather than all the orbitals and the total π-electron energy, as is usually done when discussing resonance effects.

It seems as if further studies of these large cyclophanes and their dianions could give valuable experimental results to test present theories of π-electron delocalization, ring current effects, and resonance stabilization in large conjugated π-systems.

EXPERIMENTAL

cis,cis,trans,cis,cis,trans-[2_6]*Paracyclophanehexaene*. *trans*-Stilbene-4,4′-dicarboxylic acid diethylester[10] was reduced with lithiumaluminum hydride in refluxing diethylether to give 4,4′-bis(hydroxymethyl)-*trans*-stilbene (75 %, m.p. 268–270 °C).[11] MS (50 eV): *m/e* 240 (M^+, 100 %), 238 (26), 179 (42, 178 (42), 113 (14), 103 (14), and 91 (12). Abs. mass 240.1154; calc. for $C_{16}H_{16}O_2$ 240.1150. NMR (270 MHz, pyridine-d_5): δ 7.62 (m) 8H, 7.31 (s) 2H, 4.95 (s) 4H.

The diol was oxidized by a solution of chromium trioxide in pyridine–dichloromethane to *trans*-stilbene-4,4′-dicarbaldehyde (82 %, m.p. 165 °C[12]). MS (50 eV): *m/e* 236 (M^+, 100 %), 235 (37), 179 (50), 178 (60), 176 (12), 152 (10), 89 (11). Abs. mass 236.078; calc. for $C_{16}H_{12}O_2$ 236.084. NMR (270 MHz $CDCl_3$): δ 9.99 (s) 2 H, 7.86 (m) 4 H, 7.68 (m) 4 H, 7.27 (s) 2 H. The dialdehyde (13 mmol) was reacted with the bistriphenylphosphonium salt from 1,4-bis(bromomethyl)benzene (13 mmol) and lithium ethoxide in dry DMF at −30 °C under the standard conditions.[4] The product mixture in DMF was diluted with water and extracted with diethylether and dichloromethane. The combined organic phases were washed with water, dried ($MgSO_4$) and the solvents evaporated. The residue was stirred with ethanol to remove triphenylphosphine oxide and recrystallized from chloroform–methanol to give *cis,cis,trans,cis,cis,trans*-[2_6]-paracyclophanehexaene (122 mg, 5 %, m.p. 278–280 °C). IR (KBr): 3000 (m), 1598 (m), 1505 (s), 1420 (s), 960 (s), 940 (s), 890 (s) and 830 (vs) cm^{-1}. UV (ethanol): λ_{max} 344 nm ε 116 000 and 244 nm 52 000. NMR (270 MHz, $CDCl_3$): d 7.34 and 7.27 (16 H, AA′BB′-pattern, *J*8 Hz, H_A and H_B), 7.20 (8 H, s, H_C), 7.03 (4 H, s, H_D), 6.58 and 6.54 (8 H, AB-pattern, *J* 12 Hz, H_E and H_F). MS (50eV): *m/e* 612 (M^+, 100 %), 306

(M^{2+}, 14). Abs. mass 612.289; calc. for $C_{48}H_{36}$ 612.282.

[2_6]*Paracyclophanetetraenediyne.* 4,4′-Dibromotolan[13] was treated with butyl-lithium in diethylether, followed by dry DMF, and, finally, dilute hydrochloric acid to give tolan-4,4′-dicarbaldehyde (58 %, m.p. 213–214 °C).[14] MS (50 eV): *m/e* 234 (M^+, 100 %), 233 (63), 176 (38), 151 (14). Abs. mass 234.063; calc. for $C_{16}H_{10}O_2$ 234.068. NMR (270 MHz, $CDCl_3$): δ 10.03 (s) 2 H, 7.87 (m) 4 H, 7.67 (m) 4 H.

The aldehyde (10 mmol) was reacted with the bistriphenylphosphonium salt from 1,4-bis(bromomethyl)benzene (10 mmol) and lithium ethoxide in dry DMF at −40 °C under the standard conditions.[4] The reaction mixture was diluted with water and extracted with diethyl ether.

The organic phase was dried and the solvent distilled off. Careful chromatography of the residue on a silica gel column with tetrachloromethane as eluant gave [2_6]paracyclophanetetraenediyne (363 mg, 12 %, m.p. 265–270 °C) IR (KBr): 3000 (w), 1595 (w), 1505 (m), 1410 (m), 890 (s) and 830 (vs). UV (ethanol): λ_{max} 324 nm ε 86 000 and 237 nm ε 48 600. NMR (270 MHz, $CDCl_3$): d 7.39 and 7.27 (16 H, AA′BB′-pattern, *J* 8 Hz, H_A and H_B), 7.19 (8 H, s, H_C), 6.58 and 6.53 (8 H, AB-pattern, *J* 12 Hz, H_D and H_E). MS (34 eV): *m/e* 608 (M^+, 100 %), 304 (M^{2+}, 7). Abs. mass 608.253; calc. for $C_{48}H_{32}$ 608.250.

[2_8]*Paracyclophanetetraenetetrayne* 3. Tolan-4,4′-dicarbaldehyde was reduced with sodium borohydride in ethanol to 4,4′-bis(hydroxymethyl)tolan (93 %, m.p. 214–215 °C). MS (50 eV): *m/e* 238 (M^+, 100 %), 237 (12), 221 (15), 191 (14), 179 (20), 178 (35). Abs. mass 238.095; calc. for $C_{16}H_{14}O_2$ 238.099. NMR (270 MHz, DMSO-d_6): δ 7.51 (m) 4 H, 7.37 (m) 4 H, 5.24 (br tr) 2 H, 4.53 (br d) 4 H.

The diol was treated with hydrobromic acid (30 %) in acetic acid to give 4,4′-bis(bromomethyl)tolan (83 %, m.p. 187–190 °C). MS (50eV): *m/e* 366 (5 %), 364 (10), 362 (6) M^+, 285 (61), 283 (62) M^+ − Br, 205 (30), 204 (M^+ − 2 Br, 100), 202 (26), 102 (37). NMR (270 MHz, $CDCl_3$): δ 7.64 (m) 4 H, 7.38 (m) 4 H, 4.50 (s) 4 H.

The bisbromomethyl compound was converted to its bistriphenylphosphonium salt by treatment with triphenylphosphine in dry DMF up to 154 °C (87 %). Tolan-4,4′-dicarbaldehyde (5 mmol) and the bisphosphonium salt (5 mmol) were suspended in dry DMF and lithium ethoxide was added dropwise at −40 °C under the standard conditions.[4] The usual work-up and separation on a silica gel column gave small amounts of an unstable product identified as the all-*cis*-isomer of [2_8]paracyclophanetetraenetetrayne. NMR (270 MHz, $CDCl_3$): d 7.39 and 7.24 (32 H, AA′BB′-pattern, *J* 8 Hz, aromatic protons), 6.60 (8 H, s, olefinic protons). MS (50 eV): *m/e* 808 (M^+, 100 %), 404 (M^{2+}, 30). Abs. mass 808.303; calc. for $C_{64}H_{40}$ 808.313.

On standing, the cyclophane gave rise to a new compound, tentatively assigned as the *cis,trans*,-*cis, trans*-isomer *4* on the basis of its NMR spectrum (270 MHz, $CDCl_3$): δ 7.44 and 7.42 (16 H, AA′BB′-pattern, aromatic protons), 7.29 and 6.94 (16 H, AA′BB′-pattern, *J* 8 Hz, aromatic protons), 7.01 (4 H, s, olefinic protons), 6.81 (4 H, s, olefinic protons).

From a preliminary experiment, another cyclophane was isolated and identified as [2_6]paracyclophanetrienetriyne, *5*, from its simple NMR and MS. ^{1}H NMR (270 MHz, $CDCl_3$): d 7.32 and 7.04 (24 H, AA′BB′-pattern, *J* 8 Hz, aromatic protons) and 6.70 (6 H, s, olefinic protons). MS (50 eV): *m/e* 606 (M^+, 100 %), 303 (M^{2+}, 13). Abs. mass 606.230; calc. for $C_{48}H_{30}$ 606.235.

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Synthesis of Some Bicyclophanes

HANS-ERIK HÖGBERG[a] and OLOF WENNERSTRÖM[b]

[a] Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm and [b] Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg, Sweden

Bicyclic aromatic compounds, bicyclophanehexaenes of the general structure A, have been obtained from sixfold Witting reactions between aromatic bisphosphonium salts and 1,3,5-benzenetricarbaldehyde. Hydrogenation gave bicyclophanes of structure B. The synthesis of bicyclophanes from ylids prepared from the bisphosphonium salts from 1,3- and 1,4-bis(bromomethyl)benzene, 1,3-bis(bromomethyl)-5-bromobenzene, and 2,5-bis(chloromethyl)thiophene is described and the structures of the bicyclophanes discussed.

Cage compounds having a framework large enough to define a sizeable cavity in the centre of the molecule have been found to possess unusual properties, for example as host molecules in host–guest complexes.[1,2] Whether such a cavity would be stable or collapse in the absence of guest molecules should depend on the rigidity of the bonding framework. Studies of rigid cage compounds might provide information on the factors governing formation and stability of cavities in molecules.

Molecules containing aromatic rings linked by double bonds are conformationally restricted. We have recently prepared a number of [2$_4$]cyclophanetetraenes.[3,4]

The macrocyclic ring in [2$_4$]paracyclophanetetraene, *7A*, is relatively planar and the rotation of the benzene rings around the single bonds in *7A* and some closely related compounds is rapid.[5] On hydrogenation of the olefinic bonds, the cyclophanes show increased flexibility and conformational isomerism occurs, arising from *gauche* and sometimes *anti* orientations of the substitutents at the ethane bridges.[6] In line with these observations, cage cyclophanes or bicyclophanes with bridging double bonds might be compounds possessing stable cavities.

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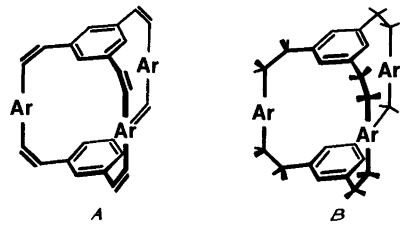
The [2$_4$]cyclophanetetraenes mentioned above can be synthesized by fourfold Witting reactions between aromatic dialdehydes and biphosphonium salts.[3,4] The conditions have been optimized to yield the highest possible *cis/trans* ratio.[3,4]

RESULTS AND DISCUSSION

We found that a mixture of 1,3,5-benzenetricarbaldehyde (2 equiv. and a biphosphonium salt from a bis(bromomethyl)arene (3 equiv.) in dry dimethylformamide at −40 °C on treatment with lithium ethoxide gave a crude product which, on chromatography followed by sublimation, afforded a bicyclophane* with unsaturated bridges (*A*, 1.4–

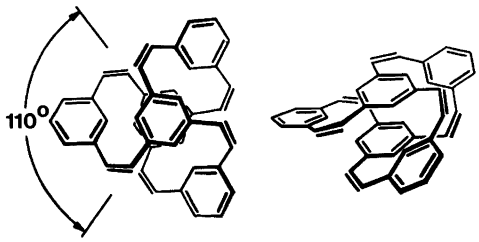


A *B*

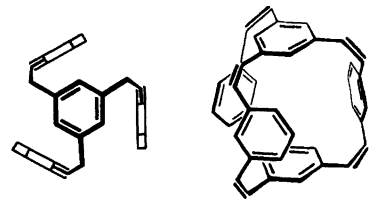
* We suggest the name bicyclophane for compounds in which two aromatic rings are joined by three bridges, all containing aromatic rings. The name should contain the types and number of bridges between the aromatic rings, followed by the types and number of aromatics, starting with the triply-bridged rings. The name should end with the suffix -bicyclophane. This suggestion does not lead to an unambiguous naming of bicyclophanes, for which the IUPAC nomenclature must be used, but is intended to simplify the naming of complex multicyclic compounds of this type. According to this suggestion, the compounds *1A*, *2A* and *3A* should be named [2$_6$](1,3,5)$_2$(1,3$_3$-bicyclophanehexaene, [2$_6$](1,3,5)$_2$(1,4)$_3$bicyclophanehexaene and [2$_6$](1,3,5)$_2$(2,5-thiopheno)$_3$bicyclophanehexaene, respectively. The IUPAC name of *2A* is heptacyclo[12.12.8^{1,14}.2^{6,9}.2^{19,21}.2^{29,32}.1^{3,25}.1^{12,16}]dotetraconta-1,3(41),4,6,8,10,12(42),13,15,17,19,21,23,25,27,29,31,33,35,37,39-heneicosaene.

2 % yield).[7] Although the yields were low, the simplicity of this one-step synthesis makes it an easy route to this type of compound. Catalytic hydrogenation of the "unsaturated" bicyclophanes furnished the "saturated" bicyclophanes of type *B* in quantitative yield. The bisphosphonium salts from 1,3- and 1,4-bis(bromomethyl)benzene, 1,3-bis(bromomethyl)-5-bromobenzene, and 2,5-bis(chloromethyl)thiophene gave, together with 1,3,5-benzenetricarbaldehyde, the bicyclophanes *1A*, *2A*, *4A* and *3A*, respectively. Hydrogenation of the bicyclophanes *1A*, *2A* and *3A* afforded the bicyclophanes *1B*, *2B* and *3B*, respectively.

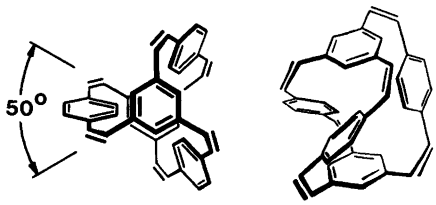
The mass spectra of the bicyclophanes of type *A* confirmed their proposed gross structures, showing base peaks at *m/e* values of the expected molecular



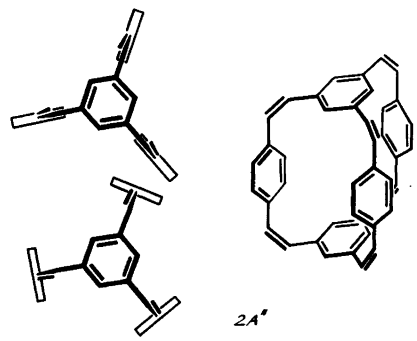
1A'



1A''



2A'



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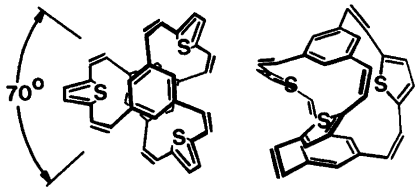
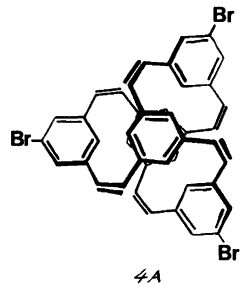
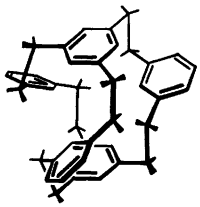


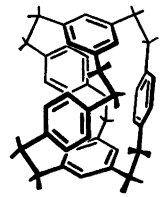
3A



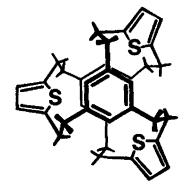
4A



1B



2B



3B

weights. Doubly-charged molecular ions were the second most abundant in these spectra, while almost no fragmentation was observed. The bicyclophanes of type *B* gave rise to molecular ion base peaks at *m/e* twelve units higher than those of type *A* and fragmentation from cleavage of the sp^3-sp^3 C–C bonds.

Inspection of molecular models (CPK and Dreiding) revealed that the "unsaturated" bicyclophanes (type *A*) are conformationally mobile to a certain extent and able to adopt conformations ranging between two extremes. In one of these, the distance between the two trisubstituted rings is as short as possible and a maximum is reached for the twist angle (*i.e.* the angle between the start and end of a bridge when viewing the molecule from a point on the threefold axis of symmetry). A large twist angle leads to a minimal cavity in the centre of the molecule (see *1A'*, *2A'* and *3A*). In the other extreme conformation, the twist angle is zero and the distance between the trisubstituted rings as well as the size of the cavity in the centre are as large as possible (see *1A"* and *2A"*).

The *m*-phenylene bridged bicyclophane *1A* thus has two extreme conformations *1A'* and *1A"* (two projections are shown for each of these with twist angles of 110 and 0°, respectively).

The conformation *1A"* with D_3 symmetry is chiral and the models show that the enantiomers can interconvert *via* *1A"* which has C_{3h} symmetry.

In the ^{1}H NMR spectrum of the bicyclophane *1A* one peak appeared at unusually low field (δ 8.86). This peak arises from the three *m*-phenylene protons *ortho* to both the ethylene bridges (H_A in Table 1). A similar low field shift has been observed for the corresponding H_A-protons in [2$_2$]metacyclophanediene *5A* (δ 7.90),[8] which has the *anti* conformation depicted in *5A*.[9] The average value of the shifts

Table 1. Chemical shifts for the protons in bicyclophanes *1–4* and some closely related cyclophanes *5–9*.

Compound	H_A	H_B	H_C	H_D	H_E	H_F	H_G	and	$H_{G'}$	H_H	and	$H_{H'}$
1A	8.86	7.08	7.33	7.33			6.31		6.18			
1B	6.05	7.04	7.24	6.36						2.71		2.65
2A				6.98	7.08		6.65		6.42			
2B				6.67	6.51						2.81	
3A				7.10		6.81	6.55		6.28			
3B				6.66		6.46				2.83		2.65
4A	8.70	7.32		7.26				6.22				
5A	7.90	7.01	6.60					6.22				
5B	4.20	7.30	7.00									
6A	7.89	7.13	7.07					6.32				
7A					7.32			6.45				
7B					6.72						2.85	
8A					7.24	6.80	6.55		6.38			
8B					6.75	5.69				2.97		2.86
9B	5.88	6.93	7.13		6.81							2.73

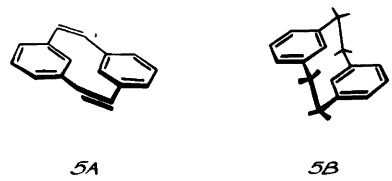
of the peaks due to the H_A and H_D protons in *1A* is $(\delta_{H_A}+2\delta_{H_D})/3 = 7.84$. Of the possible conformations of *1A* only the compressed one *1A'* has the H_A and H_D protons located in an environment similar to that of the H_A protons in the cyclophane *5A*. Because the *m*-phenylene rings in *1A'* are tilted, the H_D protons are more shielded than the H_A protons in *5A*. However, since the H_A protons in *1A'* are deshielded by two aromatic rings their resonances appear at a lower field than those of the H_A protons in the cyclophanediene *5A*. It seems clear that the chiral conformation *1A'* best represents the structure of the bicyclophane *1A*. Whether the activation energy of the interconvertion between the enantiomers is large enough to determine by NMR methods remains to be tested on a properly substituted derivative.

It is interesting to note that the resonances of the H_A protons in [2$_4$]metacyclophanetetraene (*6A*) appear at δ 7.89.[4] The favoured conformation of compound *6A* must therefore be closely related to that of the bicyclophane conformation *1A'*.

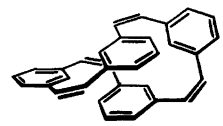
The tribromo-derivative of the *m*-phenylene bridged bicyclophane, compound *4A*, shows essentially the same NMR spectrum as *1A* and should behave similarly and prefer the conformation shown in *4A*.

Models of the *p*-phenylene bridged bicyclophane *2A* show that the compressed chiral D_3 conformation *2A'* is virtually strain-free if the bond angles in the bridging double bonds are close to 120°. However, the corresponding bond angles in crystalline [2$_4$]paracyclophanetetraene *7A* are 132°.[5] If the bond angles are of similar magnitude in the bicyclophane *2A* the conformation *2A''* with D_{3h} symmetry is favoured. The rotation of the *p*-phenylene rings should be more restricted in the compressed conformation *2A'* than in *2A''*. It may be noted that if the *p*-phenylene rings are oriented tagentially in the D_{3h} conformation *2A''*, the cavity in the centre of the molecule is large enough to accommodate small molecules such as methane or ethane.

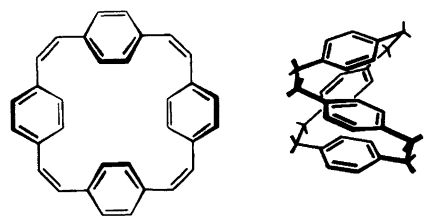
The NMR spectrum of the bicyclophane *2A* (see Table 1) does not show any anomalous shifts. The protons H_E of the *p*-phenylene rings resonate at a δ-value very similar to that of the corresponding protons in [2$_4$]paracyclophanetetraene *7A*. The chemical shifts of the aromatic protons in various conformations of the bicyclophane *2A* were calculated using the Bovey-Johnson nomogram.[10] Satisfactory agreement between calculated and



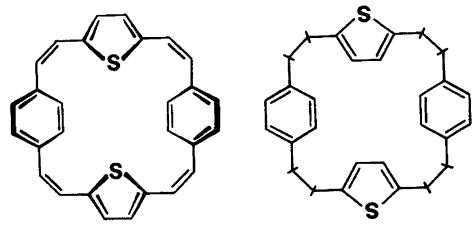
5A 5B



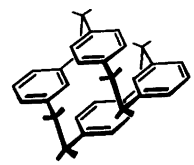
6A



7A 7B



8A 8B



9B

observed shift values was obtained for both the D_{3h} conformation *2A''* and for the twisted, compressed D_3 conformation with twist angles up to 60 – 70°. Larger angles lead to large upfield shifts of the calculated value for H_D. Thus it is not possible, from NMR data alone, to establish which of the possible conformations the bicyclophane *2A* prefers. In order to solve this problem, an X-ray structure determination is necessary.

The NMR spectrum of the thiophenylene bridged bicyclophane *3A* showed singlets for the arene protons at δ 6.80 and 7.10. The former is assigned to the thiophene protons H_F by analogy with the shifts in the cyclophane *8A*[11] and the latter to the benzene protons H_D. The shift of the H_D-protons in *3A* lies between the shifts of the same type of proton in *1A* and *2A* (see Table 1). Space filling models (CPK) show that in the compressed conformation with D_3 symmetry, which is depicted in *3A*, the sulfur atoms point towards the centre of the molecule. In the other extreme conformation, with maximum separation of the trisubstituted rings (twist angle 0°), the thiophene rings can rotate.

Since the bicyclophane *1A* most probably adopts the twisted compressed conformation *1A'*, it seems likely that the bicyclophanes *2A* and *3A* behave similarly and adopt their respective compressed conformations depicted in *2A'* and *3A*. The twist angles in the virtually strain-free conformations are approximately 110° (*1A'*), 70° (*3A*) and 50° (*2A'*) as judged from space filling (CPK) models. It is interesting to note that the observed chemical shifts for the H_D protons of these bicyclophanes are roughly linearly dependant on the estimated twist angle.

All the "unsaturated" bicyclophanes (type *A*) are extremely high-melting colourless solids and are only slightly soluble in organic solvents. These facts indicate that the bicyclophanes are rather rigid with ordered structures of high symmetry. The "saturated" bicyclophanes (type *B*) have much lower melting points, are fairly soluble, and should therefore be more flexible.

The NMR spectrum of the *m*-phenylene bridged bicyclophane *1B* showed that the resonances of the protons *ortho* to the bridges, H_A and H_D, are shifted upfield on hydrogenation of *1A*, indicating that these protons become shielded by the neighbouring aromatic rings (see Table 1). The effect is larger for H_A which is sandwiched between the trisubstituted benzene rings. A similar effect is observed in [2$_4$]-metacyclophane *5B* and [2$_4$]metaparametapara-cyclophane *9B*[4] (see Table 1). In the bicyclophane *2B* two of the *m*-phenylene rings exert deshielding effects on the nearby H_A protons of the third ring. A similar effect is operative in compound *9B*. Therefore, although the overall shielding effects in compounds *1B* and *9B* are smaller than in [2$_2$]metacyclophane, *5B*, the former two compounds most likely adopt compressed conformations closely analogous to that observed for compound *5B*.

The *p*-phenylene bridged bicyclophane *2B* should have a helix structure similar to *1B* but less compressed. The NMR spectrum shows smaller but significant upfield shifts of the aromatic protons (see Table 1), indicative of mutual shielding from the di- and trisubstituted benzene rings.

The same upfield shift of the aromatic protons is observed in the thiophene bridged bicyclophane *3B* as compared to *3A*. The effect is smaller for the thiophene protons, H_F, than for the protons in the benzene rings, H_D, which is consistent with the assumption that the sulfur atoms point towards the centre of the molecule. The shift difference for the thiophene protons in the [2$_4$](2,5)thiophenoparacyclophanes *8A* and *8B* is much larger[11] (see Table 1) and this is interpreted as being due to different orientations of the thiophene rings in the two compounds.

Several conformations are possible for the bicyclophanes *1B*, *2B* and *3B*. The orientation of the aromatic rings linked by $-CH_2-CH_2-$ bridges must be *gauche* in most cases. Conformations with *anti*-orientations at some bridges are possible in *1B* but seem to be of higher energy than the all-*gauche* conformations, due to steric interactions. Neglecting *anti*-orientations at the $-CH_2-CH_2-$ bridges, the number of different conformations due to *gauche*$^+$ or *gauche*$^-$ orientations is still sixteen (including mirror images). The interconversion of conformers should occur mainly over *syn* barriers, as the other possibility, over *anti* conformations, is less plausible. The barriers to rotation of the aromatic rings are assumed to be low.

The temperature-dependence of the ^{1}H NMR spectra of the bicyclophanes *1B* and *2B* has been investigated and reported elsewhere.[12] Both bicyclophanes show the same type of behaviour. The AA'BB'-patterns for the protons in the saturated bridges broaden on cooling the samples and reappear as ABCD-patterns. The signals for the aromatic protons in *1B* also broaden and then resharpen to the original pattern. These observations are consistent with the assumption of

rapidly interconverting symmetrical conformations (D_3-symmetry).

The analysis of the ^{1}H NMR spectra of the bicyclophanes *1*–*4*, by comparison with spectra of similar but simpler cyclophanes, has led us to the conclusion that these molecules adopt conformations in which the central cavity is minimized. Furthermore, several of the cyclophanes prefer one or only a few, often highly symmetrical, conformations out of the large number of theoretically possible ones.

EXPERIMENTAL

The *bistriphenylphosphonium* salts were prepared from 1,3-bis(bromomethyl)benzene, 1,4-bis(bromomethyl)benzene, 1,3-bis(bromomethyl)-5-bromobenzene (*cf.* below) or 2,5-bis(chloromethyl)thiophene[11] by warming with triphenylphosphine (2 mol equiv.) in DMF up to *ca.* 150 °C. The salts crystallized on cooling and were collected and dried under vacuum at 110 °C before use.

1,3,5-Benzenetricarbaldehyde was prepared *via* Rosenmund reduction of 1,3,5-benzenetricarbonyl chloride using a slightly modified procedure as compared with that previously described.[13] The acid chloride (100 g) was dissolved in dry xylene (1 l). Palladium on barium sulphate catalyst (5 %, 25 g) and finely powdered thiourea (500 mg) were added. The mixture was stirred under nitrogen using a high speed stirrer. A stream of hydrogen was passed directly into the reaction mixture which was then heated to reflux temperature whereupon evolution of hydrogen chloride began. Addition of hydrogen, heating and stirring were continued until no further hydrogen chloride was evolved (approx. 10 h). On cooling, crystals were slowly formed. These and the catalyst were filtered off (filtrate: A) and boiled with water (3.5 l) until all the volatile components had steam distilled. After hot filtration the solution was cooled. The trialdehyde crystallized as long colourless needles, which were collected by filtration (filtrate: B), to give 27 g, m.p. 152 °C (Lit[13] 152 °C). Filtrate A was evaporated to 75 ml volume. The solid formed was collected by filtration (filtrate: C) and dissolved in the hot filtrate B which was then evaporated to approx. 1 l volume. On cooling, an additional 9 g of the trialdehyde was obtained, m.p. 150–151 °C (total yield 59 %). Filtrate C on evaporation gave 1,3-benzenedicarbaldehyde (9.5 g, 19 %).

Synthesis of bicyclophanehexaenes. 1,3,5-Benzenetricarbaldehyde (8.1 g, 0.05 mol) and a bisphosphonium salt from a bis(halomethyl)arene (0.075 mol) were added to dry DMF (250 ml) and the mixture was stirred under nitrogen at −40 °C. A freshly prepared solution of lithium ethoxide (approx. 0.3 M) was added dropwise at such a rate that the coloured ylid was consumed before the next drop was added. When no further colour was observed on addition of the base (after several hours) the reaction mixture was poured into water (*ca.* 250 ml) and filtered. The solid obtained was triturated with hot ethanol which dissolved the triphenylphosphineoxide formed. The aqueous phase was extracted with dichloromethane which was then washed with water, dried and the solvent evaporated. The residue together with the solid above was chromatographed on silica gel (tetrachloromethane as eluent except for the trithiabicyclophane, *A*, where dichloromethane was used). The bicyclophanehexaenes were eluted first. On concentration of the eluate to a small volume (*ca.* 10 ml) the cage cyclophanes crystallized. Filtration furnished essentially pure bicyclophanehexaenes, 1.5–2 %, which could be further purified by gradient vacuum sublimation.

[2$_6$](1,3,5)$_2$(1,3)$_3$Bicyclophanehexaene, 1A. Prisms (xylene), m.p. >360 °C. MS (70 eV): *m/e* 534 (M^+, 100 %), 277 (10, M^{2+}), 91 (2). Mol. wt., obs. 534.237; calc. for $C_{22}H_{30}$ 534.235. UV ($CHCl_3$): 297 nm (ε 83500), 265 sh (48700).

[2$_6$](1,3,5)$_2$(1,4)$_3$Bicyclophanehexaene, 2A. Prism (subl. or recryst. from quinoline), m.p. >550 °C. Anal. $C_{42}H_{30}$:C,H. MS (70 eV): *m/e* 534 (M^+, 100 %), 277 (M^{2+}, 6), 115 (4), and 91 (4). Mol. wt., obs. 534.233; calc. for $C_{42}H_{30}$ 534.235. UV ($CHCl_3$): 288 nm (ε 32100) and 260 sh (36000).

[2$_6$](1,3,5)$_2$(2,5-Thiopheno)$_3$bicyclophanehexaene, 3A. M.p. >360 °C from sublimation. MS (36 eV): *m/e* 552 (M^+, 100 %). Mol. wt. obs. 552.109; calc. for $C_{36}H_{24}S_3$ 552.108. UV ($CHCl_3$): 272 nm (ε 36300), 300 sh (19000), and 343 (15500).

[2$_6$](1,3,5)$_2$(5-Bromo,1,3)$_3$bicyclophanehexaene, 4A, was prepared in a small amount from 1,3,5-benzenetricarbaldehyde and the bistriphenylphosphonium salt from 1,3-bis(bromomethyl)-5-bromobenzene (see below) and its ^{1}H NMR spectrum recorded. (see Table 1).

1-Bromo-3,5-dimethylbenzene was prepared from diazotized 3,5-dimethylaniline. B.p. 80–82 °C/10 mmHg (Lit.[14] 88–89 °C/12 mmHg). ^{1}H NMR (60 MHz, $CDCl_3$): δ 2.18 (6H, broad s), 6.75 (1H, m), 7.00 (2H,m).

1,3-Bis(bromomethyl)-5-bromobenzene. 1-Bromo-3,5-dimethylbenzene (13,1 g), *N*-bromosuccinimide (recrystallized from acetic acid, 27.4 g) and benzoyl peroxide (700 mg) were reflexed in analytical grade tetrachloromethane (150 ml) under nitrogen. When all the bromosuccinimide was consumed the reaction mixture was filtered. The filtrate was evaporated to give an oil. On addition of ligroin, crystals separated which were recrystallized from ethanol, 5.7 g (22 %), m.p. 97–99 °C. Anal. $C_8H_7Br_3$: C, H.

^{1}H NMR (60 MHz, $CDCl_3$); δ 4.41 (4 H, s), 7.30 (1H, broad t, J 1.5 Hz), 7,47 (2H, d, J 1.5 Hz).

Synthesis of the bicyclophanes with saturated bridges, B. A small amount of a bicyclophanehexaene (*ca.* 10 mg) was mixed with palladium on charcoal (10 %, 5 mg) in benzene and stirred under hydrogen at atmospheric pressure until all the starting material had disappeared (usually 24 h). The mixture was filtered and the solvent evaporated to give a quantitative yield of the bicyclophane.

[2$_6$](1,3,5)$_3$Bicyclophane, 1B. M.p. 205–206 °C from acetic acid. MS (36 eV): *m/e* 546 (M^+, 100 %), 441 (16), 427 (11), 233 (16), 231 (13), 223 (10), 221 (13), 220 (11), 219 (44), 218 (10), 217 (14), 207 (21), 206 (16), 205 (48), 204 (10) and 203 (12), only fragments with *m/e* > 200 are listed. Mol. wt., obs. 546.323; calc. for $C_{42}H_{42}$ 546.329.

[2$_6$](1,3,5)$_2$(1,4)$_3$Bicyclophane, 2B. M.p. 207–208 °C, from acetic acid. MS (70 eV): *m/e* 546 (M^+, 100 %), 455 (8), 441 (7), 233 (13), 221 (11), 219 (22), 207 (12), 205 (18), 131 (9), 119 (21), 117 (18), 105 (28), 91 (13) and metastables for *m/e* 546→455 and 546→441. Mol. wt., obs. 546.328; calc. for $C_{42}H_{42}$ 546.329.

Calculation of the chemical shifts of the H_E and H_D protons of [2$_6$](1,3,5)$_2$(1,4)$_3$bicyclophanehexaene, 2A. The Bovey-Johnson nomogram[10] was used to calculate the expected chemical shifts of the protons on the aromatic rings at various twist angles. The influence of the bridging double bonds was considered to be small and was neglected. The distances from one H to the neighbouring aromatic rings were measured using Dreiding models. In case of the D_{3h} conformation (twist angle 0°) the mean values for δ_{H_E} and δ_{H_D} were used as calculated from the two extreme conformations depicted in *2A''*. δ_{H_E} is 7.2 for *p*-divinylbenzene and δH_D is 7.3 for *m*-divinylbenzene.[15] These were taken as standard values for unperturbed shifts. δ_{H_E} was then calculated to be: δ_{H_E} (twist angle): 7.05(0°), 6.95(50°), 6.85(70°) and 6.65(90°); δ_{H_D} (twist angle): 7.30(0°), 7.25(50°), 7.05(70°) and 6.38(90°).

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Syntheses of Some Cyclic Amino Acids Structurally Related to the GABA Analogue Homo-β-proline

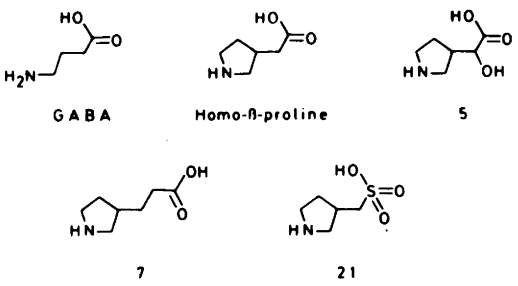
IBRAHIM M. LABOUTA,* POUL JACOBSEN, PIA THORBEK,** POVL KROGSGAARD-LARSEN and HANS HJEDS

Department of Chemistry BC, Royal Danish School of Pharmacy, DK-2100 Copenhagen Ø, Denmark

The synthesis of (±-α-hydroxy-3-pyrrolidineacetic acid (*5*), (*RS*)-3-pyrrolidinepropionic acid (*7*), (*RS*)-3-pyrrolidinemethanesulfonic acid (PMSA) (*21*) are described. Furthermore, an alternative route to (*RS*)-3-pyrrolidineacetic acid (homo-β-proline) (*13*) and attempts to prepare 3-pyrroline-3-ylacetic acid (*15*) and (±)-α-amino-3-pyrrolidinepropionic acid (*24*) are described.

A Doebner condensation of the protected 3-pyrrolecarbaldehyde *1* followed by catalytic hydrogenation gave *7*. A cyanohydrin synthesis with *1* performed under acylating conditions gave *2*, which was converted into *5 via 3* and *4*. A Wittig reaction on *8* gave a separable mixture of the enamide *11* and the *Z* and *E*-ethyl 1-methoxycarbonyl-$\Delta^{3,\alpha}$-pyrrolidineacetates (*9*, *10*). Catalytic hydrogenation of *11* and subsequent hydrolysis gave *13*. Alkaline hydrolysis and rearrangement of *9* or *10* gave *14*. Cleavage of *14* under a variety of conditions to give *15* were unsuccessful. KSCN treatment of *18* followed by oxidative chlorination and hydrolysis gave *21*.

The heterocyclic GABA analogue 3-pyrrolidineacetic acid (homo-β-proline) (*13*) is a very potent competitive inhibitor of the glial as well as of the presynaptic GABA uptake systems,[1,2] responsible for the termination of the GABA mediated synaptic transmission in the central nervous system.[3,4] Inhibitors of these uptake mechanisms have considerable pharmacological interest[4,5] and in an attempt to develop such compounds we have synthesized the following amino acids, (±)-α-hydroxy-3-pyrrolidineacetic acid (*5*), (*RS*)-3-pyrrolidinepropionic acid (*7*) and (*RS*)-3-pyrrolidinemethanesulfonic acid (PMSA) (*21*) structurally related to homo-β-proline. Attempts to prepare 3-pyrrolin-3-ylacetic acid (*15*) and α-amino-3-pyrrolidinepropionic acid (*24*) were unsuccessful.



A cyanohydrin synthesis performed under acylating conditions with the 3-pyrrolecarbaldehyde (*1*) gave compound *2*. Hydrolysis under Pinner conditions[6] followed by catalytic rhodium-Al_2O_3 hydrogenation afforded the hydroxyester *4*, which was converted into the amino acid *5*. 3-Pyrrolidinepropionic acid (*7*) was prepared from *1 via E*-3-pyrrolepropenoic acid (*6*).

Reaction of the pyrrolidone derivative *8* with triethylphosphonoacetate under Wittig conditions[7] gave a complex mixture from which the *Z*- and *E*-isomers *9* and *10* of ethyl 1-methoxycarbonyl-$\Delta^{3,\alpha}$-pyrrolidineacetate together with the enamine *11* were isolated by column chromatography. Low pressure hydrogenation of *11* and subsequent deprotection gave homo-β-proline (*13*). This synthetic sequence represents an alternative route to homo-β-proline (*13*). Alkaline treatment of *9*, *10* gave a

* Present address: Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

** Present address: Dumex A/S, Prags Boulevard 37, DK-2300 Copenhagen S, Denmark.

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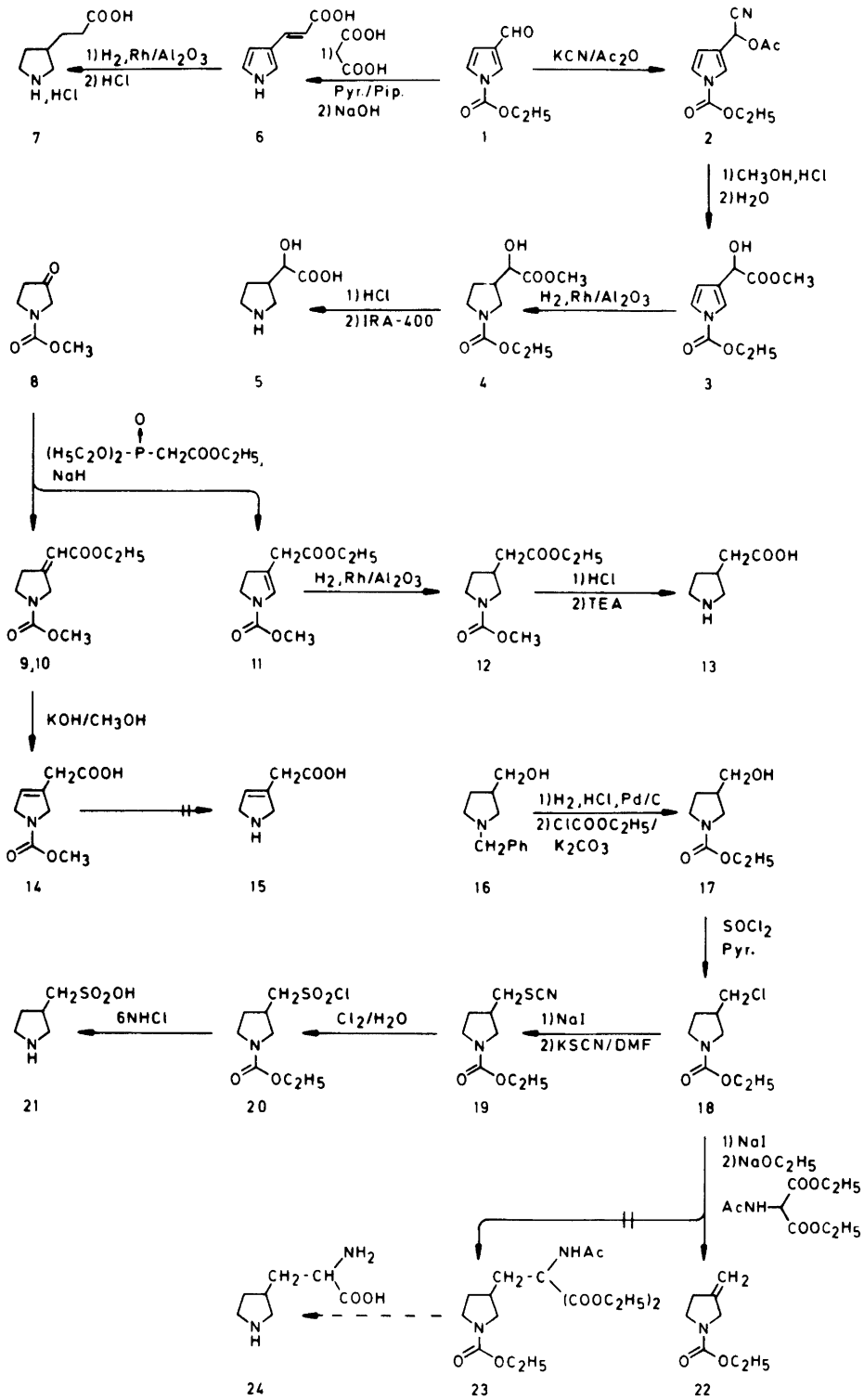

Scheme 1.

single compound, which was assigned the structure *14*. Attempts to deprotect *14* resulted in excessive decomposition.

Ethyl 3-chloromethylpyrrolidine-1-carboxylate (*18*) was prepared from *16* as outlined in Scheme 1. However, attempts to convert *18* into the acetaminomalonic ester *23* were unsuccessful, the dehydrohalogenated compound *22* being the only reaction product.

Compound *18* was converted into the thiocyanate *19*, which in turn was oxidized with chlorine to give the sulfonylchloride *20*. Hydrolysis and deprotection of *20* gave the sulfonic acid analogue of homo-β-proline, (*RS*)-3-pyrrolidinemethanesulfonic acid (PMSA) (*21*).

The structure determinations of the novel compounds were based on spectroscopic data supported by elemental analysis.

As generally accepted, the Doebner modification of the Knoevenagel reaction on compound *1* led to the formation of the unsaturated carboxylic acid *6*, of which the *trans* configuration corresponded with a strong UV absorption at 299 nm. Furthermore, the *trans* configuration was unequivocally shown by a coupling constant of 15.6 Hz in the ^{1}H NMR spectrum of *6*.

The enamine structure of compound *11* was supported by a UV absorption at 233 nm and a one proton singlet at δ 6.48 in the ^{1}H NMR spectrum.

The ^{1}H NMR spectra of *9* and *10* allow a tentative determination of the configuration at the double bonds. The signals originating in the C-2 and C-4 protons of compound *9* are found as a quartet centered at δ 4.24 and a multiplet centered at 3.1, respectively. The corresponding signals of compound *10* are found at δ 4.55 and δ 2.82. The anisotropy effect of the ester carbonyl group is assumed to cause shielding of the C-2 protons of the *Z*-form and the C-4 protons of the *E*-form, leading to upfield shifts of the C-2 proton signal of the *Z*-form and of the C-4 proton signal of the *E*-form. Consequently, *9* and *10* are assigned the *Z*- and *E*-configuration, respectively.

EXPERIMENTAL

Melting points determined in capillary tubes are corrected. Elemental analyses were made by Mr. G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark. A Perkin-Elmer grating infrared spectrophotometer model 402, and a JEOL JMN-C-60HL (60 MHz) ^{1}H NMR instrument were used. ^{1}H NMR spectra were recorded using TMS as an internal standard. Compounds dissolved in D_2O were referenced to TSP. Thin-layer chromatography (TLC) and column chromatography (CC) were accomplished using silica gel F_{254} plates (Merck) and silica gel (Woelm 0.063 – 1.00 mm), respectively. Columns were developed by stepwise gradient elution. The pK_A values were determined as described in a previous paper.[8]

(±)-α-Acetoxy-1-ethoxycarbonyl-3-pyrroleacetonitrile (2). To a solution of *1*[9] (3.1 g; 18.7 mmol) in glacial acetic acid (9 ml) was added potassium cyanide (1.82 g; 28 mmol). After stirring for 40 min acetic anhydride (2.04 g; 20 mmol) was added and the mixture was heated to 50 °C for 24 h. After cooling to room temperature, water (40 ml) was added and the mixture was extracted with ether (50 ml). The organic layer was washed with an aqueous solution of sodium carbonate (40 ml; 1 M), dried ($MgSO_4$) and evaporated *in vacuo* to give crude *2*, which submitted to CC [silica gel: 70 g; eluent: toluene containing ethyl acetate (5 – 15 %)] gave *2* (850 mg; 20 %) as an oil. An analytical sample was purified by ball-tube distillation at 5×10^{-3} Pa (oven temp. 200 °C). Found: C 55.80; H 5.25; N 11.80. Calc. for $C_{11}H_{12}N_2O_4$: C 55.93; H 5.12; N 11.86. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.59 (1 H, m), 7.38 (1 H, m), 6.6 – 6.3 (2 H, broad signal), 4.44 (2 H, q, *J* 7 Hz), 2.13 (3 H, s), 1.38 (3 H, t, *J* 7 Hz). IR (film): 3150 (m), 2980 (m), 2940 (m), 2320 (m), 1800 – 1660 (s), 1495 (s), 1420 (s), 1380 (s), 1345 (s), 1280 (s) cm^{-1}.

Methyl (±)-α-hydroxy-1-ethoxycarbonyl-3-pyrroleacetate (3). To an ice-cooled solution of methanol (6 ml) saturated with hydrogen chloride was added *2* (800 mg; 3.5 mmol) and the mixture was left at 4 °C for 16 h with stirring. The solution was evaporated *in vacuo* to give an oil, which was dissolved in water (4 ml) and extracted with ether (3 × 30 ml). The combined and dried ($MgSO_4$) organic phases were evaporated *in vacuo* to give crude *3* (0.71 g), which was submitted to CC [silica gel: 70 g; eluent: toluene containing ethyl acetate 15 – 20 %)] to give *3* (400 mg; 52 %). An analytical sample was purified by ball-tube distillation at 5×10^{-3} Pa (oven temp. 220 °C). Found: C 52.83; H 5.72; N 6.25. Calc. for $C_{10}H_{13}NO_5$: C 52.86; H 5.77; N 6.17. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.40 (2 H, m), 6.40 (1 H, m), 5.23 (1 H, d, *J* 6 Hz), 4.46 (2 H, q, *J* 7 Hz), 3.83 (3 H, s), 3.28 (1 H, d, *J* 6 Hz), 1.40 (3 H, t, *J* 7 Hz). IR (film): 3480 (s), 3140 (m), 2980 (s), 2950 (s), 1800 – 1650 (s), 1580 (m), 1490 (s) cm^{-1}.

Methyl (±)-α-hydroxy-1-ethoxycarbonyl-3-pyrrolidineacetate (4). A solution of *3* (700 mg; 3.0 mmol) in ethanol (50 ml) was hydrogenated (*ca.* 300 kPa) for 20 h in a PARR low-pressure hydrogenation apparatus using 5 % Rh – Al_2O_3 (500 mg)

as a catalyst. Evaporation *in vacuo* gave a crude product which was submitted to CC [silica gel: 70 g; eluent: toluene containing ethyl acetate (15–25 %) and formic acid (1 %)] to give *4* (500 mg; 73 %) as an oil. Found: C 52.04; H 7.47; N 6.12. Calc. for $C_{10}H_{17}NO_5$: C 51.94; H 7.41; N 6.06. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.16 (2 H, q, *J* 7 Hz), 4.4–3.9 (1 H, m), 3.83 (3 H, s), 3.8–3.0 (4 H, m), 3.0–2.2 (2 H, m), 2.2–1.5 (2 H, m), 1.23 (3 H, t, *J* 7 Hz). IR (film): 3400 (s), 2950 (s), 2880 (s), 1735 (s), 1710–1600 (s, several bands), 1435 (s), 1380 (s) cm^{-1}.

(±)-α-Hydroxy-3-pyrrolidineacetic acid (5). A mixture of *4* (262 mg; 2 mmol) and hydrochloric acid (10 ml; 6 M) was refluxed for 2 h. After evaporation *in vacuo* the residue was dissolved in water (5 ml), passed through a column containing ion exchange resin [Amberlite IRA-400 (OH) (40 ml)] using acetic acid (6 %) as an eluent. The eluate was evaporated *in vacuo* to give *5* (120 mg; 39 %) as a glassy compound. Found: C 45.63; H 7.45; N 8.22. Calc. for $C_6H_{11}NO_3$,$\frac{1}{2}H_2O$: C 46.75; H 7.97; N 9.09. ^{1}H NMR (60 MHz, D_2O and acetonitrile as an internal standard): δ 4.2–3.6 (1 H, m), 3.6–2.7 (5 H, m), 2.3–1.3 (2 H, m). IR (KBr): 3700–2000 (s, several bands), 1740–1380 (s, several bands), 1360 (s), 1355–1140 (s) cm^{-1}.

E-*3-Pyrrolepropenoic acid (6).* To a solution of *1*[9] (3.0 g; 18 mmol) and malonic acid (3.8 g; 36 mmol) in pyridine (30 ml) was added piperidine (1.5 ml) with stirring while the solution was heated to 100 °C for 8 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was taken up in sodium hydroxide (25 ml; 1.5 M). After stirring for 1.5 h the reaction mixture was washed with ether (3 × 25 ml). The aqueous phase was acidified with hydrochloric acid (*ca.* 10 ml; 4 M) to pH 3 and was extracted with ether (5 × 30 ml). The pooled extracts were dried (Na_2SO_4) and concentrated to about 10 ml, after which *6* (1.2 g; 54 %) crystallized. An analytical sample was recrystallized (ethyl acetate) to give *6*, *m.p.* 180–184 °C. Anal. $C_7H_7NO_2$: C, H, N. ^{1}H NMR (DMSO-d_6): δ 11.18 (1 H, s), 7.58 (1 H, d, *J* 15.6 Hz), 7.22 (1 H, s), 6.82 (1 H, m), 6.43 (1 H, m), 5.97 (1 H, d, *J* 15.6 Hz), 5.69 (1 H, broad signal). IR (KBr): 3440 (m), 3150–2300 (m–w, several bands), 1690 (s), 1660 (s), 1600 (s), 1540 (w), 1500 (w), 1410 (m) cm^{-1}. UV [methanol (log ε)]: 211 (4.10), 299 (4.26) nm.

(RS)-3-Pyrrolidinepropionic acid hydrochloride (7). A mixture of *6* (2.0 g; 16 mmol), hydrochloric acid (4 ml; 16 mmol, 4 M), water (300 ml) and 5 % Rh–Al_2O_3 (1.5 g) was hydrogenated at *ca.* 300 kPa H_2-pressure in a PARR low-pressure hydrogenation apparatus for 18 h. The filtered mixture was evaporated to dryness *in vacuo* and the residue was crystallized (water–ethanol) to give *7* (1.2 g; 45 %), m.p. 104–105 °C. Anal. $C_7H_{14}ClNO_2$: C, H, Cl, N. ^{1}H NMR (60 MHz, D_2O): δ 3.7–2.7 (4 H, m), 2.7–2.2 (3 H, m), 2.2–1.5 (4 H, m). IR (KBr): 3420 (m), 3200–2300 (s–w, several bands), 1720 (s), 1460 (m), 1400 (m) cm^{-1}.

Z- *and* E-*Ethyl 1-methoxycarbonyl-*$\Delta^{3,\alpha}$*-pyrrolidineacetate (9,10) and ethyl 1-methoxycarbonyl-2-pyrroline-3-ylacetate (11).* To a suspension of sodium hydride (1.92 g; 80 mmol) in 1,2-dimethoxyethane (DME) (15 ml) was added dropwise a solution of triethylphosphonoacetate (17.92 g; 80 mmol) in DME (15 ml) with stirring, which was continued for 1 h. To the reaction mixture was added a solution of *8*[2] (11.44 g; 80 mmol) in DME (50 ml) and the resulting solution was stirred at room temperature for 12 h. The solvent was removed *in vacuo* to give a crude product, which was submitted to CC [silica gel: 1000 g; eluent: cyclohexane containing ethyl acetate (25–50 %)] to give *9* (710 mg; 3.9 %), *10* (800 mg; 4.5 %), and *11* (4.4 g; 24 %). Analytical samples of *9* and *10* were purified by recrystallization (cyclohexane–light petroleum) and *11* was purified by ball-tube distillation at 5×10^{-3} Pa (oven temperature 150 °C).

9: M.p. 80.0–81.5 °C. Found: C 55.77; H 7.04; N 6.57. Calc. for $C_{10}H_{15}NO_4$: C 56.32; H 7.09; N 6.57. ^{1}H NMR (60 MHz, $CDCl_3$): δ 6.04–5.79 (1 H, m), 4.42–4.05 (2 H, q. *J* 7 Hz), 4.24 (2 H, q, *J ca.* 2 Hz), 3.79 (3 H, s), 3.86–3.49 (2 H, m), 3.38–2.71 (2 H, m), 1.32 (3 H, t, *J* 7 Hz). IR (KBr): 3450 (m), 3000–2850 (n), 1720–1680 (s), 1660 (m), 1450 (s), 1400 (s), 1380 (s), 1340 (s), 1230 (s) cm^{-1}. UV [methanol (log ε)]: 215 (3.92) nm.

10: M.p. 62.5–65.5 °C. Found: C 56.20; H 7.09; N 6.46. Calc. for $C_{10}H_{15}NO_4$: C 56.32; H 7.09; N 6.57. ^{1}H NMR (60 MHz, $CDCl_3$): δ 6.00–5.79 (1 H, m), 4.55 (2 H, q, *J ca.* 2 Hz), 4.23 (2 H, q, *J* 7 Hz), 3.78 (3 H, s), 3.87–3.36 (2 H, m), 3.03–2.61 (2 H, m), 1.20 (3 H, t, *J* 7 Hz). IR (KBr): 4350 (m), 3000–2860 (m), 1730–1680 (s), 1640 (s), 1400 (s), 1380 (s), 1340 (m), 1300 (m), 1220 (s) cm^{-1}.

11: Found: C 55.38; H 6.98; N 6.64. Calc. for $C_{10}H_{15}NO_4$: C 56.32; H 7.09; N 6.57. ^{1}H NMR (60 MHz, $CDCl_3$): δ 6.48 (1 H, s), 4.16 (2 H, q, *J* 7 Hz), 3.92–3.36 (2 H, m), 3.10 (2 H, s), 2.92–2.36 (2 H, m), 1.26 (3 H, t, *J* 7 Hz). IR (film): 3450 (w), 3050–2800 (m), 1735–1680 (s), 1645 (s), 1460 (s), 1390 (s), 1280 (m) cm^{-1}. UV [methanol (log ε)]: 233 (4.20) nm.

(RS)-Ethyl 1-methoxycarbonyl-3-pyrrolidineacetate (12). A solution of *11* (225 mg; 1 mmol) in ethanol (50 ml) was hydrogenated (*ca.* 300 kPa) for 20 h in a PARR low-pressure hydrogenation apparatus using Rh–Al_2O_3 (200 mg) as a catalyst. After evaporation *in vacuo* the crude product was submitted to CC [silica gel: 20 g; eluent: toluene containing ethyl acetate (25–50 %)] to give *12* (120 mg; 53 %) as an oil. Found: C 55.50; H 7.99; N 6.29. Calc. for $C_{10}H_{17}NO_4$: C 55.80; H 7.96;

N 6.51. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.18 (2 H, q, *J* 7 Hz), 3.68 (3 H, s), 3.8–2.7 (7 H, m), 2.7–2.2 (2 H, m), 1.25 (3 H, t, *J* 7 Hz). IR (film): 3700–3200 (m), 3100–2800 (s), 1760–1600 (s), 1450 (s), 1395 (s) cm^{-1}.

(RS)-3-Pyrrolidineacetic acid (homo-β-proline) (13). A solution of *12* (880 mg; 4.1 mmol) in hydrochloric acid (40 ml; 5 M) was refluxed for 2 h. Evaporation *in vacuo* gave an oil, which was dissolved in ethanol (5 ml). To this solution was added a solution of triethylamine (412 mg; 4.1 mmol) in ethanol (3 ml). Filtration gave *13* (210 mg; 40 %) as crystals, m.p. 128–130 °C (decomp.). Found: C 55.26; H 8.84; N 10.51. Calc. for $C_6H_{11}NO_2$: C 55.79; H 8.58; N 10.85. ^{1}H NMR (60 MHz, D_2O): δ 3.8–3.0 (3 H, m), 3.0–2.5 (2 H, m), 2.6–1.4 (4 H, m). IR (KBr): 3700–3150 (s), 3150–2800 (s), 2800–2000 (m, several bands), 1405 (s), 1280 (s) cm^{-1}.

1-Methoxycarbonyl-3-pyrroline-3-ylacetic acid (14). To a solution of *9* or *10* (225 mg; 1 mmol) in methanol (5 ml) was added a solution of potassium hydroxide (60 mg; 1.2 mmol) in methanol (10 ml) with stirring, which was continued at room temperature for 4 days followed by evaporation *in vacuo*. The residue was dissolved in water (15 ml) and pH was adjusted to 4 using acetic acid. The reaction mixture was extracted with chloroform (5 × 10 ml). The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo* to give an oil, which was submitted to CC [silica gel: 20 g; eluent: toluene containing ethyl acetate (50–80 %) and formic acid (1 %)] to give *14* (70 mg; 36 %) as an oil. Found: C 49.46; H 6.26; N 7.16. Calc. for $C_8H_{11}NO_4,\frac{1}{2}H_2O$: C 49.48; H 6.23; N 7.14. ^{1}H NMR (60 MHz, $CDCl_3$–D_2O:99–1): δ 6.3–5.8 (1 H, m), 4.26 (2 H, s), 3.83 (s) and 3.77 (s) (a total of 5 H), 3.23 (2 H, s). IR (film): 3600–2500 (s, several bands), 1770 (s), 1700 (s), 1400 (s), 1250 (m) cm^{-1}.

(RS)-1-Ethoxycarbonyl-3-hydroxymethylpyrrolidine (17). A solution of *16*[10] (8 g; 26 mmol) in a mixture of aqueous hydrochloric acid (260 ml; 0.1 M) and aqueous ethanol (120 ml; 50 %) was hydrogenated (*ca.* 300 kPa) for 20 h in a PARR low-pressure hydrogenation apparatus using 5 % Pd–C (2.5 g) as a catalyst. The reaction mixture was concentrated *in vacuo* to 100 ml and washed with methylene chloride (2 × 20 ml). The aqueous phase was evaporated *in vacuo* to give an oil (7 g). To an ice-cooled solution of the oil and potassium carbonate (9 g; 65 mmol) in water (90 ml) was added ethylchloroformate (7.8 g; 65 mmol) with stirring, which was continued for 1 h at 0 °C and then for 1 h at room temperature. The reaction mixture was extracted with ether (6 × 100 ml) and the combined and dried ($MgSO_4$) organic phases were evaporated *in vacuo* to give *17* (3.4 g; 80 %). An analytical sample was purified by ball-tube distillation at *ca.* 100 Pa (oven temperature 250 °C). Found: C 55.17; H 8.51; N 7.95. Calc. for $C_8H_{15}NO_3$: C 55.47; H 8.73; N 8.09. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.3–3.9 (3 H, m), 3.8–3.0 (6 H, m), 2.82–2.25 (1 H, m), 2.15–1.65 (2 H, m), 1.26 (3 H, t). IR (film): 3440 (m), 2955 (m), 2870 (w), 1740 (m), 1680 (s), 1460 (s), 1405 (s), 1270 (s) cm^{-1}.

(RS)-Ethyl 3-chloromethylpyrrolidine-1-carboxylate (18). To a stirred solution of *17* (2.0 g; 12.5 mmol) and pyridine (1.0 g; 12.5 mmol) in chloroform (50 ml) was added dropwise a solution of thionyl chloride (3.0 g; 25 mmol) in chloroform (10 ml). The reaction mixture was refluxed for 2.5 h and then evaporated *in vacuo* to dryness. Water (50 ml) was added followed by extraction with chloroform (3 × 75 ml). The combined and dried ($MgSO_4$) organic phases were evaporated *in vacuo* to give an oil, which was submitted to CC [silica gel: 200 g; eluent: toluene containing ethyl acetate (40–60 %)] to give *18* (1.12 g; 50 %). An analytical sample was purified by ball-tube distillation at *ca.* 100 Pa (oven temperature 100 °C). Found: 50.43; H 7.44; Cl 17.25; N 7.39. Calc. for $C_8H_{14}ClNO_2$: C 50.13; H 7.36; Cl 18.50; N 7.31. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.15 (2 H, q, *J* 7 Hz), 3.8–3.0 (6 H, m), 2.8–2.4 (1 H, m), 2.2–1.7 (2 H, m), 1.25 (3 H, t, *J* 7 Hz). IR (film): 2950 (s), 2870 (s), 1695 (s), 1450 (m), 1420 (s), 1380 (s), 1350 (s) cm^{-1}.

(RS)-Ethyl 3-thiocyanomethylpyrrolidine-1-carboxylate (19). A solution of *18* (6.0 g; 31.2 mmol) and sodium iodide (4.7 g; 31.2 mmol) in acetone (180 ml) was stirred at room temperature for 12 h, filtered and evaporated *in vacuo* to give an oil (5.5 g). To a solution of the oil in dimethylformamide (80 ml) was added potassium thiocyanate (16.6 g; 171 mmol) and the reaction mixture was heated to 85 °C for 5 days. The solvent was removed *in vacuo*, and the residue was submitted to CC [silica gel: 300 g; eluent: toluene containing ethyl acetate (50 %)] to give *19* (5.54 g; 83 %) as an oil. Found: C 50.61; H 6.68; N 12.71; S 14.18. Calc. for $C_{19}H_{14}N_2O_2S$: C 50.46; H 6.59; N 13.08; S 14.94. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.15 (2 H, q, *J* 7 Hz), 3.9–3.0 (6 H, m), 2.5 (1 H, m), 2.3–1.8 (2 H, m), 1.25 (3 H, t, *J* 7 Hz). IR (film): 3700–3300 (w), 2950 (s), 2910 (s), 2850 (s), 2150 (s), 1680 (s), 1500–1400 (s, several bands), 1380 (s) cm^{-1}.

(RS)-1-Ethoxycarbonyl-3-pyrrolidinemethanesulfonyl chloride (20). Chlorine gas was passed through an ice-cooled solution of *19* (2.0 g; 10.4 mmol) in water (40 ml) for 2.5 h, and then oxygen was bubbled through the reaction mixture for 10 min. The reaction mixture was extracted with ether (3 × 60 ml). The combined and dried ($MgSO_4$) organic phases were evaporated *in vacuo* to give *20* (2.3 g; 92 %) as an oil. Found: C 38.71; H 5.64; Cl 12.96; N 5.21; S 11.59. Calc. for $C_8H_{14}ClNO_4S$: C 37.58; H 5.52; Cl 13.87; N 5.48; S 12.51. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.08 (2 H, q, *J* 7 Hz), 3.9–2.6 (7 H, m),

2.5–1.7 (2 H, m), 1.25 (3 H, t, *J* 7 Hz). IR (film): 3500–3100 (m), 2990 (s), 1800–1600 (s), 1450 (m), 1430 (s), 1380 (s) 1260 (s) cm^{-1}.

(RS)-3-Pyrrolidinemethanesulfonic acid (21). A solution of *20* (1.0 g; 4.14 mmol) in aqueous hydrochloric acid (10 ml); 6 M) was refluxed for 2 h. Evaporation *in vacuo* and recrystallization (methanol–ether) gave *21* (190 mg; 41.3 %), m.p. 268 °C (decomp.). Found: C 36.36; H 6.73; N 8.48; S 19.37. Calc. for $C_5H_{11}NO_3S$: C 36.36; H 6.71; N 8.48; S 19.38. ^1H NMR (60 MHz, D_2O): δ 3.7–2.8 (m), 2.5–2.0 (m) and 1.9–1.5 (m) (a total of 13 H). IR (KBr): 3700–3300 (m), 3230–2850 (s), 2850–2200 (m), 1610 (s), 1420 (s), 1300–1100 (s, several bands) cm^{-1}.

Ethyl 3-methylenepyrrolidine-1-carboxylate (22). To a stirred solution of sodium iodide (4.0 g; 27 mmol) and sodium thiosulfate (10 mg) in acetone (20 ml) was added a solution of *18* (4.0 g; 20 mmol) in acetone (20 ml). Stirring was continued at room temperature for 12 h. The reaction mixture was diluted with ether (100 ml), filtered and evaporated *in vacuo* to give an oil, which was dissolved in ethanol (10 ml). To a solution of sodium (460 mg; 20 mmol) and diethyl acetaminomalonate (4.34 g; 20 mmol) in ethanol (70 ml) was added the above solution and the reaction mixture was refluxed for 48 h, until it became neutral. The mixture was cooled, filtered and evaporated *in vacuo*, and the residue was dissolved in water (100 ml) followed by extraction with methylene chloride (5 × 30 ml). The combined and dried (Na_2SO_4) organic phases were evaporated *in vacuo* to give an oil, which was submitted to CC [silica gel: 200 g; eluent: toluene containing ethyl acetate (10–25 %)] gave *22* (3.0 g; 96.7 %). An analytical sample was purified by ball-tube distillation at 1.5×10^{-3} Pa (oven temperature 80 °C). Found: C 61.62; H 8.46; N 9.19. Calc. for $C_8H_{13}NO_2$: C 61.91; H 8.44; N 9.03. ^1H NMR (60 MHz, $CDCl_3$): δ 5.06 (2 H, s), 4.21 (4 H, m, *J* 7 Hz), 3.56 (2 H, t, *J* 7 Hz), 2.8–2.2 (2 H, m), 1.29 (3 H, t, *J* 7 Hz). IR (film): 3550–3150 (s), 3040–2750 (s, several bands), 1680 (s), 1580–1500 (m), 1420 (s), 1375 (s) cm^{-1}.

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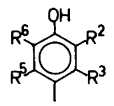
Electrophilic Chlorination of 4-Methylphenols with Molecular Chlorine. Synthesis of Dimethoxy Aromatics by Methanolysis of 4-Chloro-4-methylcyclohexa-2,5-dienones

KARL-ERIK BERGQUIST, ANDERS NILSSON and ALVIN RONLÁN

Organic Chemistry 2, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund, Sweden

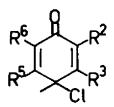
The chlorination of *p*-cresol (*1a*), 2,4-dimethylphenol (*1b*), 3,4-dimethylphenol (*1c*), 2,4,5-trimethylphenol (*1d*), 2,4,6-trimethylphenol (*1e*) and the various possible mono- and dichloro derivatives of these phenols (*3a–d*, *6a* and *6c*) with molecular chlorine was investigated in dichloromethane or dimethyl formamide solution. The 2,6-substituted *p*-cresol derivatives all give quantitative yields of 4-chloro-4-methylcyclohexa-2,5-dienones (*5b*, *5d*, *7a*, *7c* and *2e*), whereas the less substituted *p*-cresol derivatives give a 20–50% yield of the corresponding 4-chloro-4-methylcyclohexa-2,5-dienones (*2a–d*, *5a* and *5c*), the other products being chlorinated phenols. Treatment of 4-chloro-4-methylcyclohexa-2,5-dienones with at least one of the double bonds of the ring unsubstituted (*2a–c* and *4a*) with methanol gave a quantitative yield of a 1,3-dimethoxybenzene derivative (*9a–c* and *10*), whereas similar treatment of 4-chloro-2,4,5-trimethylcyclohexa-2,5-dienone (*2d*) gave 5-methoxy-2,3-dimethylbenzyl methyl ether (*11*). Treatment of the other 4-chlorodienones with methanol gave the corresponding 4-methoxydienones. Electrophilic chlorination of certain *p*-cresol derivatives followed by methanolysis hence is a facile although low-yield method for 3-methoxylation or selective side chain oxidation of these *p*-cresol derivatives.

In a previous publication,[1] we described a novel high yield synthesis of 4-chloro-4-methylcyclohexa-2,5-dienone (*2a*) from *p*-cresol (*1a*) by chlorination with antimony pentachloride in dichloromethane (DCM) at low temperature. In the same publication a number of reactions with *2a* were described which demonstrated that *2a* is a useful synthon. However, the work-up of the reaction mixture from chlorination with antimony pentachloride was cumbersome, and therefore a search for alternative chlorination reagents was initiated. Attempts were made to oxidize 4-alkylphenols electrochemically in the presence of chloride ions, in the hope that anodically generated phenoxonium ion would react with chloride ion to give the desired chlorodienone. From previous studies[2] we know that 4-alkylphenoxonium ions reacts with nucleophiles preferentially in the 4-position. Actually, anodic oxidation of *p*-cresol in dimethyl formamide (DMF) containing hydrogen chloride gave a modest yield (20%) of *2a*. However, electroanalytic studies re-

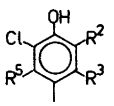


1a-e

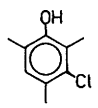
a: $R^2=R^3=R^5=R^6=H$
b: $R^2=Me; R^3=R^5=R^6=H$
c: $R^2=R^5=R^6=H; R^3=Me$
d: $R^2=R^5=Me; R^3=R^6=H$
e: $R^2=R^6=Me; R^3=R^5=H$



2a-e



3a-d



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vealed that no oxidation of *p*-cresol occurred in this system. Only chlorid ion was oxidized to molecular chlorine, which in turn reacted with *p*-cresol to give a mixture of *2a* and 2-chloro-*p*-cresol (*3a*). We therefore started a detailed study of the chlorination of *p*-cresol and other 4-alkylphenols (*1b–e*) by molecular chlorine to see if it was possible to use this method for the preparation of 4-alkyl-4-chlorocyclohexa-2,4-dienones. In the course of this study a similar investigation of the *ipso* chlorination of 4-alkylphenols in acetic anhydride has been published.[3]

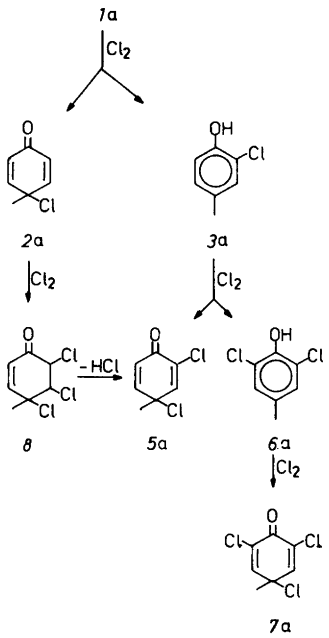
RESULTS AND DISCUSSION

The effect of the amount of chlorine, solvent, temperature, and added Friedel-Crafts catalyst on the product distribution in the chlorination of *p*-cresol was studied in detail, and the results are given in Table 1. The products formed and their

Table 1. Chlorination of p-cresol (*1a*) with molecular chlorine. Effects of amount of chlorine, solvent, temperature add added catalyst on product distribution.[a]

Exp. No.	Molar ratio (Cl_2:*p*-cresol)	Solvent	Temp. (°C)	Catalyst (mol)[b]	Products and yields (%) *2a*	*8*	*5a*	*7a*	*3a*	*6a*	Ratio dienones–phenols (*2a*+*8*+*5a*+*7a*)/(*3a*+*6a*))
1	1.0	DCM[c]	18	–	22	0	0	0	78	0	0.28
2	1.0	DCM	0	–	27	0	0	0	73	0	0.37
3	1.2	DCM	−40	–	8	10	14	0	26	42	0.47
3	1.5	DCM	−40	–	2	12	26	0	20	40	0.67
4	1.9	DCM	−40	–	0	21	29	0	6	44	1.0
5	2.1	DCM	−40	–	0	18	34	0	0	48	1.1
6	4.0	DCM	−40	–	0	18	35	0	0	47	1.1
7	8.0	DCM	−40	–	0	17	27	6	0	50	1.0
8	2.9	DCM	0	–	0	18	36	0	0	46	1.2
9	2.9	DCM	−20	–	0	19	31	0	0	50	1.0
10	2.9	DCM	−35	–	0	20	35	0	0	45	1.2
11	1.0	DMF[d]	−40	–	24	0	0	0	76	0	0.32
12	1.0	DMF	−20	–	27	0	0	0	73	0	0.37
13	2.0	DMF	−40	–	27	0	16	0	23	24	0.75
14	2.0	DMF	20	–	23	0	24	3	7	43	1.0
15	2.9	DMF	−20	–	22	0	22	56	0	0	
16	9.0	DMF	−20	–	20	0	30	50	0	0	
17	1.0	CS_2	19	–	0	0	0	0	>95		
18	1.0	CS_2	−40	–	5	0	0	0	95		0.05
19	1.0	DCM	−40	BF_3OEt_2 (1)	25	0	0	0	75		0.33
20	1.0	DCM	−40	BF_3OEt_2 (2)	35	0	0	0	65		0.54
21	1.0	DCM	−20	BF_3OEt_2 (2)	34	0	0	0	66		0.52
22	1.0	DCM	20	BF_3OEt_2 (2)	36	0	0	0	64		0.56
23	1.0	DCM	−40	BF_3OEt_2 (3)	28	0	0	0	72		0.39
24	1.0	DCM	0	$TiCl_4$ (0.1)	25	0	0	0	75		0.33
25	1.0	DCM	0	$TiCl_4$ (0.25)	34	0	0	0	66		0.42
26	1.0	DCM	15	$TiCl_4$ (0.25)	28	0	0	0	72		0.39
27	1.0	DCM	0	$TiCl_4$ (0.5)	39	0	0	0	61		0.64
28	1.0	DCM	0	$TiCl_4$ (1.0)	35	0	0	0	65		0.54
29	1.0	DCM	0	$TiCl_4$ (2.0)	35	0	0	0	65		0.54
30	1.0	DCM	22	CF_3COOH (5)	26	0	0	0	74		0.32
31	1.0	DCM	22	CF_3SO_3H (1)	20	0	0	0	80		0.25
32	1.0	CS_2	−20	$TiCl_4$ (0.5)	31	0	0	0	69		0.45
33	1.0	CS_2	0	$TiCl_4$ (1.0)	24	0	23	0	53		0.89

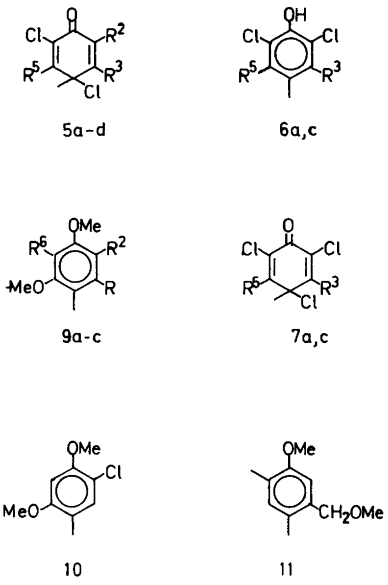
[a] The yields were determined from the NMR spectrum of the crude product mixture (for details see Experimental). [b] Molar ratio between *p*-cresol and the catalyst. [c] DCM = dichloromethane. [d] DMF = dimethyl formamide.



Scheme 1.

formation paths are shown in Scheme 1. As can be seen, the reaction temperature is of little importance whereas the other variables are of great importance. Addition of one mol of chlorine per mol of *p*-cresol always gives the highest yield of *2a*, as *p*-cresol turns out to be far more reactive towards chlorine than any of the products in Scheme 1. As long as *p*-cresol is present, the formations of *2a* and *3a* are the only reactions occurring. If more than one mol of chlorine is added, all the other reactions in Scheme 1 begin to take place. That is, addition of Cl_2 to *2a* to give 2,3,4-trichloro-4-methyl-cyclohex-5-enone (*8*), chlorination of *3* to give a mixture of 2,4-dichloro-4-methylcyclohexa-2,5-dienone (*5a*) and 2,6-dichloro-*p*-cresol (*6a*), and finally chlorination of *6a* to give 2,4,6-trichloro-4-methylcyclohexa-2,5-dienone (*7a*) [NMR indicates that the addition of chlorine to *2a* is stereospecific, and only the *trans*- or the *cis*-3,4-dichloro isomer is formed.] That product formation actually occurs as shown in Scheme 1 was confirmed by reacting pure *2a*, *3a*, *5a* and *6a* with chlorine in DCM. On standing, *8* eliminates hydrogen chloride to give *5a* and exhaustive chlorination of *p*-cresol in either DCM or DMF (no catalyst added) eventually gives a 1:1 mixture of the dienones *5a* and *7a*.

In both DCM and DMF *2a* and *3a* are formed in approximately a 1:3 ratio on chlorination with one mol of chlorine. However, in DMF the addition of chlorine to *2a* does not occur, probably because of the buffering effect of DMF which diminishes the acidity of the HCl catalyst formed in the chlorination. In CS_2 essentially only ortho chlorination occurs. Addition of trifluoroacetic acid (TFA) or trifluoromethane sulfonic acid (TFMS) does not affect the yield of *2a*, whereas boron trifluoride etherate, and especially titanium tetrachloride, gives a marked increase of the yield of *2a* (exps. 22 and 27). At least two mols of boron trifluoride etherate and at least half a mol of titanium tetrachloride must be added to obtain the maximum yield of *2a*. This indicates that the actual specied being chlorinated is a *p*-cresol – metal halide complex, where the metal halide is attached to the phenolic hydroxy group and thereby causes steric hindrance to ortho-chlorination. Changes of the electron density in the benzenoid ring caused by the increased electrophilicity of the oxygen substituent might also be important.



The results of chlorination of 2,4-dimethylphenol (*1b*), 3,4-dimethylphenol (*1c*), 2,4,5-trimethylphenol (*1d*), 2,4,6-trimethylphenol (*1e*), and the chlorinated phenols, *3a*, *6a*, *3b–d* and *6c* (formed in the chlorination of the phenols *1a–d*) are given in Table 2. No attempts to study the effect of added catalyst or solvent were made. The 2,6-substituted *p*-cresol derivatives, with the exception of 2,4,6-trimethylphenol (*1e*), all give quantitative yields of the cor-

Table 2. Chlorination of 2,4-dimethylphenol (*1b*), 3,4-dimethylphenol (*1c*), 2,4,5-trimethylphenol (*1d*), 2,4,6-trimethylphenol (*1e*), 4-chloro-4-methylcyclohexa-2,5-dienone (*2a*), 2-chloro-*p*-cresol (*3a*), 2,6-dichloro-*p*-cresol (*6a*), 6-chloro-2,4-dimethylphenol (*3b*), 2-chloro-3,4-dimethylphenol (*3c*), 2,6-dichloro-3,4-dimethylphenol (*6c*), and 6-chloro-2,4,5-trimethylphenol (*3d*) with molecular chlorine in DCM.[a]

Substrate	Products and yields (%)	Ratio dienones – chlorophenols
1b	*2b* (11); *5b* (16); *3b* (73)	0.27
1c	*2c* (47); *7c* (28); *3c* (5); *5c* (20)	3.0
1d	*2d* (67); *5d* (11); *3d* (22)	3.5
1e	*2e* (65); *4* (35)	---
2a	*8* (quant.)	---
3a	*5a* (45); *6a* (55)	0.8
6a	*7* (quant.)	---
3b	*5b* (quant.)	---
3c	*6c* (quant.)	---
6c	*7c* (quant.)	---
3d	*5d* (quant.)	---

[a] All experiments were carried out at 20 °C with addition of one mol of Cl_2 per mol of substrate. The conversions were 100% and the reaction time 20 min in all cases. The yields were determined from the NMR spectrum of the crude product (for details see Experimental).

responding 4-chlorodienones. With *1e* a mixture of the chlorodienone *2e* and 3-chloro-2,4,6-trimethylphenol (*4*) was obtained. The latter compound is probably formed by acid catalysed dienone-phenol rearrangement of *2e*. Protonation of *2e* gives a carbonium ion similar to *19c* (a hydroxy group instead of the methoxy group) in which chlorine migrates from C-4 to C-3. This rearrangement was not observed for any other chlorodienone in the chlorination experiments described in Table 2. However, the chlorodienones *2a–d*, *5a–b*, and *5c* all underwent the dienone-phenol rearrangement to the corresponding 3-chlorinated phenols by treatment with TFMS in DCM.[4]

In our previous communication [1] we showed that the chlorodienone *2c* can readily be transformed to the corresponding 4-hydroxy-, 4-methoxy-, or 4-acetoxy-4-methylcyclohexa-2,5-dienones by silver catalysed solvolysis in presence of water, methanol, or acetic acid – sodium acetate. In our continued investigation we discovered that *2a* on standing in pure methanol rearranges to 2,4-dimethoxytoluene (*9a*). In other words chlorination of *p*-cresol with molecular chlorine in DCM followed by metha-

Table 3. Reaction of the 4-chloro-4-methylcyclohexa-2,5-dienone derivatives, *2a-e*, *5a-b*, *5d*, and *7c* with methanol.[a]

Dienone	Reaction time (h)	Products and yields[b] (%)
2a	8	2,4-dimethoxytoluene (*9a*, quant.)
2b	24	1,3-dimethoxy-4,6-dimethylbenzene (*9b*, quant.)
2c	8	1,3-dimethoxy-5,6-dimethylbenzene (*9c*, quant.)
2d	24	5-methoxy-2,3-dimethylbenzyl methyl ether (*11*, quant.)
2e	168	4-methoxy-2,4,6-trimethyl cyclohexa-2,5-dienone (*12e*, 42); 3-chloro-2,4,6-trimethyl phenol (*4*, 58)
5a	24	5-chloro-2,4-dimethoxytoluene (*10a*, quant.)
5b	48	6-chloro-4-methoxy-2,4-dimethylcyclohexa-2,5-dienone (*13b*, quant.)
5b	168	6-chloro-4-methoxy-2,4,5-trimethylcyclohexa-2,5-dienone (*13d*, quant.)
7a	168	2,6-dichloro-4-methoxy-4-methylcyclohexa-2,5-dienone (*14a*, quant.)
7c	168	2,6-dichloro-4-methoxy-3,4-dimethylcyclohexa-2,5-dienone (*14c*, quant.)

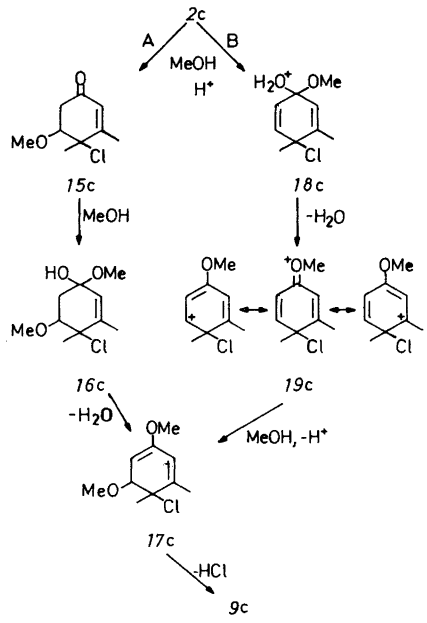
[a] The dienone was simply dissolved in pure methanol (large excess) and left at room temperature for the length of time indicated. [b] The yields were determined from the NMR spectrum of the crude product.

nolysis affords a facile synthetic method for the introduction of an oxygen in the 3-position of *p*-cresol, a transformation which otherwise requires several steps and cumbersome isomer separations. Previously[5] 2,4-dimethoxytoluene has been prepared in very low yield (3.6 %) by treatment of 4-hydroxy-4-methylcyclohexa-2,5-dienone with methanol containing approximately 2 % by weight of concentrated sulfuric acid. The major products were 2-methoxy-5-hydroxytoluene (54.7 %) and 2,4,4'-trimethoxy-5,2'-dimethylbiphenyl (24.3 %). The mechanism for the formation of these products will be discussed later.

In order to test the generality of our methoxylation reaction, all of the 4-chloro-4-methylcyclohexa-2,5-dienones prepared in this study (Tables 1 and 2) were subjected to methanolysis. The results are shown in Table 3. 1,3-Dimethoxy-4,6-dimethylbenzene (*9b*) has also been obtained in almost quantitative yield from 4-hydroxy-2,4-dimethylcyclohexa-2,5-dienone (which most conveniently is prepared by electrochemical oxidation of 2,4-dimethylphenol[7]) by treatment with methanol containing either concentrated sulfuric acid (4 %) or hydrogen chloride (2 %).[6] In this study[6] it was also shown that no *9b* was formed if the methanolysis was carried out in a 1:2 (vol/vol) mixture of concentrated sulfuric acid and methanol. In the latter case only 4-methoxy-3,6-dimethylphenol and dimers were obtained. From Table 3 it can be seen that 2,6-substituted chlorodienones do not give any dimethoxyaromatics on methanolysis. The only reaction observed is substitution of the 4-chlorine with a methoxy group (*5b*, *7a* and *7c–d*) or rearrangement to the 3-chlorophenol derivative (*2e*). Furthermore we can see that a chlorine or methyl substituent deactivates the adjacent 3- or 5-position and thus prevents methoxylation in this position. Finally the results of the methanolysis of *2a–b*, *2d*, *5a*, and *5c–d* show that migration of chlorine is only prevented by an adjacent chlorine substituent not by a methyl substituent.

The methanolysis of *2d* gives only side chain methoxylation and no ring methoxylation. This amounts to a selective oxidation of the least reactive of the three methyl groups in *2d*. The benzylic methoxy group in *11* can easily be oxidised further to an aldehyde or carboxylic acid function. It should be noted that only ring methoxylation (in the 3-position) occurs by methanolysis of the chlorodienone *2c*.

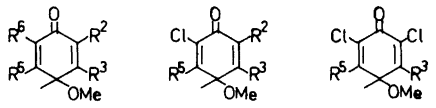
Two slightly different mechanisms can be envisioned for the formation of the dimethoxyaromatics (*9* and *11*) by methanolysis of the 4-chloro-4-methylcyclohexa-2,5-dineones *2a–d* and *4a*. In the first (mechanism A in Scheme 2), the initial reaction is either 1,2-addition of methanol to one of the double bonds of the cyclohexadienone ring or 1,4-addition of methanol to the α,β-unsaturated carbonyl system of the cyclohexadienone followed by enol-keton tautomerisation. In both cases the product formed is *15c*. In Scheme 2 compound *2c* is used as an example. *2a* and *2b* react similarly. The intermediate *15c* then adds methanol to the carbonyl double bond with formation of the hemiketal *16c*, which eliminates water (to *17c*) and hydrogen chloride to finally give the 1,3-dimethoxybenzene derivative *9c*. It should be noted that the order of the two first steps in mechanism A could be reversed.



Scheme 2.

In the other mechanism (B in Scheme 2), formation of the protonated hemiketal *18c* is assumed to be the first step. By elimination of water from *18c*, the carbonium ion *19c* is formed. From the three resonance structures shown in Scheme 2, it can be seen that *19c* should react with methanol in either the 3- or the 5-position of the ring. From our experimental data we know that methoxylation of *2c* only occurs in the 5-position although the 3-methyl substituent should stabilise a positive charge at

C-3. This can be explained by a rapid irreversible elimination of hydrogen chloride from the intermediate *17c*. An intermediate formed by nucleophilic attack of methanol on the 3-position of *19c* cannot directly eliminate hydrogen chloride. The methanolysis of *2d* (see Scheme 3) indicates that such an intermediate undergoes an intramolecular rearrangement/elimination reaction leading eventually to a side chain methoxylated aromatic compound. This recation is expected to be slower than the direct elimination/aromatisation reaction observed for *2c*. The present data does not allow



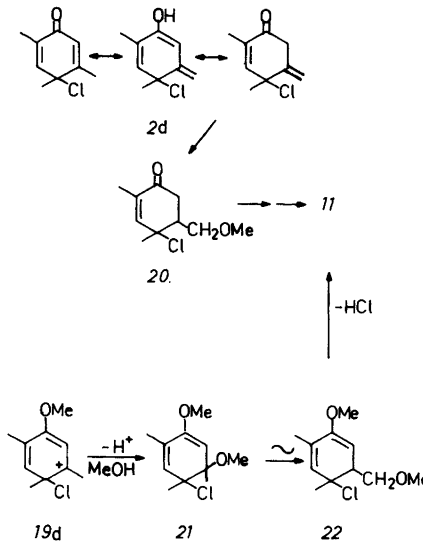
12 a-e *13*a-d *14* a,c

any conclusions concerning the relative importance of mechanisms A and B.

The chlorodienone *2d* reacts with methanol to give the side-chain methoxylated product *11*. Also in this case we believe that a mechanism similar to A or B in Scheme 2 is in operation. In this case mechanism A, however, involves addition of methanol to the tautomeric form of *2d* with an exocyclic double bond (see Scheme 3). The carbonium ion path (B) is also shown in Scheme 3. The initial formation of the hemiketal and the subsequent elimination of methanol to form the carbonium ion *19d* are not shown. The rearrangement/elimination reaction leading from *21* to *11* is shown as a stepwise sequence in Scheme 3. However, a concerted mechanism is probably in operation.

The substituent effects described above on the methanolysis reaction of the 4-chloro-4-methylcyclohexa-2,5-dienones are very well explained by both mechanism A or B in Scheme 2 or 3, since both the addition of methanol to a double bond (or α,β-unsaturated corbonyl system) of the cyclohexa-2,5-dienone or the exocyclic double bond of the tautomeric form of *2d* (mechanism A) and the reactivity of the intermediate carbonium ion (*19c* or *19d*) towards methanol should be strongly affected by steric hindrance from the substituents of the cyclohexadienone ring and by changes in electron density caused by electron withdrawing substituents (chlorine).

The 2,6-substituted 4-chloro-4-methylcyclohexa-2,5-dienones (*2e*, *5b*, *5d*, *7a* and *7c*) yield 4-methoxy-4-methylcyclohexa-2,5-dienones (*12e*, *13b*, *13d*, *14a*



Scheme 3.

and *14c*) on treatment with methanol (Table 3). One might therefore suspect that 4-methoxydienones could be intermediates in the methanolysis of the 4-chlorodienones *2a–d* and *4a*. However, the 4-methoxydienones *12a–d* and *13a* obtained by silver ion assisted methanolysis[2] of the corresponding 4-chlorodienones were all stable in pure methanol. Treatment of these 4-methoxydienones with methanol containing concentrated sulfuric acid (2 %) resulted in the formation of both 1,3-dimethoxy- and 1,4-dimethoxyaromatics and dimeric products as would have been expected from the previous studies of the corresponding 4-hydroxydienones.[5,6] The 1,3-dimethoxyaromatics obtained by acid catalysed methanolysis of the 4-methoxydienones are probably formed by a mechanism similar to A or B (Scheme 2 or 3). However, the formation of the 1,4-dimethoxyaromatics (and the formation of the hydroquinone monomethyl ethers from 4-hydroxydienones[5,6]) must involve migration of the 4-methyl group in a carbonium such as *19* (with a methoxy or a hydroxy group instead of the 4-chloro substituent). This indicates that in presence of strong acid (>2 %) mechanism B is most important. Mechanism B also explains very well that strong acid in large excess suppresses the formation of the 1,3-dimethoxyaromatics from both the 4-methoxy- and the 4-hydroxydienones[5,6] as the formation of *17c* from *19c* (Scheme 2) involves a de-

Table 4. Yields of dimethoxy aromatic compounds obtained from the *p*-cresol derivatives *1a–e* and *3a* by chlorination with molecular chlorine in DCM followed by methanolysis (method A) and by direct chlorination with molecular chlorine in methanol (method B).[a]

Phenol	Products and yields (%) by method A[b]	Products and yields (%) by method B[b,c]
1a[d]	*9a* (33)	*9a* (16); *10a* (13)
1b	*9b* (16)	*9b* (21)
1c	*9c* (36)	*9c* (28)
1d	*11* (51)	*11* (53)
1e	None (see Table 2)	None[e]
3a	*10a* (43)	*10a* (40)

[a] Preparative scale experiments. In all cases 0.2 mol of chlorine dissolved in DCM was added to 0.2 mol of the phenol dissolved either in DCM (method A) or in methanol (method B). Chlorination of the chlorophenols *3c–d*, *6a*, or *6c* gave the corresponding chlorodienones (see Table 2) by method A and mixtures of chlorodienones and the corresponding methoxydienones by method B (see Table 3). [b] The yields refer to products isolated and purified. [c] The chlorination in methanol is less selective than that in DCM, and all possible chlorination products (see Tables 1 and 2) as well as the product of reaction of methanol with the various chlorodienones (see Table 3) are observed. [d] Titanium tetrachloride was added as catalyst (0.5 mol). [e] The products were *12e* (28%) and *4* (68%).

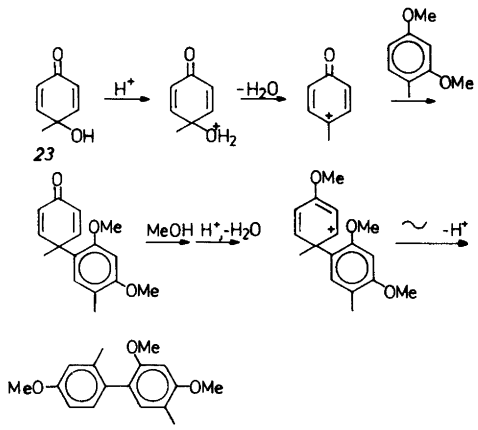
protonation step. At high acid concentration methyl group migration occurs with final formation of a hydroquinone derivative.

In conclusion, 4-methoxydienones can be excluded as intermediates in the methanolysis of the 4-chlorodienones described here (Table 3). Furthermore the much higher reactivity of the 4-chlorodienones compared to the 4-hydroxy- or 4-methoxydienones and the absence of methyl group migration in the methanolysis of 4-chlorodienones (at high acid concentrations chlorine migration actually occurs) is remarkable.

Preparative experiments. In a series of preparative scale experiments aiming at the synthesis of the dimethoxyaromatics *9a–c* and *11*, we discovered that isolation and purification of the 4-chlorodienones (*2a–d*) were not necessary. Directly after the chlorination a large excess of methanol was added to the reaction mixture and, when all of the 4-chlorodienone had reacted with methanol, the solvents were removed, the residue was dissolved in ether and extracted with 1 M sodium hydroxide, which removed all of the phenolic compounds from the product. Left in the ether solution was almost pure *9a–c* or *11* (see Table 4). We also discovered that the dimethoxy compounds *9a–c* and *11* are formed directly from the corresponding phenols *1a–d* when the chlorination is carried out in pure methanol at low temperature (Table 4). None of the 4-chlorodienones *2a–d* or *5a*, which give dimethoxyaromatics on methanolysis, were observed after the chlorination in methanol. However, all the other 4-chlorodienones (*5b*, *5d–e*, *7a* and *7c*), the chloro compound *4*, and to some extent also the 4-methoxydienones *12e*, *13b*, *13d*, *14a* and *14c* were observed, showing that the chlorination in methanol is less selective than that in DCM. Although the 4-chlorodienones *2a–d* could not be observed on chlorination in methanol, we still believe that they are intermediates. However, the hydrogen chloride formed in the chlorination reaction catalyzes the addition of methanol to the double bond of the cyclohexa-2,5-dienone and the further ketalisation (see Schemes 2 and 3).

The yields of dimethoxy aromatics from the various *p*-cresol derivatives by the method described in this study are quite low. However, the low yields are compensated for by the convenience of the method and the simplicity of the isolation procedure. Furthermore, our attempts to improve the yields by adding different Friedel-Crafts catalysts (Table 1) indicate that improvements in yield could be achieved in this way.

Another interesting development would be to treat the 4-chlorodienones with other nucleophiles than methanol either in the presence of acid or silver ions. In the latter case the 4-chlorodienone is converted to a phenoxonium ion which reacts with the nucleophile to give a new dienone with the nucleophile in the 4-position. The 2,4,4′-trimethoxy-5,2′-dimethylbiphenyl obtained by Bamberger[5] on methanolysis of *23* is probably formed in a sequence like that in Scheme 4. Therefore silver ion catalysed reaction of 4-chloro-4-methylcyclohexa-2,5-dienones



Scheme 4.

with a phenol ether in presence of acid should be a feasible synthesis of unsymmetrical biphenyls.

EXPERIMENTAL

The phenols *1a–e* were commercial products. The chlorophenols *3a–d*, *6c* and *6d* were prepared by chlorination of *1a–d*, *3a* and *3c* in carbon disulfide with one mol of chlorine and purified as described in the literature. The NMR spectra were recorded with a 60 MHz instrument in deuterio chloroform solutions with Me_4Si as internal standard.

Chlorination of the phenols 1a–d, 3a–d, 6a *and* 6c. *Analytical experiments. General.* The phenol (0.01 mol) was dissolved in DCM, DMF or CS_2, 10 ml, and when applicable the Friedel-Crafts catalyst was added and the resulting mixture cooled to the reaction temperature indicated in Tables 1 and 2. Chlorine dissolved in DCM (1 M solution) at 0 °C was then added rapidly with stirring. The amount of chlorine added is shown in Tables 1 and 2. The mode of addition of the molecular chlorine did not affect the product distribution: whether chlorine was added in gaseous form or in solution, slowly or rapidly or even if the addition was reversed, lead to no change in the products. After the chlorination the solvent was removed by distillation at reduced pressure (50 °C) and the residue was analysed by NMR. The different products in the reaction mixture were identified by comparison with the NMR of the pure compounds obtained as described below. The results of the analytical chlorination experiments are given in Tables 1 and 2.

Synthesis of the 4-chloro-4-methyl-cyclohexa-2,5-dienones 2a-e, 5a-b, 5d, 7a, *and* 7c. *General.* The chlorination was carried out at room temperature using one mol of chlorine as described above. When DCM was used as a solvent all phenolic compounds were extracted from the reaction mixture with a phosphate buffer (pH 12, 0.5 M, made from Na_3PO_4 and half an equivalent of HCl). Evaporation of the DCM yielded the crude 4-chlorodienone which was further purified by chromatography on silica gel (DCM eluent). The yields of pure 4-chlorodienones in general were about 5 % lower than the NMR yields reported in Tables 1 and 2. The physical and spectroscopic data of the 4-chlorodienones *2a–c*, *2e* and *5a*, have been reported previously (Refs. 1 and 3) and are in accordance with those obtained in this study.

2,3,4-Trichloro-4-methylcyclohexa-5-enone (8). Obtained by addition of 1 mol of chlorine to *2a* in DCM at room temperature. Liquid. M^+ 212 m/e. NMR δ 7.10 (H5, d, $J = 10$ Hz), 6.05 (H6, d, $J = 10$ Hz), 4.55 (H2,H3, m), 1.86 (3H, s, Me-4) ppm.

4,6-Dichloro-2,4-dimethylcyclohexa-2,5-dienone (5b). Obtained from chlorination of *3b* with one mol of chlorine in DCM as described above. Liquid. M^+ 190 *m/e*. NMR δ 7.17 (H5, d, J = 3 Hz), 6.82 (H3, m), 1.92 (Me-2,bs), and 1.82 (Me-4, s) ppm.

2,4,6-Trichloro-4-methylcyclohexa-2,5-dienone (7a). Obtained by chlorination of *6a* with one mol of chlorine in DCM as described above. M.p. 53–54 °C. M^+ 210 *m/e*. NMR δ 7.18 (2H, s) and 1.93 (3H, s) ppm.

4,6-Dichloro-2,4,5-trimethylcyclohexa-2,5-dienone (5d). Obtained by chlorination of *3d* as described above. M.p. 38–40 °C. M^+ 204 *m/e*. NMR δ 6.83 (H3,bs), 2.28 (Me-5-s), 1.96 (Me-2,bs), and 1.83 (Me-4,s) ppm.

2,4,6-Trichloro-3,4-dimethylcyclohexa-2,5-dienone (7c). Obtained by chlorination of *6c* as described above. M.p. 47–48 °C. M^+ 238 *m/e*. NMR δ 7.23 (H5,s), 2.40 (Me-3,s) and 1.87 (Me-4,s) ppm.

4-Chloro-2,4,5-trimethylcyclohexa-2,5-dienone (2d). Obtained by chlorination of *1d* as described above. Liquid. M^+ 170 *m/e*. NMR δ 6.80 (H3,bs), 6.10 (H6,bs), 2.13 (Me-5,s), 1.85 (Me-2,s), and 1.71 (Me-4,s) ppm.

3-Chloro-2,4,6-trimethylphenyl (4). 1e was chlorinated with one mol of chlorine in DCM as described above. Analysis of the reaction mixture 15 min after the chlorine addition showed that *1e* had been converted to a 13:7 mixture of *2e* and *4*. The reaction mixture was left at room temperature for 24 h. During this period all of *2e* rearranged to *4* which could be isolated in quantitative yield by evaporation of the solvent. White needles, m.p. 81–83 °C, Lit[8] 81–83 °C M^+ 170 *m/e*. NMR δ 6.80 (H5,s), 4.58 (OH,s), 2.26 (6H,s), and 2.13 (3H,s) ppm.

Methanolysis of the 4-chloro-4-methylcyclohexa-2,5-dienone derivatives. Analytical experiments. General. The pure 4-chlorodienone (obtained as

described above) was dissolved in a 100-fold molar excess of methanol and left standing at room temperature until all of the 4-chlorodienone had reacted (NMR). Then the methanol was removed by distillation at 50 °C and reduced pressure and the resulting product analysed by NMR. The different compounds in the product were identified by comparison with the NMR spectra of the pure compounds obtained as described below. The yields were determined from the integrated NMR spectra and are given together with reaction times in Table 3.

The products from methanolysis of the pure 4-chlorodienones were isolated from the reaction mixture either directly by recrystallisation from ether (crystalline compounds) or by chromatography on silica gel (DCM eluent). In this manner the following compounds were obtained:

2,4-Dimethoxytoluene (9). From methanolysis of *2a*. Oil. M^+ 152 *m/e* NMR δ 7.00 (H6,d, J=9 Hz), 6.45 (H3,H5,m), 3.80 (6H,s), and 2.15 (3H,s) ppm.

1,3-Dimethoxy-4,6-dimethylbenzene (9b). From methanolysis of *2b*. M.p. 75–76 °C (lit.[6] M.p. 76 °C). M^+ 166 *m/e*. NMR δ 6.87 (H5,s), 6.43 (H2,s), 3.76 (6H,s), and 2.10 (6H,s) ppm.

1,3-Dimethoxy-5,6-dimethylbenzene (9c). From methanolysis of *2c*. Oil; b.p. 117–118 °C/11 mmHg (lit.[9] 117–118 °C/11 mmHg). M^+ 152. NMR δ 6.32 (H2,H6,s), 3.77 (6H,s), 2.22(Me-5,s), and 2.06 (Me-4,s) ppm.

5-Methoxy-2,3-dimethylbenzyl methyl ether (11). From methanolysis of *2d*. Oil; M^+ 166 *m/e*. NMR δ 6.87 (1H,s), 6.83 (1H,s), 4.37 (Ar-CH_2-O-, s), 3.77 (Ar-CMe,s), 3.36 (-CH_2-O*Me*,s) and 2.18 (6H,s) ppm.

4-Methoxy-2,4,6-trimethylcyclohexa-2,5-dienone (22e). From methanolysis of *2e*. M.p. 111.5–112 °C (lit[7] 111.5–112 °C). Spectroscopic data has been published previously.[7]

5-Chloro-2,4-dimethoxytoluene (10a). From methanolysis of *5a*. M.p. 74–75 °C. M^+ 186 *m/e*. NMR δ 7.07 (H6,s), 6.47 (H3,s), 3.83 (3H,s), 3.76 (3H,s), and 212 (3H,s) ppm.

6-Chloro-4-methoxy-2,4-dimethylcyclohexa-2,5-dienon (13b). From methanolysis of *5b*. M.p. 138–140 °C. M^+ 136 *m/e*. NMR δ 6.98 (H5,d, J= 3 Hz), 6.67 (H3,m), 3.20 (O*Me*,s), 1.97 (Me-2,bs), and 1.43 (Me-4,s) ppm.

6-Chloro-4-methoxy-2,4,5-trimethylcyclohexa-2,5-dienone (13d). From methanolysis of *5d*. M.p. 118–120 °C. M^+ 200 *m/e*. NMR δ 6.63 (H3,bs), 3.46 (OMe,s), 2.10 (Åe-5,s), 1.96 (Me-2, bs), and 1.40 (Me-4,s) ppm.

2,6-Dichloro-4-methoxy-4-methylcyclohexa-2,5-dienone (14a). From methanolysis of *7a*. M.p. 161–162 °C. M^+ 206 *m/e*. NMR δ 7.03 (H3,H5,s), 3.27 (OMe,s), and 1.50 (Me-4,s) ppm.

2,6-Dichloro-4-methoxy-3,4-dimethylcyclohexa-2,5-dienone (14c). From methanolysis of *7c*. M.p. 148–150 °C. M^+ 220 *m/e*. NMR δ 7.10 (H5,s), 3.13 (OMe,s), 2.17 (Me-3,s), and 1.50 (Me-4,s) ppm.

Preparative experiments. Large scale synthesis of the dimethoxy aromatics 9a–b, 10a, *and* 11. *Method* A (see Table 4). The starting phenol (*1a–e* or *3a*, 0.2 mol) was dissolved at room temperature in DCM, 100 ml, and in the case of *1a* $TiCl_4$, 12 g, 0.5 mol, dissolved in DCM, 100 ml, was added before the chlorine, 0.2 mol, 14 g, dissolved in DCM, 200 ml. The chlorine solution was kept at 0 °C and added in one batch with rapid stirring. Stirring was continued until all chlorine had been consumed. This required only a few minutes. Then methanol, 100 ml, was added and the solution was left at room temperature for 24 h after which the solvents were evaporated by distillation at reduced pressure and 50 °C. The residue was dissolved in ether, 200 ml, and extracted with 1 M sodium hydroxide, 4 × 150 ml, and water, 3 × 100 ml. The residue obtained by evaporation of the ether solution was almost pure dimethoxy compound and was further purified by chromatography on silica gel (DCM eluent) or by recrystallisation from ether (see above).

Method B (see Table 4). The starting phenol was dissolved in methanol, 100 ml, and this solution was cooled to −60 °C in a dry ice bath before the chlorination was carried out in the same way as described above. The reaction mixture was allowed to warm up to room temperature before the solvent was evaporated. The resulting residue was worked up as described under method A.

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Antibodies to Ornithine Decarboxylase. Immunochemical Cross-reactivity

LO PERSSON

Department of Physiology and Biophysics, University of Lund, Sölvegatan 19, S-223 62 Lund, Sweden

L-Ornithine decarboxylase was purified to apparent homogeneity from the kidneys of testosterone-treated mice. Antibodies to ornithine decarboxylase were raised in a rabbit using the purified enzyme. Ouchterlony double diffusion technique revealed a single precipitin line between the antiserum and purified mouse kidney ornithine decarboxylase. The antibodies inhibited ornithine decarboxylase from various tissues of mice and rats to the same extent, indicating a close immunological relationship. S-Adenosyl-L-methionine decarboxylase and L-histidine decarboxylase from mouse kidney as well as ornithine decarboxylase from *Escherichia coli* were unaffected by the antibodies.

L-Ornithine decarboxylase (ODC) (EC 4.1.1.17) catalyzes the first and rate-limiting step in the biosynthesis of polyamines. This enzyme has been intensely studied since an increase in ornithine decarboxylase activity has been observed in response to growth stimuli in various tissues.[1,2]

Ornithine decarboxylase of rat liver has been purified to what was considered to be homogeneity.[3,4] However, using radioactive α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, the specific activity of pure rat liver ornithine decarboxylase was calculated to be about 100-fold higher than the value reported for purified rat liver ornithine decarboxylase.[5] Recently, ornithine decarboxylase was purified to apparent homogeneity from kidneys of testosterone-treated mice.[6] The purified enzyme exhibited a specific activity in agreement with the theoretical value for the pure enzyme.[7] Similar results were obtained for rat liver ornithine decarboxylase by Kameji *et al.*,[8] who succeeded in purifying the enzyme 350 000 times to the same high specific activity.

Antibodies to rat liver ornithine decarboxylase have been prepared in several laboratories.[9–12] Since the enzyme preparations used for immunization must have contained considerable amounts of protein impurities, the antisera to ornithine decarboxylase so far reported should be considered nonspecific. Using purified ornithine decarboxylase from kidneys of testosterone-treated mice, the present work describes the generation of what apparently are monospecific antibodies to ornithine decarboxylase.

METHODS

Ornithine decarboxylase was purified from kidneys of testosterone-treated NMRI mice as described previously.[6] The purification resulted in an enzyme with a specific activity of 1.4 mmol/mg h.[7] To stabilize the enzyme 0.3 % Tween 80 was added to the purified ornithine decarboxylase.[13] The enzyme preparation was concentrated, using an Amicon YM 10 membrane, and resuspended in a small volume of 0.1 M phosphate buffer (pH 7.2) containing 0.1 mM EDTA, 2.5 mM dithiothreitol and 0.3 % Tween 80. The purified enzyme (0.15 mg) was emulsified in Freund's complete adjuvant and given to a rabbit by the multiple site, intradermal technique as described by Vaitukaitis *et al.*[14] A booster injection (0.1 mg) in complete adjuvant was given after 3 months. Antiserum to ornithine decarboxylase (code No. 8111) was collected every two weeks and stored at −20 °C. The specificity of the antiserum was tested by the Ouchterlony double diffusion technique.[15]

To examine the cross-reactivity of the antiserum, ornithine decarboxylase obtained from kidney of

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testosterone-treated mice (200 μg daily for 7 days), liver of carbon tetrachloride-treated mice (1.5 ml/kg; 14 h before death), kidney of carbon tetrachloride-treated rats (1.5 ml/kg; 14 h before death), liver of thioacetamide-treated rats (150 mg/kg; 18 h before death), ovary of female rats treated with human chorionic gonadotrophin (400 IU/kg; 5 h before death) and ventral prostate of male rats were used. The mice and rats were of the NMRI and Sprague-Dawley strains, respectively.

Immunoprecipitation of ornithine decarboxylase was carried out in 0.5 ml of 0.1 M Tris-HCl (pH 7.5), 0.1 mM EDTA and 2.5 mM dithiothreitol (buffer A) containing 0.25% of bovine serum albumin at 4 °C overnight. To precipitate the antigen-antibody complexes 0.25 ml of buffer A containing 0.25% of bovine serum albumin and 10 μl of goat anti-rabbit IgG was added and incubated overnight at 4 °C. The solution was then centrifuged at 20000 × g at 4 °C for 20 min. Remaining ornithine decarboxylase activity was measured in aliquots of the supernatant.

Ornithine decarboxylase activity was measured by determining the release of $^{14}CO_2$ from carboxyl-labelled ^{14}C-ornithine in a mixture containing 0.1 mM pyridoxal 5'-phosphate and 0.5 mM L-1-^{14}C-ornithine (0.4 or 4.0 MBq/mmol) in buffer A as described earlier.[6,7] One unit of ornithine decarboxylase was defined as the amount of enzyme giving rise to 1 nmol CO_2 per h.

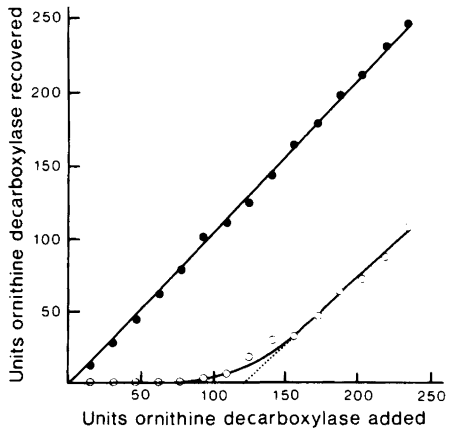

Fig. 1. Immunotitration of mouse kidney ornithine decarboxylase. Various amounts of ornithine decarboxylase from kidneys of testosterone-treated mice were incubated with 0.2 μl of control (●) or anti-ornithine decarboxylase (○) serum as described in Methods.

RESULTS

The antibodies raised against purified mouse kidney ornithine decarboxylase were shown to inhibit completely the enzyme in extracts from kidneys of testosterone-treated mice (Fig. 1). One μl of the antiserum was estimated to inhibit nearly 600 units of ornithine decarboxylase. The addition of normal rabbit serum to the enzyme extracts did not affect the enzyme activity. Utilizing the Ouchterlony double diffusion technique it was shown that the antibodies gave a sharp single precipitin line against purified mouse kidney ornithine decarboxylase (Fig. 2).

The immunochemical cross-reactivity of the antibodies with ornithine decarboxylase from various tissues of mice and rats was also studied. Ornithine decarboxylase from kidney and liver of mice and from kidney, liver, ovary and ventral prostate of rats was diluted to about the same activity and then incubated with different amounts of antiserum against the enzyme. After precipitating the antigen-antibody complexes with goat anti-rabbit IgG the solution was centrifuged and the residual ornithine decarboxylase activity determined. As seen in Fig. 3 the antibodies against purified mouse kidney ornithine decarboxylase inhibited ornithine decarboxylase from liver of mice and from kidney, liver, ovary and ventral prostate of rats.

To compare the capability of the antibodies to inhibit ornithine decarboxylase from these tissues the amounts of antiserum required to inactivate 50% of 1 unit of enzyme (Ab^{50}) were calculated from Fig. 3. As shown in Table 1 the antiserum was found to inhibit ornithine decarboxylase from each of the tissues examined to the same extent, indicating a

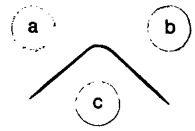

Fig. 2. Ouchterlony double diffusion precipitin analysis of ornithine decarboxylase antiserum and purified mouse kidney ornithine decarboxylase. The immunoplate (3% agar) was incubated for 48 h in a moist chamber at room temperature and then washed with saline and stained with 0.1% Coomassie brilliant blue. Wells a and b contained 4 μg of purified enzyme. Well c contained 5 μl of ornithine decarboxylase antiserum.

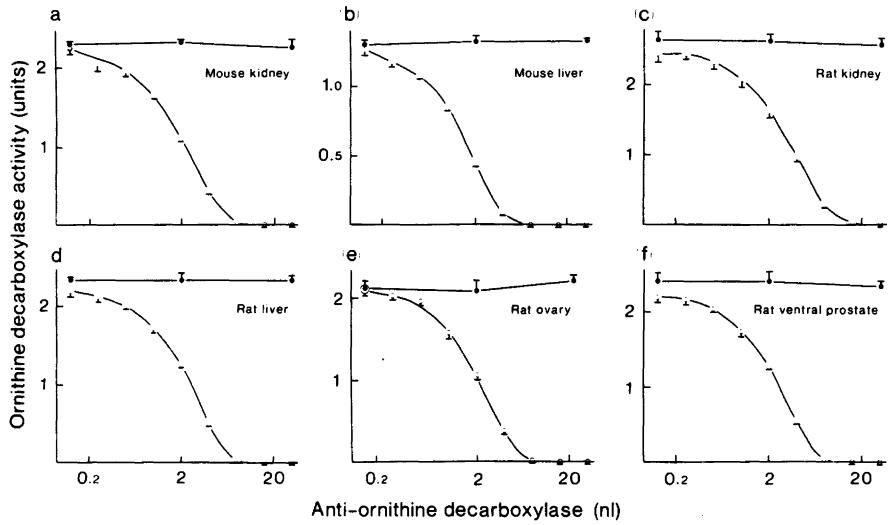

Fig. 3. Immunoprecipitation of ornithine decarboxylase from various tissues with mouse kidney ornithine decarboxylase antiserum. Increasing amounts of control (●) or anti-ornithine decarboxylase (○) serum were added to a fixed quantity of enzyme activity. After incubation, as described in Methods, remaining ornithine decarboxylase activity was determined in aliquots of the supernatant. Ornithine decarboxylase from mouse kidney (a), mouse liver (b), rat kidney (c), rat liver (d), rat ovary (e) and rat ventral prostate (f). Each point represents the mean ±S.E. of the mean, $n=5$.

close, if not identical, immunological relationship. Because of the low amounts of ornithine decarboxylase in these tissues the immunological relationship could not be determined by the Ouchterlony double diffusion technique. It should be mentioned that the antibodies did not inhibit ornithine decarboxylase from *Escherichia coli*, nor did they affect *S*-adenosylmethionine decarboxylase or histidine decarboxylase from mouse kidney (results not shown).

Table 1. Immunochemical cross-reactivity of antiserum to mouse kidney ornithine decarboxylase.

Origin of ornithine decarboxylase	Ab^{50} [a] (nl/unit)
Mouse kidney	0.85
Mouse liver	1.12
Rat kidney	1.13
Rat liver	0.96
Rat ovary	0.98
Rat ventral prostate	0.93

[a] Ab^{50} is defined as the amount of antiserum needed for 50% inactivation of 1 unit of ornithine decarboxylase. Ab^{50} is calculated from data of Fig. 3.

DISCUSSION

The antiserum against ornithine decarboxylase was shown to have a much higher titre than those reported previously.[10,12] It would appear that this is due to the fact that the preparations used for immunization until now have been impure. Using the calculated value of the specific activity of a pure ornithine decarboxylase[5] as confirmed for the purified enzyme,[7,8] it can be concluded that previous preparations used for immunization must have contained less than 2% ornithine decarboxylase. The kidneys of testosterone-treated mice seem to be most useful for purification of ornithine decarboxylase since they contain 100-fold more ornithine decarboxylase activity than reported for the rat liver.[16-18] In fact, it even seems to be difficult to obtain adequate amounts of the pure enzyme for immunization from stimulated rat livers.[8] Until monoclonal antibodies to ornithine decarboxylase are available, the generation of ornithine decarboxylase antisera could easily be achieved using purified mouse kidney enzyme.

The finding that ornithine decarboxylase from mouse kidney and rat liver were inactivated to the same degree by the antibodies indicated that the molecules of ornithine decarboxylase in these

tissues are equally effective in decarboxylating ornithine. During the progress of the present work, this suggestion was confirmed by the reports of Pritchard *et al.*[5] and Kameji *et al.*,[8] who demonstrated that the specific activity of pure rat liver ornithine decarboxylase was in accordance with the specific activity of purified ornithine decarboxylase from mouse kidney. This seems to apply also to ornithine decarboxylase from mouse liver, rat kidney, rat ovary and rat ventral prostate since the enzymes from these tissues were equally inhibited by the ornithine decarboxylase antiserum.

The available antibodies to ornithine decarboxylase have successfully been employed in an immunofluorescent technique for the histochemical localization of ornithine decarboxylase in the kidneys of testosterone-treated mice.[19] The demonstrated capability of the antibodies to cross-react with ornithine decarboxylase from other tissues indicates that these antibodies may be of general use for the immunohistochemical localization of ornithine decarboxylase.

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A Sensitive and Rapid Method for Determination of Pyrophosphatase Activity

YURI A. SHAKHOV* and PÅL NYRÉN

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

A new, rapid and sensitive method of measuring the rate and amount of PP_i hydrolysis is described. The method is based on registration of small pH changes, caused by decrease of hydrogen-ion concentration during the pyrophosphatase reaction. With this detected value of hydrogen-ion concentration change and the buffering capacity of each reaction mixture determined by titration, the rate and amount of PP_i hydrolysis can be calculated. The results obtained by pH method are in good agreement with values determined by direct phosphate analysis. The described method can be applied to the continuous determination of pyrophosphatase activity in chromatophores, mitochondria, crude extracts or purified enzymes.

ABBREVIATIONS

P_i, inorganic orthophosphate; PP_i, inorganic pyrophosphate; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; MES, 2-(*N*-morpholino)-ethanesulfonic acid; MOPS, morpholinopropane sulfonic acid; PIPES, piperazine-*N,N'*-bis[2-ethanesulfonic acid]; TCA, trichloroacetic acid; Bchl, bacteriochlorophyl.

It is well known that many tissues and cells contain inorganic pyrophosphatases (E.C. 3.6.1.1.). Two forms of inorganic pyrophosphatase, which are customarily called soluble and membrane-bound have been intensively investigated during the last decade. The recent interest in membrane-bound pyrophosphatases is due to the fact that this enzyme has PP_i-synthesizing activity. It has been shown that synthesis of PP_i coupled to the electron-transport chain is carried out in the chromatophores of *Rhodospirillum rubrum* [1] and mitochondria of animal tissues and yeast.[2,3] Solubilization and characterization of the membrane-bound pyrophosphatases, as well as the purification and characterization of the soluble ones, are being carried out in few laboratories.[4–7] However, investigations of pyrophosphatases are inhibited by the absence of the simple and efficient method for measuring the pyrophosphatase activity. All today's methods of pyrophosphatase assay are based on determination of P_i as a product of the enzyme reaction. Some of the P_i-determination methods include an extraction procedure of the phosphomolybdate complex, which takes time and is rather complicated.[8,9] The other known methods have limitations with regard to the purity of the pyrophosphatase preparation that is used, or require delicate tools or enzymes.[10–14]

Saris and Nishamura *et al.*[15,16] were the first who applied the pH recording method to the study of phosphorylation and ATPase reaction in bacterial chromatophores and animal mitochondria. According to reaction (1), a certain amount of H^+ is

$$ADP + P_i + nH^+ \rightleftarrows ATP + H_2O \qquad (1)$$

released or bound in the ATPase reaction or in phosphorylation, respectively. The *n*-value in eqn. (1) is dependent on pH and ionic strength.

We have developed a rapid method for determination of the rate and amount of PP_i hydrolysis by chromatophore pyrophosphatase, with the use of a sensitive recording pH-meter. This method has been applied to the continuous

*Present address: A. N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117 234, U.S.S.R.

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measuring of the pyrophosphatase activity in chromatophores, mitochondria, crude extracts or partly purified enzymes.

EXPERIMENTAL

General. The principle of this method is to measure small pH changes by the decrease of hydrogen-ion concentration when the reaction (2) takes place in the physiological pH range; the value of $n = \Delta H^+/\Delta PP_i$ is calculated theoretically and the buffering capacity (added $H^+/\Delta pH$) is determined experimentally by titration.

$$PP_i + nH^+ \rightleftarrows 2\ P_i \qquad (2)$$

From the Debye-Hückel theory,[17] it is expected that the values of pK_a decrease as the ionic strength increases in dilute solution. It has been shown that there is a considerable decrease of pK_a for ATP- and ADP-magnesium complexes, compared to pK_a for ATP and ADP, respectively. The values of pK_a which have been found in the literature, are shown in Table 1. As is seen in Table 1, differences between pK_{aATP} and $pK_{aATP \cdot Mg}$, as well as between pK_{aADP} and $pK_{aADP \cdot Mg}$, reach about 2 pH units. Proceeding from similar structure and charge distribution in molecules of ATP, ADP and PP_i[18,19] we propose the decrease of pK_a for $PP_i \cdot Mg$ complex up to values about 7.5 and 5.1 for the first and second proton dissociation. We do not consider the difference between the pK_{aPi} and $pK_{aPi \cdot Mg}$ because, according to Ref. 21, phosphate should remain in uncomplexed form. Then in a certain pH range (up to pH 8.0), the reactions (3) should be predominant for hydrolysis of the $PP_i \cdot Mg$ complex which is the true substrate for the enzyme.[22]

$$\begin{pmatrix} MgP_2O_7^{2-} \\ \Updownarrow \\ MgHP_2O_7^{-} \end{pmatrix} + H_2O + nH^+ \rightleftarrows \begin{pmatrix} HPO_4^{2-} \\ \Updownarrow \\ H_2PO_4^{-} \end{pmatrix} + Mg^{2+} \qquad (3)$$

At pH > 8.0 hydrolysis of PP_i does not need protons *e.g.* the reaction appears as neutral; reaction (4).

$$MgP_2O_7^{2-} + H_2O \rightleftarrows 2HPO_4^{2-} + Mg^{2+} \qquad (4)$$

The buffering capacity of the reaction mixture was determined experimentally: a known amount of hydrochloric acid (usually 25 μl of 10 mN HCl = 250 ng ions H^+) was added to the reaction mixture after each measurement and the pH change induced by this addition was recorded.

The amount of protons (n) in eqn. (3) can be calculated as (number of hydrogen ions disappeared)/(number of PP_i ions hydrolyzed) by assuming the relationship (5) for weak acid HA.

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \qquad (5)$$

Chromatophore isolation. For pyrophosphatase activity measurements chromatophores isolated from the non-sulfur purple bacterium *R. rubrum* strain Sl were used. The bacteria was grown anaerobically in light at 30 °C in the medium described by Bose *et al.*[23] After 40 h of growth (the end of the logarithmic phase) cells were harvested, washed and chromatophores were prepared by mechanical disruption in a Ribi cell fractionator in 0.2 M glycylglycine, pH 7.4 at 138 MPa. Cell debris was removed by centrifugation at 10 000*g* for 60 min and the supernatant was further centrifuged at 100 000*g* for 90 min. The pellet was washed twice by 0.3 M NaCl in 0.2 M glycylglycine pH 7.4 and by 0.2 M glycylglycine pH 7.4, respectively, with following centrifugations at 100 000*g* for 60 min. The preparation was stored at 0 °C in 0.2 M glycylglysine pH 7.4.

Pyrophosphatase activity measurement. The pyrophosphatase assay was carried out in a medium with weak buffering action, containing 1 mM PIPES buffer, 1 mM $Na_4P_2O_7$ and 1 mM $MgSO_4$ at 30 °C. Total volume 3 ml, chromatophores 2–40 μl (0.8 mg Bchl/ml, 35 mg protein/ml) were added and pH adjusted to 6.80.

Table 1. Values for pK_a for ATP, ADP, PP_i and P_i.

Acid	pK_a	Ref.	Acid	pK_a	Ref.
ATP^{-3}	7.68	18, 19	$ATPMg^{-1}$	5.44	18, 19
ADP^{-2}	7.2	18, 19	ADPMg	5.38	18, 19
P_i^{-1}	7.21	20	P_i^{-2}	12.38	20
PP_i^{-3}	8.95	20	PP_iMg^{-1}	7.5	Assumed by us
PP_i^{-2}	6.1	22	PP_iMg	5.1	Assumed by us

An open cylindrical thermostatic cuvette, a glass electrode GK 2322C and pH meter model 26 both from Radiometer and recorder model 2210 from LKB were used. The sensitivity of measurements could be changed, but a full-scale reading on the recorder shart corresponding to 0.1 – 0.15 pH units was the most frequent. Each measurement was usually done in a pH range of 6.80 ± 0.05 and there was no detectable change of the linear, rate of the reaction caused by pH change. If, during the reaction, pH changes surpassed 0.1 pH units some changes in the velocity of the PP_i hydrolysis could be detected.

For comparison, the pyrophosphatase activity was determined by direct analysis of phosphate by using the method described in Ref. 10. In this case the assay medium contained 50 mM PIPES buffer pH 6.80, 1 mM $Na_4P_2O_7$, 1 mM $MgSO_4$ and 2 – 4 μl chromatophores. Total volume 2 ml. The reaction was carried out for 10 min at 30 °C and arrested by addition of 1 ml of 10% cold TCA.

RESULTS AND DISCUSSION

A typical example of pH change during PP_i hydrolysis is shown in Fig. 1. The vertical basic line shows the absence of pH changes in the assay medium, caused by ingradient interactions of atmospheric carbonation. The fast shift of the pH is due to addition of chromatophores, suspended in 0.2 M glycylglycine buffer pH 7.4. This method has been applied to verify the effects of uncoupler and inhibitor on pyrophosphatase activity in chromatophores. As is seen in Fig. 1 the pyrophosphatase is stimulated by 1 μM FCCP from Boehringer Mannheim GmbH) and inhibited by 0.1 mM DCCD (from Fluka AG).

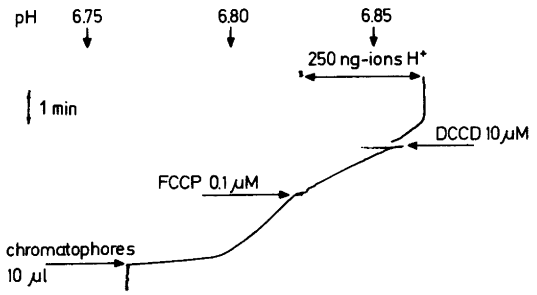

Fig. 1. Recordings of the pH change during the pyrophosphatase reaction in chromatophores of *R. rubrum* and the effects of FCCP and DCCD. Chromatophores corresponding to 0.35 mg of protein were added to 3 ml of assay medium.

It follows from eqn. (3) and values of pH presented in Table 1, that the low pH should be optimal for measuring the Δ pH which appears during the pyrophosphatase reaction. As is shown in Fig. 2, pH optimum for this method was about pH 6.0 – 6.3, when various buffers were used. In contrast to the P_i-determination method of pyrophosphatase activity (see Fig. 3), the buffers, obviously, have a strong effect on pyrophosphatase assay using pH method. The highest sensitivity occurred with MOPS, but in this case there was some change of rate of the reaction caused by pH-change. Therefore we used PIPES, in the presence of which there practically is no change in the reaction velocity in the pH range 6.0 – 7.0 as seen in Fig. 2.

To understand the cause of the dramatic decrease of pyrophosphatase activity at a pH lower than 6.0 we studied the pH optimum of its activity by the P_i-

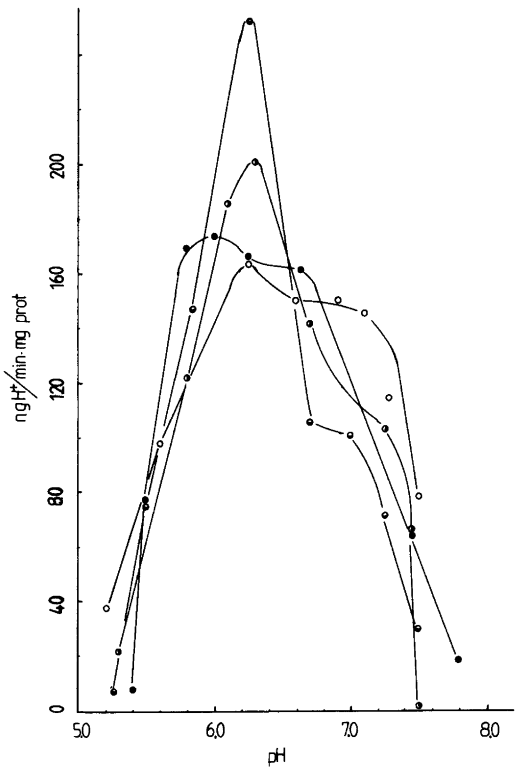

Fig. 2. pH optima for pyrophosphatase activity determined by pH method. 1 mM buffers were used: ●, tris-HCl; ○ PIPES-NaOH) ◑, MES-NaOH; ◒, MOPS-NaOH. Chromatophores corresponding to 1.4 mg of protein were added to 3 ml of assay medium. FCCP 0.1 μM.

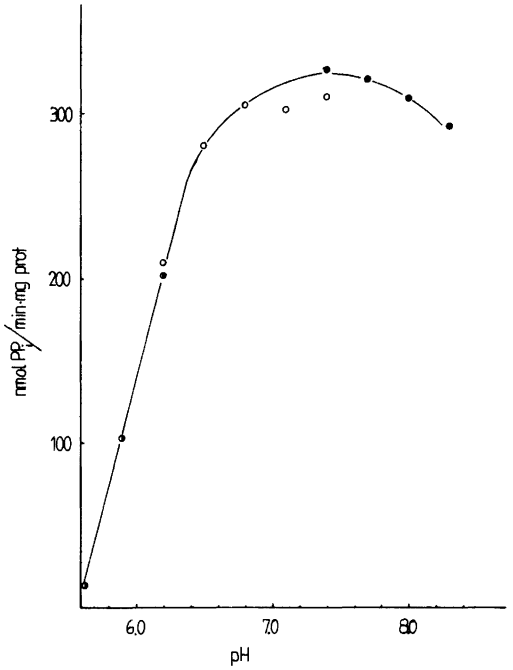

Fig. 3. Activity of chromatophore pyrophosphatase as a function of pH. Determination by direct phosphate analysis. 50 mM buffers were used: ●, tris-HCl; ○, PIPES-NaOH; ◑, MES-NaOH. Chromatophores corresponding to 0.07 mg of protein were added to 3 ml of assay medium. FCCP 0.1 μM.

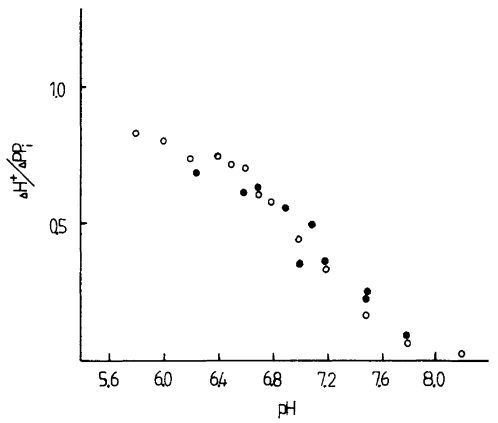

Fig. 4. Values of $n = \Delta H^+/\Delta PP_i$ used for calculations of rate of PP_i hydrolysis. Points ○ are derived from theoretical calculations using eqns. (3)–(5) and values of pK, presented in Table 1. Points ● correspond to the experimentally determined values of n at various pH from the comparison of PP_i hydrolysis rate obtained by two independent methods (pH recording and direct P_i determination).

determination method. As seen in Fig. 3, the pyrophosphatase activity has a pH optimum at pH 6.8–8.2 and decreased rapidly at low pH. Thus the observed decrease in the activity as measured by the pH-method actually occurs at low pH. At a pH range of 7–8 the pH change should be decreased according to eqn. (4).

The rates of PP_i hydrolysis, as determined by the two independent methods (P_i-determination and pH measurement), with the same chromatophore samples at pH 6.80±0.01 are compared in Table 2. In the first and the second columns, the rates of photophosphorylation determined by the P_i analysis and pH measurement are indicated. The determination of $n = \Delta H^+/\Delta PP_i$ is possible in

Table 2. Comparison of pyrophosphatase activity rates determined by two methods and experimental determination of $n = \Delta H^+/\Delta PP_i$. All experiments done at pH 6.80±0.01.

Experiment No.	Rate of PP_i hydrolysis (ΔPP_i/min mg prot)		$n = \frac{\Delta H^+}{\Delta PP_i}$ determined experimentaly
	P_i determination	pH measurement	
1	76.05	47.8	0.62
2	69.14	40.5	0.58
3	70.25	37.2	0.52
4	133.5	81.0	0.60
5	133.9	78.5	0.58
6	113.0	80.8	0.71
7	304.0	172.1	0.57
8	106.2	75.8	0.71

parallel experiments from the chemically analyzed amount of P_i released, from one side, and the measurements of pH change and buffering capacity, from the other. The *n*'s determined by this method with no assumptions of pK_a or equations are presented in the last column of Table 2. The mean value of *n*, obtained in 17 experiments, was 0.59 (standard deviation 0.09) at pH 6.8 ± 0.01. The *n*-values were calculated also at different pH values by assuming eqns. (3) and (5) and using the pK_a values, presented in Table 1. The result of the calculation of $n = \Delta H^+/\Delta PP_i$ is presented in Fig. 4, as well as the results of the experimental determination of *n*-values at the same pH. It is seen that *n*-values calculated theoretically and obtained experimentally are in good agreement with each other. According to theoretical calculations, changes of ionic strength (when 2 or 40 μl chromatophores were added) shall not exceed 7% of *n*-coefficient changes to a lower value.

Moyle *et al.*[23] studied proton-translocating pyrophosphatase of *R. rubrum* by measuring changes in the pH value. In the presence of 1 μM FCCP the hydrolysis of PP_i by the chromatophore pyrophosphatase produced an alkalization of the media. This effect was not discussed, but we think it may be explained by the same H^+-binding as in our experiments. Using 3.3 mM glycylglycine buffer pH 7.0 – 7.1 in the presence of FCCP, Moyle *et al.* have obtained the coefficient *n* about 0.3 (g ion H^+ disappeared)/(mol PP_i hydrolyzed). In our case *n*-values for this pH level are 0.39, 0.36 and 0.34 for 1 mM PIPES, MES and MOPS buffers, respectively.

It should be remembered that consumption of H^+ may be accompanying some reactions and as a result the $\Delta H^+/\Delta PP_i$ ratio may be changed. Actually, one example is in 23, where gradient of H^+ was formed in the vesicle preparation during PP_i hydrolysis. In our experiments with chromatophores we abolished the H^+-gradient formation by addition of uncoupler.

It may be mentioned that the described pH method was successfully used by us for measurement of pyrophosphatase activity in rat liver mitochondria which were isolated as described by Ernster and Löw[24] and suspended in 0.25 sucrose.

Extensive application of this method should contribute to a fuller understanding of the role of pyrophosphatases in cell metabolism, as its rapidity and sensitivity make it suitable both for quantitative, kinetic and screening experiments.

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Arylglycerol Glucosides from *Pinus sylvestris**

LENNART N. LUNDGREN, THOMAS POPOFF** and OLOF THEANDER

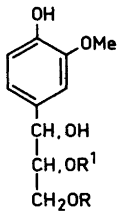
Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

The 2- and 3-*O*-β-D-glucopyranosides of 1-(4-hydroxyphenyl)-1,2,3-propanetriol [1-*C*-(*p*-hydroxyphenyl)glycerol] and 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol [1-*C*-guaiacylglycerol] have been isolated from needles of *Pinus sylvestris* L. and identified. Syntheses of the (1*S*, 2*R*)- and (1*R*, 2*R*)-forms (D-*erythro*- and D-*threo*, respectively) of the above aglycones are reported. The results indicate the presence of both D- and L-forms of the respective aglycones in the plant material.

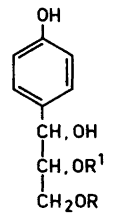
Using chromatography on Sephadex LH-20 and silicic acid, a series of dilignol and flavonoid glycosides have previously been isolated from needles of *Pinus sylvestris* L. and *Picea abies*, respectively, and identified.[1,2] The isolation of the 1- and 2-*O*-β-D-glucopyranosides of 1-*C*-guaiacylglycerol[3] is also reported. By treating heartwood of *Pinus resinosa* with *p*-dioxane under acetylating conditions, acetates of D,L-*threo*- and D,L-*erythro*-1-*C*-guaiacylglycerol have been isolated in trace amounts.[4] These were suggested to be lignin degradation products. The compounds were subsequently isolated from the cambium of *Tsuga heterophylla*[5] and, after mild hydrolytic treatment, from wood of *Picea excelsa*[6] and *Picea jezoenesis*.[7] In the latter publication, the isolation of 1-*C*-(*p*-hydroxyphenyl)glycerol was also reported. It has also been shown that arylglycerols, obtained in the form of *threo*- and *erythro*-isomers, can be formed enzymatically from cinnamyl alcohols,[8] which are proposed to be lignin precursors. A dimer of guaiacylglycerol and a series of lignans in which guaiacylglycerol is linked by ether bonds were recently isolated[9,10] from *Larix leptolepis*. The present publication reports the isolation of 1-*C*-(*p*-hydroxyphenyl) glycerol (*6*), 1-*C*-guaiacylglycerol (*5*) and their 2- and 3-*O*-β-D-glucopyranosides *1* – *4* from needles of *Pinus sylvestris* L. and their identification, and the synthesis of the D-*erythro*- and D-*threo*-forms of *5* and *6*. The configuration of the isolated compounds is discussed.

* Part 9 in the series *The Constituents of Conifer Needles*. Part 8, see Ref. 2.

** Present address: Waters Associates AB, Sommarvägen 5, S-171 40 Solna, Sweden.



1 R = β-D-glucopyranoside; R¹ = H
2 R = H; R¹ = β-D-glucopyranoside
5 R = R¹ = H



3 R = β-D-glucopyranoside; R¹ = H
4 R = H; R¹ = β-D-glucopyranoside
6 R = R¹ = H

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RESULTS AND DISCUSSION

The compounds *1*–*6* were all obtained amorphous but chromatographically homogeneous after subfractionation on anion-exchange resins and silicic acid columns. The positive colour reactions towards spray *a* – orange for compounds *1*, *2*, and *5* and yellow for *3*, *4*, and *6* – indicating free phenolic hydroxyl group (s), were in accordance with those previously reported [7] for guaiacylglycerol and *p*-hydroxyphenylglycerol, respectively. Enzymatic hydrolysis (β-glucosidase) of compounds *1* and *2* yielded D-glucose and a mixture of *erythro*- and *threo*-1-*C*-guaiacylglycerol (*5*) (shown by borate buffer paper electrophoresis [11] and ^{1}H NMR of their acetates [12,13]; identical with those of authentic samples). Periodate oxidation [14] of compound *1* gave vanillin, indicating that the glucose was linked to the 3-hydroxyl group in the glycerol chain. Compound *2* was chromatographically and electrophoretically identical with the previously isolated β-glucoside [3] and, since periodate oxidation of compound *2* did not give vanillin, the glucose must be linked to either the 1- or the 2-hydroxyl group in the glycerol chain. In the ^{1}H NMR comparison of compound *2* with that of its acetate, a downfield shift for the benzylic proton was noted, indicating an acetyl on the 1-position. Glucose must hence be linked to the 2-hydroxyl group. The sugars in compounds *1* and *2* are linked as β-glucopyranosides, which was shown by ^{1}H NMR ($J_{1,2}$ 7 Hz) and by hydrolysis with β-glucosidase.

In the same way, compounds *3* and *4* were identified as 3-*O*- and 2-*O*-β-D-glucopyranosides, respectively, of 1-*C*-(*p*-hydroxyphenyl)glycerol (*6*). By ^{1}H NMR and electrophoresis, compound *3* was found to be a mixture of *erythro*- and *threo*-isomers while compound *4* was found, by ^{1}H NMR, to be a pure *threo*-isomer ($[\alpha]_D$ +18.7°).

Small amounts of pure *threo-5* and *-6* ($[\alpha]_D$ +12.8 and $[\alpha]_D$ +19.5°) were isolated by preparative electrophoresis. The optical rotations for these *threo* compounds differ markedly from those for the synthetic D-*threo-5* and *-6* ($[\alpha]_D$ −26.1 and $[\alpha]_D$ −33.8°) and that previously [3] found for *threo-5* ($[\alpha]_D$ −18°). To check these results, pure *erythro*- and *threo-5* and *-6* were isolated from a larger batch of an enzymatically hydrolyzed glucoside mixture by LC on Sephadex LH-20 and silicic acid and by preparative HPLC. Before hydrolysis, only traces of the free aglycones *5* and *6* could be detected in the aqueous extract remaining after extraction with chloroform and ethyl acetate, respectively. The isolated aglycones released by hydrolysis showed almost no optical rotation ($[\alpha]_D < |2°|$). A check was also made on pure *erythro-6* that the mild conditions used during the isolation experiment B had no influence on its optical rotation. These results support the notable existence of both D- and L-forms of compounds *5* and *6*, glycosidically linked in the plant. Enantiomeric mixtures of procyanidin polymers in Palmae species were also reported recently. [15]

The D-*erythro*- and D-*threo-5* and *-6* were prepared by addition of 2,3-*O*-isopropylidene-D-glyceraldehyde to (4-benzyloxy-3-methoxyphenyl)magnesium bromide and (4-benzyloxyphenyl)magnesium bromide, respectively. All isomers were crystalline, as previously reported for D,L-mixtures of *erythro-* [16] and *threo-5* [6] and *erythro-6*. [17]

EXPERIMENTAL

General. ^{1}H NMR spectra were determined at 89.60 MHz (TMs as internal reference); s, d, m and q denote singlet, doublet, multiplet and quartet, respectively. Data for the strongly coupled protons in *threo-5* and *-6* were obtained by ABX analysis, checked by computer simulation, and adjusted if necessary. [20] TLC studies were performed on silica gel HF_{254} plates with (*a*) 2-butanone – MeOH – H_2O, (9:0.5:1) as solvent, and for further deter-

Table 1. Mobilities on paper electrophoresis in borate buffer and retention on HPLC on a RAD-PAK C_{18} column.

Compound[a]	*1*t	*1*e	*2*t,*2*e	*3*t	*3*e	*4*t	*5*t	*5*e	*6*t	*6*e
Mg-value[b]	0.50	0.27	0.18	0.52	0.28	0.20	0.59	0.43	0.65	0.46
HPLC[c]	–	–	–	–	–	–	3.0	2.4	3.7	2.8

[a] t = *threo* and e = *erythro*. [b] Mobilities compared with glucose (1.0) and 5-hydroxymethylfurfural (0). [c] Retention times in min. Mobile phase: H_2O – MeOH – HOAc, 100:10:1 for *5* and H_2O – HOAc, 100:1, for *6*, respectively. Flow rate for both: 1 ml/min.

mination of the purities of the compounds, also with (*b*), $CHCl_3$ – MeOH – H_2O, (7:3:0.5). TLC plates (after inspection in UV light) were sprayed with (*a*) 0.1 % diazotized sulfanilic acid in 10 % Na_2CO_3, followed by 50 % H_2SO_4 or (*b*) anisaldehyde – H_2SO_4 – EtOH (1:1:18). Electrophoresis was performed on Whatman 1 paper with 0.05 M borate buffer, pH 9.2, as electrolyte, for 1.5 h at 1500 V. The mobilities are given in Table 1. The visualizing agents used were spray *a* (without subtreatment with 50 % H_2SO) and (*c*) $AgNO_3$ [1.5 % in aq. $(CH_3)_2CO$], followed by 2 N NaOH. HPLC was performed on a RAD-PAK C_{18} high-sensitivity column (Waters) and the UV-absorbance at 254 nm was measured. The mobile phase consisted of H_2O – MeOH – HOAc, 100:10:1 for compound 5 and H_2O – HOAc, 100:1 for compound 6. The retention times are given in Table 1. All reagents were commercial samples of good grade. 1-Hydroxy-2-methoxybenzene was brominated as described for hydroxybenzene.[18] The benzyl derivatives of 1-bromo-4-hydroxy-3-methoxybenzene and 1-bromo-4-hydroxybenzene were prepared by reaction with benzylbromide in the presence of tetrabutylammonium hydrogen sulfate.[19] Melting points are corrected.

Syntheses

(1S, 2R)- and (1R, 2R)-1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol [D-erythro-5 *and* D-threo-5]. 1-Benzyloxy-4-bromo-2-methoxybenzene (6.9 g, 25 mmol) was allowed to react with magnesium metal (0.52 g, 21.3 g-atom) and 2,3-*O*-isopropylidene-D-glyceraldehyde (1.8 g, 16 mmol) was added to the Grignard reagent as described for bromobenzene in the synthesis of D-*erythro*- and D-*threo*-1-phenyl glycerol ($[\alpha]_D$ +19.6 and $[\alpha]_D$ −38.6°),[21] but with tetrahydrofuran as solvent. The reaction mixture was poured onto ice-water, neutralized with dilute hydrochloric acid and extracted with ether. LC of the evaporation residue on silica acid [light petroleum (60 – 70 °C) – EtOAc, 4:1] yielded a mixture of (1*S*, 2*R*)- and (1*R*, 2*R*)-1-(4-benzyloxy-3-methoxyphenyl)-2,3-*O*-isopropylidene-1,2,3-propanetriol. The mixture was debenzylated by catalytic hydrogenation on 5 % Pd/C in methanol for 1 h. After filtration and evaporation, it was hydrolyzed in 0.05 M sulfuric acid for 1 h at room temperature. The solution was neutralized with barium carbonate, centrifuged and evaporated. By LC of the residue on Sephadex LH-20 (elution with water) and preparative HPLC, D-*erythro*-5 and D-*threo*-5 were separated. Recrystallization was performed from acetone – dichloromethane. D-*erythro*-5 contained 1 mol water as previously reported.[16] It was not intended to optimize the preparations for yield, but rather to obtain pure reference specimens.

Compound 5. D-*erythro*-Isomer. (96 mg, 2.8 %), m.p. 82 – 84 °C (Lit.[16] D,L-mixture 83 – 84 °C), $[\alpha]_D^{23}$ +9.4° (*c* 3.0, EtOH). Anal. $C_{10}H_{14}O_5$, H_2O; C, H, O. MS, m/e (rel. int.): 214 (7, M), 197 (4), 154 (13), 153 (100), 152 (36), 151 (19), 137 (17), 125 (31), 110 (13), 93 (82), 65 (51). ^{1}H NMR (CD_3OD): δ 3.4 – 3.9 m (3 H), 3.85 s (3 H), 4.52 d, J 5.9 Hz (1 H), 6.7 – 7.0 m (3 H). Tetraacetate of D-*erythro*-5 (Ac_2O-Pyr): ^{1}H NMR ($CDCl_3$): δ 2.00 s (3 H), 2.03 s (3 H), 2.13 s (3 H), 2.30 s (3 H), 3.84 s (3 H), 4.24 d, *J* 5.0 Hz (2 H), 5.39 q (1 H), 6.01 d, *J* 5,6 Hz (1 H), 6.8 – 7.1 m (3 H).

Compound 5. D-*threo*-Isomer. (60 mg, 1.7 %), m.p. 133 – 134 °C (Lit.[6] D,L-mixture, 133 – 134 °C), $[\alpha]_D^{23}$ −26.1° (*c* 1.4, EtOH). Anal. $C_{10}H_{14}O_5$; C, H, O. MS was identical with MS for the D-*erythro*-isomer. ^{1}H NMR ($CDCl_3$): δ 3.32 dd, *J* 11.2 and 6.3 Hz (1 H), 3.45 dd, *J* 11.2 and 3.4 Hz (1 H), 3.65 m (1 H), 3.85 s (3 H), 4.51 d, *J* 6.2 Hz (1 H), 6.7 – 7.0 m (3 H). Tetraacetate of D-*threo*-5 (Ac_2O-Pyr): ^{1}H NMR ($CDCl_3$): 2.05 s (3 H), 2.07 s (3 H), 2.09 s (3 H), 2.30 s (3 H), 3.81 dd, *J* 5.6 Hz and 12.0 Hz (1 H), 3.84 s (3 H), 4.29 dd, *J* 3.7 Hz and 12.0 Hz (1 H), 5.3 – 5.6 m (1 H), 5.96 d, *J* 7.5 Hz (1 H), 6.8 – 7.1 m (3 H).

(1S, 2R)- and (1R, 2R)-1-(4-hydroxyphenyl)-1,2,3-propanetriol [D-erythro-6 *and* D-threo-6] were prepared from 1-benzyloxy-4-bromobenzene (8.9 g, 34 mmol) in the same way as the isomers of 5. The compounds were recrystallized from acetone – dichloromethane.

Compound 6. D-*erythro*-Isomer. (320 mg, 9.7 %, m.p. 140 – 141 °C (Lit.[17] D,L-mixture, 149 – 151 °C), $[\alpha]_D^{23}$ +21.3° (*c* 0.6, EtOH). Anal. $C_9H_{12}O_4$; C, H, O. MS, *m/e* (rel. int.): 166 (4, M − 18), 124 (13), 123 (100), 122 (12), 121 (25), 107 (26), 95 (58), 77 (58), 65 (14). ^{1}H NMR (CD_3OD): δ 3.4 – 3.9 m (3 H), 4.51 d, *J* 5.7 Hz (1 H), 6.74 d, *J* 8.5 Hz (2 H), 7.21 d, *J* 8.5 Hz (2 H). Tetraacetate of D-*erythro*-6 (Ac_2O – Pyr): ^{1}H NMR ($CDCl_3$): δ 2.00 s (3 H), 2.03 s (3 H), 2.12 s (3 H), 2.30 s (3 H), 4.23 d, *J* 5.0 Hz (2 H), 5.38 q (1 H), 6.02 d, *J* 5.5 Hz (1 H), 7.08 d, *J* 8.5 Hz (2 H), 7.39 d, *J* 8.5 Hz (2 H).

Compound 6. D-*threo*-Isomer. (570 mg, 17.2 %), m.p. 144 – 146 °C, $[\alpha]_D^{23}$ −33.8° (*c* 1.2, EtOH). Anal. $C_9H_{12}O_4$; C, H, O. MS was identical with MS for the D-*erythro*-isomer. ^{1}H NMR (CD_3OD): δ 3.32 dd, *J* 11.2 and 6.3 Hz (1 H), 3.45 dd, *J* 11.2 and 3.4 Hz (1 H), 3.65 m (1 H), 4.50 d, *J* 6.6 Hz (1 H), 6.74 d, *J* 8.5 Hz (2 H). 7.19 d, *J* 8.5 Hz (2 H). Tetraacetate of D-*threo*-6 (Ac_2O – Pyr): ^{1}H NMR ($CDCl_3$): δ 2.05 s (3 H), 2.06 s (3 H), 2.08 s (3 H), 2.29 s (3 H), 3.79 dd, *J* 5.6 Hz and *J* 12.2 Hz (1 H), 4.29 dd, *J* 4.0 Hz and 12.2 Hz (1 H), 5.3 – 5.6 m (1 H), 5.98 d, *J* 7.2 Hz (1 H), 7.10 d, *J* 8.5 Hz (2 H), 7.39 d, *J* 8.5 Hz (2 H).

Isolation

Experiment A. Needles of *Pinus sylvestris* L. (190 g dry weight), collected in spring, were extracted without drying for 1 h with boiling Me_2CO. After filtration, the needles were dried, milled and extracted on a boiling water bath, 2 × 30 min with Me_2CO and 2 × 30 min with Me_2CO-H_2O (1:1). The extracts were combined, the Me_2CO was evaporated, and the suspension obtained was extracted several times with $CHCl_3$. The remaining H_2O fraction (24 g) was fractionated on a Sephadex LH-20 column (elution with H_2O). Twelve main fractions were collected. From fraction 1 (10.6 g), by repeated subfractionation on anion-exchange resin (Dowex 1-x8 in its acetate form), *1*, *3*, *2*, *4*, *5* and *6* were eluted with water in the order given; and on a silicic acid column (elution with $CHCl_3-MeOH-H_2O$, 7:3:0.5) compounds *1*–*6* were obtained in chromatographically pure form. The yields corresponded to 0.01 (compounds *3*, *5* and *6*), 0.02 (compounds *1* and *4*) and 0.03 % (compound *2*), respectively, of the dry weight of the needles.

Experiment B. Needles of *Pinus sylvestris* L. (360 g dry weight) collected in November, were treated as in Exp. A. The remaining H_2O fraction (48 g) was extracted with EtOAc and the aqueous residue was treated with a commercial crude enzyme (cellulase C 36, Rohm and Haas Co.) for 48 h at room temperature and then evaporated. LC of the residue on Sephadex LH-20 (elution with H_2O) and silicic acid (elution with $CHCl_3-MeOH-H_2O$, 60:15:1) separated the *erythro-threo-5* from *erythro-threo-6*. By preparative HPLC pure *erythro-5* (5 mg), *threo-5* (28 mg), *erythro-6* (6 mg) and *threo-6* (31 mg) were obtained.

Experiment C. In order to check if any isomerization could occur during the preparation and isolation under the conditions used in Exp. B, a pure sample of *erythro-6* was treated as in Exp. B. No change in the optical rotation of the compound could be observed during these treatments.

Compound 1. Mixture of the *erythro-* and *threo-*isomers in ratio 1:3. NMR (CD_3OD): δ 3.2–4.0 m (9 H), 3.84 s (3 H), 4.21 d, *J* 7.0 Hz (1 H), 4.57 d, J 6.0 Hz (1 H), 6.7–7.1 m (3 H). Heptaacetate of *1* (Ac_2O-Pyr): NMR ($CDCl_3$): δ 2.0–2.2 m (18 H), 2.29 s, (3 H), 3.48 dd, *J* 6.0 and 11.5 Hz (1 H), 3.5–3.8 m, (1 H), 3.85 s, (3 H), 3.88 dd, *J* 4.0 and 11.5 Hz (1 H), 4.08 dd, *J* 2.5 and 11.0 Hz (1 H), 4.24 dd, J 5.0 and 11.0 Hz (1 H), 4.40 d, *J* 7.5 Hz (3/4 H), 4.52 d, *J* 7.5 Hz (1/4H), 4.8–5.2 m, (3 H), 5.2–5.4 m, (1 H), 5.93 d, *J* 7.5 Hz (3/4 H), 5.95 d, *J* 5.5 Hz (1/4 H), 6.8–7.1 m, (3 H).

Compound 2. Mixture of the *erythro-* and *threo-*isomers in ratio 1:4. NMR (CD_3OD): δ 3.3–4.0 m, (9 H), 3.85 s, (3 H), 4.43 d, *J* 7.0 Hz (4/5 H), 4.45 d, *J* 7.0 Hz (1/5 H), 4.64 d, *J* 8.0 Hz (1/5 H), 4.67 d, *J* 7.5 Hz (4/5 H), 6.7–7.1 m, (3 H). Heptaacetate of *2* (Ac_2O-Pyr): NMR ($CDCl_3$): δ 1.98–2.10 m, (15 H), 2.13 s, (3 H), 2.29 s, (3 H), 3.6–4.0 m, (2 H), 3.84 s, (3 H), 4.0–4.3 m, (1 H), 4.10 dd, J 4.0 and 12.5 Hz (1 H), 4.18 dd, *J* 5.0 and 12.5 Hz (1 H), 4.68 d, *J* 7.0 Hz (1/5 H), 4.72 d, *J* 7.0 (4/5 H), 5.85 d, *J* 7.8 Hz (4/5 H), 6.00 d, *J* 5.5 Hz (1/5 H), 6.8–6.1 m, (3 H).

Compound 3. Mixture of the *erythro-* and *threo-*isomers in ratio 1:9. NMR (CD_3OD): δ 4.2–4.0 m, (9H), 4.20 d, *J* 7.0 Hz (1 H), 4.55 d, 6.5 Hz (1 H), 6.75 d, *J* 8.5 Hz (2 H), 7.21 d, *J* 8.5 Hz (2 H). Heptaacetate of *3* (Ac_2O-Pyr): NMR ($CDCl_3$): δ 2.00 s (3 H), 2.01 s (3 H), 2.05 s (3 H), 2.06 s (3 H), 2.07 s (3 H), 2.09 s (3 H), 2.29 s (3 H), 3.38 dd, *J* 5.5 and 12.0 Hz (1 H), 3.55–3.75 m (1 H), 3.88 dd, *J* 4.0 and 12.0 Hz (1 H), 4.08 dd, *J* 3.0 and 12.0 Hz (1 H), 4.25 dd, *J* 4.0 and 12.0 Hz (1 H), 4.40 d, *J* 7.0 Hz (1 H), 4.9–5.2 m (3 H), 5.2–5.4 m (1 H), 5.94 d, *J* 7.5 Hz (1 H), 7.09 d, *J* 8.5 Hz (2 H), 7.37 d, J 8.5 Hz (2 H).

*Compound 4. threo-*Isomer $[\alpha]_D^{23}$ +18.7° (*c* 0.5, EtOH). NMR (CD_3OD): δ 3.2–4.0 m (9 H), 4.45 d, *J* 7.5 Hz (1 H), 4.64 d, *J* 7.5 Hz (1 H), 6.76 d, *J* 8.7 Hz (2 H), 7.22 d, *J* 8.7 (2 H). Heptaacetate of *4* (Ac_2O-Pyr): NMR ($CDCl_3$): δ 1.99 s (6 H), 2.00 s (3 H), 2.01 s (3 H), 2.08 s (3 H), 2.11 s (3 H), 2.28 s (3 H), 3.65–3.85 m (1 H), 3.7–4.2 m (2 H), 4.11 dd, *J* 3.5 and 12.5 Hz (1 H), 4.28 dd, *J* 4.0 and 12.5 Hz (1 H), 4.71 d, *J* 7.5 Hz (1 H), 4.8–5.2 m (4 H), 5.88 d, *J* 7.5 Hz (1 H), 7.08 d, *J* 8.5 Hz (2 H), 7.34 d, *J* 8.5 Hz (2 H).

*Compound 5. erythro-*Isomer from Exp. B. $[\alpha]_D^{23}$ 0° (0.5, EtOH).

*Compound 5. threo-*Isomer from Exp. A. $[\alpha]_D^{23}$ +12.8° (*c* 1, EtOH). *threo-*Isomer from Exp. B. $[\alpha]_D^{23}$ −1.4° (*c* 2.8 EtOH).

*Compound 6. erythro-*Isomer from Exp. B. $[\alpha]_D^{23}$ −1.5° (*c* 0.6, EtOH).

*Compound 6. threo-*Isomer from Exp. A. $[\alpha]_D^{23}$ +19.5° (*c* 0.6, EtOH). *threo-*Isomer from Exp. B. $[\alpha]_D^{23}$ +1.2° (*c* 3.1, EtOH).

TLC, HPLC, paper electrophoresis, ^1H NMR and MS for compounds *5* and *6* were identical with those for the synthetic samples.

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Rearrangement of 1,2-Diazene *N*-Oxides. 3.* Photochemical Isomerization of Bicyclic *cis*-1,2-Diazene *N*-Oxides to Oxadiaziridines

HENRIK OLSEN** and CHRISTIAN L. PEDERSEN***

Department of General and Organic Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark

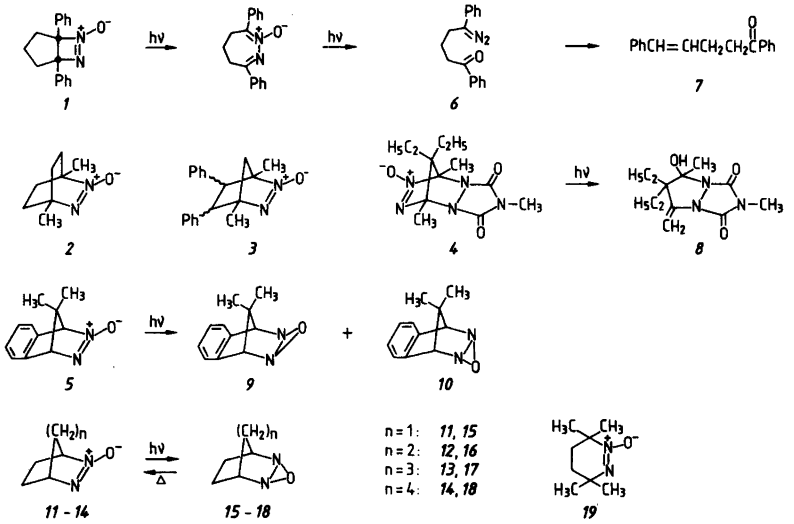
The photochemical behaviour of four bicyclic and one monocyclic aliphatic *cis*-1,2-diazene *N*-oxide was studied by means of UV- and NMR spectroscopy at low temperature. All bicyclic compounds photolyze to labile transients assigned as *cis*-oxadiaziridines. When samples of these compounds in poly(vinyl chloride) or methanol are slowly heated, 60 – 100% of the starting materials are reformed. First order time dependence is observed for these thermal reversions. The kinetic stabilities of *cis* and *trans* oxadiaziridines are compared and discussed.

The photochemical behaviour of aliphatic *trans*-1,2-diazene *N*-oxides has previously been the subject of several studies.[2-8] Irradiation with ultraviolet light may lead to any of four unimolecular reactions: (1) Elimination of dinitrogen oxide,[2] (2) *cis* – *trans* isomerization,[3] (3) deoxygenation,[4] and (4) formation of oxadiaziridines.[5-8] The *cis*-1,2-diazene *N*-oxides may share some of the properties of the *trans* compounds but they have not attracted much

* Part 1 and 2. See Ref. 1.

** Present address: The Technological Institute, Gregersensvej, DK-2630 Tåstrup, Denmark.

*** Present address: Department of Chemistry, Odense University, Campusvej 55, DK-5230 Odense M, Denmark.



Scheme 1.

0302-4369/82/100701-06$02.50

attention. Irradiation of compound *1* (Scheme 1) results in the formation of *7*, which was proposed to arise from decomposition of the diazoketone, *6*.[9] On the other hand, compounds *2*, *3* (Ref. 10) and *11* (Ref. 6) polymerize upon irradiation at room temperature whereas *4* is transformed into *8*, probably *via* a diazenoxyl diradical.[10] Recently[11,12] it was reported that dimethylisoindene may be generated by irradiation of compound *5* in frozen solution at 77 K. However, irradiation in liquid solution at 193 K produces oxadiaziridines *9* and *10*. At 253 K starting material was reformed from these after several hours.

In order to obtain further insight into the photochemistry of *cis*-diazene *N*-oxides compounds *11* – *14* (Ref. 13) and *19* (Ref. 14) were prepared and their photochemical behaviour was studied.

RESULTS

Photolysis of compounds 11, 12, 14, *and* 19 *in poly(vinyl chloride) (PVC) at 77 K. UV spectra.* Solutions (~0.05 M) in thin PVC films (see Experimental) of these compounds were irradiated (Table 1) and absorption spectra were recorded during the course of irradiation. In all cases, the absorption bands (Table 1) due to the starting materials gradually decreased in intensity with no concomitant formation of product absorptions above 200 nm. Irradiations were discontinued after 40 – 75% of conversion, and the films were slowly heated to room temperature. During the warm-up the absorption bands due to the starting materials increased in intensity.* The degrees of reversibility are given in Table 1.

Photolysis of compounds 11 – 14 *in methanol at 193 K. UV spectra.* Solutions ($\sim 2.5 \times 10^{-4}$ M) of these compounds were irradiated as described above (Table 1). The same spectroscopic behaviour was observed. After partial photolysis solutions were slowly warmed up. When the UV absorption due to the starting materials began to increase in intensity, the temperature was fixed (Table 2) and the absorbances of the signals at their respective λ_{max} values were recorded as a function of time. By plotting $\log_e(A_\infty - A_t)$ *vs.* time straight lines were obtained, *i.e.* first order time dependence is observed

* K. E. Gilbert (private communication) has studied the photolysis of *19* and has observed formation of dienes and amines.

Table 1. Photolysis of diazene *N*-oxides *11* – *14* and *19* in methanol at 193 K and in PVC at 77 K.

Compound	λ_{max}/nm	Excitation wavelength/nm	Degree of conversion/%	Reversibility/%
11[a]	225	250(15)	70	80
11[b]	229	275(20)	60	70
12[a]	227	250(20)	70	100
12[b]	230	260(20)	75	100
13[a]	232	250(20)	50	100
14[a]	234	>200	70	60
14[b]	237	250(15)	45	65
19[b]	234	250(20)	40	~2

[a] Methanol as solvent. [b] PVC as solvent.

Table 2. Kinetic data for the oxadiaziridine→diazene *N*-oxide isomerizations in methanol.

Compound	Temp./K	$t_{\frac{1}{2}}$/min.	$k/10^4$ s^{-1}	Correlation coefficient	$\Delta G^{\ddagger}$/(kJ/mol)[a]
15	223(1)	32	3.6	0.994	68.6
16	249(2)	30	3.9	0.988	77.0
17	255(1)	41	2.8	0.998	79.5
18	268(1)	38	3.0	0.998	83.3

[a] $\Delta G^{\ddagger}$ was calculated from the expression $k = kT/h \exp(-\Delta G^{\ddagger}/RT)$.

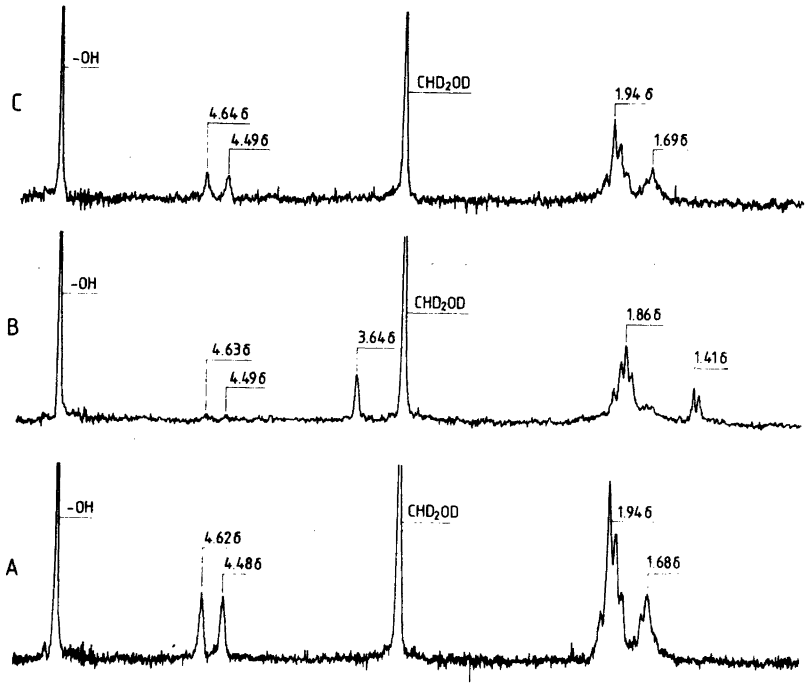


Fig. 1. 270 MHz ^{1}H NMR spectra of compound *12* in CD_3OD at 213 K. Spectrum A, before photolysis, spectrum B, after photolysis ($\lambda > 200$ nm) at 193 K, spectrum C, after heating the photolyzed sample to room temperature and recooling to 213 K.

for the thermal reversions. Least squares calculations of rate constants and $\Delta G^{\ddagger}$ values are given in Table 2.

Photolysis of compounds 12 *and* 13 *in methanol-*d_4 *at 193 K.* ^{1}*H-NMR spectra.* The UV experiments above showed that starting materials *12* and *13* were reformed quantitatively when photolyzed samples were heated to room temperature. The photolysis of these compounds (0.1 M in CD_3OD) was therefore followed by ^{1}H NMR spectroscopy.

The 270 MHz spectrum of compound *12* is shown in Fig. 1. The two bridgehead protons absorb at $\delta = 4.62$ and 4.48 (at 60 MHz in $CDCl_3$ these protons appear as a broad singlet, see Ref. 13). Upon photolysis ($\lambda > 200$ nm) the spectrum of compound *12* gradually disappeared and new resonance lines were observed at $\delta = 3.64$ (singlet) and $\delta = 1.86$ and 1.41 (multiplets). The temperature of the sample was now fixed at 248 K. This caused the signals of the intermediate to disappear with simultaneous enhancement of absorptions due to the starting material. The half-life of the intermediate was estimated from the peak areas to be approximately $\frac{1}{2}$ h. The same half-life was estimated by UV spectroscopy (Table 2) indicating that the same photolysis product had been formed at these widely different concentrations of the starting material.

NMR spectra (90 MHz) recorded during photolysis of compound *13* (Fig. 2, bridgehead protons at $\delta = 4.44$, decoupling experiments revealed a small chemical shift difference (0.09 ppm) showed the development of absorption at $\delta = 4.18$, 3.76 (ratio of areas $\sim 1:3$), and at $\delta = 1.83$ and 1.78. After heating to room temperature only signals corresponding to the starting material were observed.

DISCUSSION

The thermally unstable photoproducts formed by photolysis of compounds *11* – *14* may *a priori* be assigned any of the structures *15* – *18* and 20 – 22 (Scheme 2). Some of these candidates may, however, be ruled out since the photoproducts must possess the following chemical and spectroscopic properties: (i) Fast isomerization to starting material below

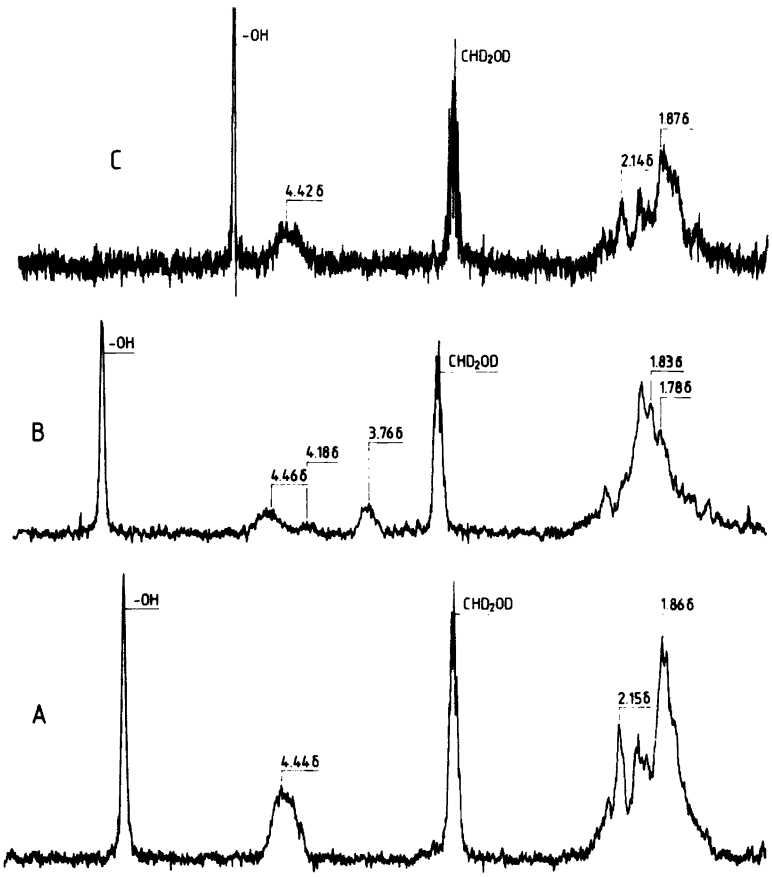
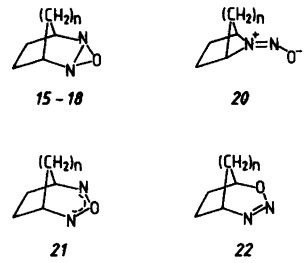


Fig. 2. 90 MHz ^{1}H NMR spectra of compound *13* in CD_3OD at 221 K. Spectrum A, before photolysis, spectrum B, after photolysis ($\lambda > 200$ nm) at 193 K, spectrum C, after heating the photolyzed sample to room temperature.

0 °C (Table 2). (ii) The photoproduct from compound *12* (Fig. 1, and probably also the photoproduct from compound *13*, Fig. 2) possesses two bridgehead protons which are chemically equivalent ($\Delta\delta < 0.02$ ppm). The resonance line of these protons is found at higher magnetic field (0.9 ppm) relative to the bridgehead protons of the starting material (0.7 ppm for the photoproduct from compound *13*). (iii) Candidates for the assignment of structure must not possess intense absorptions above 200 nm in the electronic spectrum.

The *N*-nitroso amine *20* does not obey any of these criteria since the bridgehead protons of *20* would not be expected to be chemically equivalent.[15] *N*-Nitroso amines also possess intense



Scheme 2.

$\pi-\pi^*$ transitions around 230 nm[16] and rearrangements into diazene *N*-oxides have not been described.[17,18] The reverse thermal isomerization, *i.e.* diazene *N*-oxide→*N*-nitroso amine has

recently been observed at higher temperatures.[1] The heteroallylic system *21* would be expected to absorb intensely above 200 nm and the bridgehead protons in *22* would probably be non-equivalent. Only the *cis*-oxadiaziridine system *15–18* is expected to obey the three criteria found experimentally. Thus, the *cis*-oxadiaziridines *9* and *10* isomerize rather fast at 253 K [11,12] and *trans*-oxadiaziridines (Table 3) all isomerize into diazene *N*-oxides at room temperature.[5–8] The protons α to nitrogen in *trans*-oxadiaziridines absorb at higher magnetic field relative to the same protons in the corresponding *cis*- and *trans*-diazene *N*-oxides [10] (*e.g.* $\delta = 3.37$ and 4.17 in *trans*-azoxybutane, $\delta = 2.63$ in *trans*-dibutyloxadiaziridine [6]). Furthermore, *trans*-oxadiaziridines do not absorb above 200 nm.[6]

Photolysis of the diazene *N*-oxide *5* was previously reported to yield an *exo* and *endo* oxadiaziridine in the approximate ratio of 2.5:1 [11,12] (bridgehead protons at $\delta = 4.64$ in the major product and $\delta = 4.81$ in the minor). Unfortunately, our data (Fig. 2) does not permit any conclusion about the presence of both an *exo* and an *endo* isomer of compound *17*. The difference in chemical shift values between the bridgehead protons of *exo*- and *endo*-*17* is expected to be small (see above). It is uncertain whether the peak at $\delta = 4.18$ (Fig. 2) can be assigned to compound *17*.

Besides compound *17*, compounds *15* and *18* may also be photochemically generated in both *endo* and *exo* isomers. When such mixtures decay thermally, the UV absorptions of the parent *N*-oxides may both reappear in first order reactions. However, since first order kinetics is observed (Table 2), we conclude that either one of the isomers predominate or the *endo* and *exo* isomers decay with nearly identical rate constants or, fast equilibrium exists between the isomers at the temperature of thermal reversion.

The most labile *cis*-oxadiaziridine is compound *15* which isomerizes to the parent *N*-oxide with $\Delta G^{\ddagger} = 68.6$ kJ/mol. By enlargement of the bridge $\Delta G^{\ddagger}$ increases gradually (83:3 kJ/mol for compound *18*, see Table 3). Intuitively, this order of reactivity is expected since the strain energy of the tricyclic *cis*-oxadiaziridines should decrease from compound *15* to compound *18*.[19] *trans*-Oxadiaziridines are generally less labile. *trans*-Di-*tert*-butyloxadiaziridine thus isomerizes 1200 times slower relative to compound *18* ($\Delta\Delta G^{\ddagger} = 17.5$ kJ/mol). This difference in reactivity between *cis* compounds and between *cis* and *trans* compounds might either be due to higher ground state energies or, it might be a transition state effect. Both effects could also be operative.

Similar differences in reactivity between *cis* and *trans* substituted three-membered heterocylic rings have been described in the literature. Diaziridinones decompose thermally to carbon monoxide and the corresponding diazenes.[20] *trans*-Di-*tert*-butyldiaziridinone decomposes with $\Delta G^{\ddagger} \sim 146$ kJ/mol while 2,2,5,5-tetramethyl-1,6-diazabicyclo[4.1.0]heptan-7-one (*cis* stereochemistry) decomposes with $\Delta G^{\ddagger} \sim 105$ kJ/mol. It was proposed that the difference in reactivity was due to differences in ground state stabilization.[20]

EXPERIMENTAL

Compounds. 11–14 were prepared according to Ref. 13, *19* according to Ref. 14.

Table 3. Rate constants for the oxadiaziridine→diazene *N*-oxide isomerizations.

Compound	Solvent	Temp./K	$\Delta G^{\ddagger}$/ kJ/mol	k/s^{-1} at 298 K [a]	k_{rel} at 298 K
15	CH_3OH	223	68.6	5.8	450000
16	CH_3OH	249	77.0	9.8×10^{-2}	7500
17	CH_3OH	255	79.5	7.2×10^{-2}	5500
18	CH_3OH	268	83.3	1.6×10^{-2}	1200
b	CD_3OD	298	96.2	8.4×10^{-5}	6.5
c	CD_3OD	295	98.7	3.0×10^{-5}	2.3
d	CCl_4	301	98.7	3.0×10^{-5}	2.3
e	CH_3OH	301	100.8	1.3×10^{-5}	1.0

[a] k was calculated from the expression $k = kT/h \exp(-\Delta G^{\ddagger}/RT)$. [b] *trans*-2-Cyclohexyl-3-methyloxadiaziridine, Ref. 8. [c] *trans*-2,3-Diisopropyloxadiaziridine, Ref. 7. [d] *trans*-2,3-Dibutyloxadiaziridine, Ref. 6. [e] *trans*-2,3-Di-*tert*-butyloxadiaziridine, Ref. 6.

Polymer films. Poly(vinyl chloride) (PVC), Corvic D 60/13, was used without further purification. Films (approx. 25 μm and approx. 0.05 M in *N*-oxide) were prepared using the solvent casting technique.[21] Purified tetrahydrofuran was used as solvent.

Photolyses. Irradiations were carried out using a Bausch and Lomb SP-200 mercury point source with or without monochromator. The light beam was focussed using quartz lenses.

UV spectra were recorded on a Cary 14 instrument. The low temperature UV cell, cooled with liquid nitrogen, is described in Ref. 22. Temperatures higher than 77 K were fixed using a temperature control unit. Experiments in liquid solution were carried out using approx. 2.5×10^{-4} M solutions of compounds in methanol of Uvasol quality.

^{1}H NMR spectra were recorded using a Bruker HX-90 E (continuous wave mode) and a Bruker HX-270 (Fourier transform mode) instrument. 0.1 M solutions of compounds *12* and *13* in CD_3OD (Me_4Si as internal standard) were photolyzed ($\lambda > 200$ nm) in quartz NMR tubes in a quartz dewar, cooled with dry ice. After 8 and 3 h, respectively, tubes were quickly transferred to the pre-cooled NMR apparatus.

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Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues. IX. Pathways for the Alkaline Hydrolysis of 6-Substituted 9-(1-Ethoxyethyl)purines

HARRI LÖNNBERG, PERTTI LEHIKOINEN and KARI NEUVONEN

Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku, Finland

A few 6-substituted 9-(1-ethoxyethyl)purines have been prepared and the rates of their base-catalyzed hydrolysis were measured by UV spectroscopy. The product mixtures were fractionated by preparative TLC and characterized by NMR and UV spectroscopy. The results obtained suggest that the alkaline cleavage of 9-(1-ethoxyethyl)purines generally proceeds by nucleophilic attack of hydroxide ion on C8 of the purine moiety, resulting in formation of appropriate 4,5-diaminopyrimidine and 8-methylpurine as final products. With 6-methoxy, 6-methylthio, and 6-chloro derivatives nucleophilic attack of hydroxide ion on C6 giving 9-(1-ethoxyethyl)hypoxanthine competes with this reaction.

While there have been numerous mechanistic investigations concerning the acid-catalyzed hydrolysis of purine nucleosides and related compounds,[1-11] the corresponding reactions under alkaline conditions have been much less extensively studied.[12-15] Chromatographic and UV-spectroscopic analyses of the product mixtures have been interpreted to indicate that three different pathways compete in the alkaline cleavage.[14,15] First, nucleophilic attack of hydroxide ion on C8 of the purine ring may lead to opening of the five-membered ring of the base moiety. Second, appropriate substituents on the purine ring, particularly at C6, can be displaced by hydroxide ion. Third, the purine moiety may be intramolecularly displaced by an ionized hydroxyl group of the glycon ring. Which one of the routes prevails undoubtedly depends on both the polar nature of the substrate and the concentration of hydroxide ion. Detailed mechanisms for each of the reactions are unclear. The aim of the present study is to elucidate the competition between the first and second mechanistic possibility and to examine the structures of the intermediates involved. Acyclic nucleoside analogues, 6-substituted 9-(1-ethoxyethyl)purines, have been chosen as model compounds, since with these substrates the intramolecular participation of the carbonyl moiety can be ignored. The substituents at C6 have been selected so that the electron density at C8 exhibits large variations. In other words, the ease of the nucleophilic attack on this site is greatly altered on going from one substrate to another.

RESULTS AND DISCUSSION

Table 1 records the first-order rate constants for the disappearance of some 6-substituted 9-(1-ethoxyethyl)purines in aqueous alkali. The rate of decomposition is greatly increased with the increasing electron-attracting ability of the 6-substituent, being for each compound proportional to the concentration of hydroxide ion. Both of the findings are consistent with the view that hydroxide ion performs a rate-limiting nucleophilic attack on the substrate. Table 2 summarizes the TLC and spectroscopic data for the products formed in about three half-lives. With the unsubstituted 9-(1-ethoxyethyl)purine the disappearance of the starting material is accompanied with formation of two stable products, having R_F values of 0.72 and 0.32 on Silica gel 60 ($CHCl_3 - CH_3OH$ 2:1). The chromatographic mobilities and the UV and NMR spectroscopic data for these compounds are identical

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Table 1. First-order rate constants for the decomposition of 6-substituted 9-(1-ethoxyethyl)purines in aqueous sodium hydroxide.[a]

Substituent at C6	T/K	$[OH^-]$/mol dm^{-3}	$k/10^{-4}$ s^{-1}
NH_2	363.2	0.50	0.342 ± 0.004[b]
	363.2	0.30	0.207 0.003
	363.2	0.10	0.0701 0.0004
CH_3	363.2	0.50	14.5 ± 0.2
	363.2	0.30	8.12 0.09
	363.2	0.10	3.53 0.07
	353.2	0.50	6.70 0.09
	343.2	0.50	3.22 0.02
OCH_3	333.2	0.50	2.35 ± 0.02
	333.2	0.30	1.03 0.01
	333.2	0.10	0.418 0.07
SCH_3	353.2	0.50	11.3 ± 0.1
	353.2	0.30	6.43 0.05
	353.2	0.10	1.92 0.03
	343.2	0.50	5.26 0.06
	333.2	0.50	2.31 0.01
H	333.2	0.50	9.73 ± 0.08
	333.2	0.30	5.66 0.08
	333.2	0.10	2.07 0.02
	323.2	0.50	4.07 0.04
	313.2	0.50	1.37 0.04
Cl	323.2	0.50	53.8 ± 0.8
	323.2	0.30	36.5 0.7
	323.2	0.10	12.7 0.5
	313.2	0.50	23.2 0.6
	303.2	0.50	10.8 0.1

[a] The ionic strength was adjusted to 0.50 mol dm^{-3} with sodium chloride. [b] Standard error of the mean.

with those observed for authentic samples of 8-methylpurine and 4,5-diaminopyrimidine, respectively. In addition, a third product, exhibiting R_F of 0.47, appears soon after the initiation of the reaction and disappears after five half-lives. The ^1H and ^{13}C NMR chemical shifts for this compound are listed in Table 3. Besides the signals of two aromatic protons, a doublet of three protons at δ 1.57 and a quartet of one proton at δ 5.75 is observed in the ^1H NMR spectrum in D_2O. Most probably the compound contains a group of $CHCH_3$. The ^{13}C NMR spectrum lend further support for this conclusion. Signals at δ 26.0 and 74.8 are observed, in addition to four signals in the region typical to carbons of the pyrimidine ring. The magnitude of the shift of 74.8 ppm strongly suggests that the carbon of the CH group, which with all likelihood is bonded to nitrogen, exhibits sp^3 rather than sp^2 hybridization. A compound that fulfils the structural requirement indicated above is, for example, intermediate I in Scheme 1. Accordingly, it seems reasonable to assume that rate-limiting nucleophilic attack of hydroxide ion on C8 of the purine moiety leads to opening of the imidazole ring. The pyrimidine derivative, formed possibly as a transient intermediate, then undergoes deformylation, and intramolecular displacement of the ethoxy group by the free amino group yields the observed cyclic intermediate, I. The latter partly decomposes to 4,5-diaminopyrimidine and

Table 2. Chromatographic and spectroscopic data for the main products formed in the alkaline decomposition of 6-substituted 9-(1-ethoxyethyl)purines.

Substituent at C6	Product	R_F[a]	NMR chemical shifts[b]	λ (max.)/nm	
				Acid[c]	Base[d]
H	8-Methylpurine	0.72[e]	^{1}H NMR:[e] s2.72(3H), s8.73(1H), s8.77(1H)	265[f]	276[f]
			^{13}C NMR:[e] 17.2, 131.7, 144.6, 153.5, 158.8, 162.1		
	4,5-Diamino-pyrimidine	0.32[e]	^{1}H NMR:[e] s7.70(1H), s8.23(1H)	283[f]	248,288[f]
			^{13}C NMR:[e] 128.8, 135.9, 149.5, 157.7		
CH_3	6,8-Dimethylpurine	0.67	^{1}H NMR: s2.57(3H), s2.67(3H), s8.37(1H)		
			^{13}C NMR: 16.8, 21.3, 130.0 152.9 155.5, 156.2, 159.7		
	4,5-Diamino-6-methylpyrimidine	0.47	^{1}H NMR: s2.30(3H), s7.87(1H)	286[g]	250,284[g]
			^{13}C NMR: 19.9, 125.4, 147.2, 149.3, 156.2		
	6-Methylpurine	0.71[e]	^{1}H NMR:[e] s2.67(3H), s8.37(1H), s8.43(1H)	266[f]	272[f]
			^{13}C NMR:[e] 21.3, 130.0, 148.3, 153.6, 154.8, 158.3		
NH_2	8-Methyladenine	0.64	^{1}H NMR:[h] s2.60(3H), s8.10(1H)	265	270
	4,5,6-Triamino-pyrimidine	0.34[e]	^{1}H NMR:[e] s7.72(1H)	287[e]	277[e]
	Adenine	0.52[e]	^{1}H NMR:[eh] s8.05(1H), s8.13(1H)	262[f]	268[f]
OCH_3	9-(1-Ethoxyethyl)-hypoxanthine	0.80	^{1}H NMR: t1.20(3H), d1.88(3H), q3.58(2H), q5.95(1H), s8.23(1H), s8.33(1H)	251	255
SCH_3	9-(1-Ethoxyethyl)-hypoxanthine		the data given above		
	8-Methyl-6-methyl-thiopurine	0.76[i]	^{1}H NMR: s2.67(6H), s8.24(1H)	227, 298	294
	4,5-Diamino-6-methylthiopyrimidine	0.65[i]	^{1}H NMR: s2.54(3H), s7.77(1H)	232, 318	298
	8-Methylpurine		the data given above		
	6-Methylthiopurine	0.72[i]	^{1}H NMR:[eh] s2.78(3H), s8.29(1H), s8.50(1H)	222, 294[f]	292[f]
Cl	9-(1-Ethoxyethyl)-hypoxanthine		the data given above		
	6-Chloro-4,5-di-aminopyrimidine	0.52[j]	^{1}H NMR: s7.84(1H)	268,306[g]	255,292[g]

[a] Silica gel 60 F-254, eluent $CHCl_3 - CH_3OH$ 2:1 (v/v) if not otherwise stated. [b] Recorded in D_2O at pD 7 if not otherwise stated. The shifts given are ppm values from DSS. [c] In 0.1 mol dm^{-3} HCl. [d] In 0.1 mol dm^{-3} NaOH. [e] Equal to the data for an authentic sample. [f] Consistent with the data in Ref. 16. [g] Consistent with the data in Ref. 12. [h] Recorded in 0.1 mol dm^{-3} NaOD. [i] Eluent $CHCl_3 - CH_3OH$ 3:1 (v/v). [j] Eluent $CHCl_3 - CH_3OH$ 5:1 (v/v).

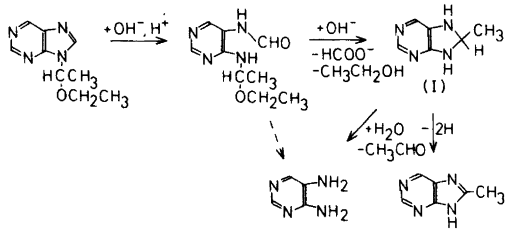
is partly oxidized to 8-methylpurine. However, the possibility that 4,5-diaminopyrimidine is formed directly from the transient intermediate cannot be excluded. 8-Methylpurine doesn't give 4,5-diaminopyrimidine under the present conditions.

The pathway described above can most probably be extended to the hydrolysis of the 6-substituted substrates. Alkaline cleavage of 9-(1-ethoxyethyl)-6-methylpurine, for example, yields both 6,8-dimethylpurine and 4,5-diamino-6-methylpyrimidine. An intermediate analogous to that in Scheme 1 is also observed, though its concentration is considerably smaller than in the hydrolysis of the unsubstituted compound.

Table 3. NMR chemical shifts for the intermediates (structure I in Scheme 1) formed in the alkaline hydrolysis of 6-substituted 9-(1-ethoxyethyl)purines in 0.10 mol dm^{-3} sodium hydroxide.

Substituent at C6	NMR chemical shifts[a]	x(I)[b]
H	^{1}H NMR: d1.57(3H), q5.75(1H), s7.07(1H), s8.10(1H) ^{13}C NMR: 26.0, 74.8, 116.4, 135.0, 148.6, 162.8	0.6
CH_3	^{1}H NMR: d1.58(3H), s2.13(3H), q5.47(1H), s7.63(1H) ^{13}C NMR: 18.2, 26.0, 73.7, 131.8, 134.4, 150.2, 161.1	0.3
NH_2	not detected	
OCH_3	not detected	
SCH_3	^{1}H NMR: d1.55(3H), s2.67(3H), q5.50(1H), s7.62(1H)	0.1
Cl	^{1}H NMR: d1.64(3H), q5.75(1H), s7.64(1H)	<0.1

[a] See footnote *b* in Table 2. [b] Approximative proportion of the intermediate of the total amount of substrate decomposed in one half-life.



Scheme 1.

Some 6-methylpurine is also formed in the reaction. Analogously, 9-(1-ethoxyethyl)adenine is decomposed to 8-methyladenine and 4,5,6-triaminopyrimidine, the expected products of the reaction described above, and free adenine. The mechanism for the production of the latter compound is unclear. Possibly intermolecular displacement of the purine base by hydroxide ion takes place, or the substrate is spontaneously decomposed to purine base and an oxocarbenium ion derived from the 1-ethoxyethyl group. The fact that 9-(1-ethoxyethyl)adenine appears to yield free purine base to a larger extent than the other nucleoside analogues studied makes the former alternative more attractive. The electropositive amino substituent increases the electron density at N9 retarding the rupture of the C – N-bond. Accordingly, it would be somewhat surprising if with this compound spontaneous decomposition of the substrate could compete with nucleophilic reactions more effectively than with those nucleoside analogues having better leaving groups. Most probably the susceptibility to polar effects is in spontaneous decomposition at least as great as in nucleophilic displacement reactions. Moreover, marked spontaneous decomposition would change the reaction order with respect to the hydroxide ion from unity at low base concentrations.

In the alkaline cleavage of the 6-methoxy, 6-methylthio, and 6-chloro compounds nucleophilic attack of hydroxide ion on C6 competes with the nucleophilic attack on C8. 9-(1-Ethoxyethyl)-6-methoxypurine is completely converted to 9-(1-ethoxyethyl)hypoxanthine. With the 6-chloro derivative this reaction represents about 60 % of the total decomposition and with the 6-methylthio derivative about 20 %. 9-(1-Ethoxyethyl)-hypoxanthine is quite stable under alkaline conditions, since it is present as N1 anion and hence not susceptible to nucleophilic attack of hydroxide ion. 6-Chloro-9-(1-ethoxyethyl)purine also yields 6-chloro-4,5-diaminopyrimidine, the product of Scheme 1, and traces of several other products. The concentration of the intermediate, I, is, however, so low that it can hardly be detected by NMR. The product mixture of the alkaline hydrolysis of 9-(1-ethoxyethyl)-6-methylthiopurine is most diverse. Besides the hypoxanthine derivative, it contains 4,5-diamino-6-methylthiopyrimidine, 8-methyl-6-methylthiopurine, 8-methylpurine and traces of 6-methylthiopurine. Possibly 8-methylpurine is formed as an oxidation product of the intermediate, I, along with 8-methyl-6-methylthiopurine.

The partial rate constants, $k(1)$, for the reaction depicted in Scheme 1 can be estimated by eqn. (1) from the rate constants observed for the decomposition of the substrates. Here [P(1)] stands for the sum concentration of the intermediates and products

$$k(1)=\frac{[\mathrm{P}(1)]}{[\mathrm{P}(\mathrm{tot.})]}k \qquad (1)$$

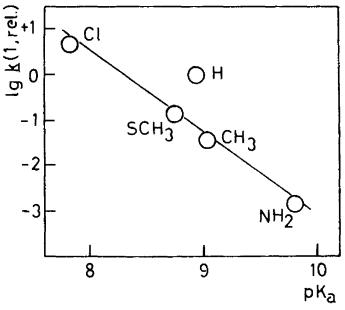


Fig. 1. The effect of the 6-substituent on the alkaline hydrolysis of 6-substituted 9-(1-ethoxyethyl)purines. The partial rate constants for the reaction depicted in Scheme 1 plotted against the pK_a-values of the correspondingly substituted purines.[17] The values of $k(1,\text{rel.})$ for the 6-CH_3 and 6-NH_2 derivatives were obtained by extrapolating $k(1)$ for the unsubstituted compound to higher temperatures *via* the Arrhenius equation.

formed by this pathway, and [P(tot.)] is the decrease in the total substrate concentration at the same moment. Estimation of the ratio [P(1)]/P(tot.)] from the ^{1}H NMR data referring to about one half-life enables the calculation of $k(1)$. As seen from Fig. 1, an approximate linear relationship exists between the logarithmic rate constants and the pK_a values of the corresponding purines. In other words, the rate of the reaction described by Scheme 1 increases with the decreasing electron density of the imidazole ring of the substrate. The value of about 2 for the slope of the straight line obtained indicates that polar effects of 6-substituents play an even more decisive role in this reaction (at C8) than in the deprotonation of the purine system (at N9). The entropy of activation, $-48\ \text{J K}^{-1}\ \text{mol}^{-1}$, for the hydrolysis of the unsubstituted compound is in the expected range for an intermolecular nucleophilic displacement reaction. The detailed mechanism cannot be deduced on the basis of the present data.

EXPERIMENTAL

Materials. 6-Substituted 9-(1-ethoxyethyl)purines were prepared from 1-chloro-diethyl ether and appropriately 6-substituted purines by the method described earlier.[18] Table 4 records the chromatographic and spectroscopic data for the compounds synthesized. The ^{13}C NMR chemical shifts observed closely resemble those reported for corresponding 9-(β-D-ribofuranosyl)derivatives, indicating that the compounds are N9 isomers. If the ethoxyethyl substituent were attached to N7, the signals for C4 and C5 would be expected to occur at about 10 ppm lower and higher field, respectively.[20]

4,5-Diaminopyrimidine and 4,5,6-triaminopyrimidine employed as reference materials were commercial products of Sigma Chemical Company. 8-Methylpurine was synthesized as described elsewhere.[17]

Table 4. Chromatographic and spectroscopic data for the 6-substituted 9-(1-ethoxyethyl)purines prepared.

Substituent at C6	R_F[a]	NMR chemical shifts[b] $\delta(2)$	$\delta(4)$	$\delta(5)$	$\delta(6)$	$\delta(8)$	δ(6-X)	$\delta(CHCH_3)$	$\delta(CH_2CH_3)$	λ(max.)/ nm[c]
H	0.84	for the ^{1}H and ^{13}C NMR chemical shifts see Ref. 18.								264
CH_3	0.86	^{1}H: s8.57				s8.13	s2.70	d1.68,q5.92	t1.07,q3.33	262
		^{13}C: 154.1	152.2	134.8	162.1	146.7	21.1	23.6,84.8	16.7,67.8	
NH_2	0.84	for the ^{1}H and ^{13}C NMR chemical shifts see Ref. 19.								262
OCH_3	0.90	^{1}H: s8.33				s8.17	s4.14	d1.85,q5.98	t1.16,q3.39	253
		^{13}C: 152.3	151.9	121.4	161.1	139.7	54.3	22.6,81.1	14.8,64.7	
		(151.7)[d]	(151.8)	(121.2)	(160.5)	(142.4)				
SCH_3	0.92	^{1}H: s8.51				s8.00	s2.70	d1.78,q5.95	t1.18,q3.41	284
		^{13}C: 152.0	148.1	131.3	161.7	140.0	11.7	22.5,80.9	14.8,64.7	
		(151.5)[d]	(148.0)	(131.3)	(160.5)	(143.1)				
Cl	0.90	^{1}H: s8.49				s8.38		d1.86,q6.01	t1.19,q3.42	267
		^{13}C: 152.1	151.0	131.6	151.6	142.9		22.5,81.7	14.8,65.0	
		(150.3)[d]	(152.4)	(132.2)	(152.4)	(146.4)				

[a] See footnote *a* in Table 2. [b] Taken as ppm from TMS. ^{1}H NMR spectra were recorded in CCl_4 and ^{13}C NMR spectra in $CDCl_3$. [c] In CH_2Cl_2. [d] The data for the corresponding 9-(β-D-ribofuranosyl) derivative.[20,21]

Kinetic measurements. The hydrolyses were carried out in stoppered bottles immersed in a thermostatted water bath. Reactions were initiated by adding the substrate in the pre-thermostatted reaction medium to give the concentration of 2×10^{-4} mol dm^{-3} and 10–12 aliquots of 2 cm^3 were withdrawn at suitable time intervals during 2–3 half-lives. The reaction was usually stopped with NaH_2PO_4 solution, the concentration of which was suitable to make the final pH 7. The unreacted starting material was extracted into methylene dichloride and the UV-absorption spectra of the organic phases were recorded. The spectra obtained at different intervals with the 6-H, 6-CH_3 and 6-NH_2 derivatives were identical with those of the starting materials and the final samples, taken at ten half-lives, exhibited no marked absorption. Accordingly, only the unreacted substrates appeared to be transferred into methylene dichloride. With the 6-OCH_3 and 6-SCH_3 derivatives the extractions were carried out directly from the cooled base solutions to keep the reaction products as their anions in the aqueous phase. The rate constants were calculated from the integrated first-order rate-equation.

Hydrolysis of 6-chloro-9-(1-ethoxyethyl)purine was too rapid to be followed by the technique described above. With this compound the determination of the rate constants was performed by continuous monitoring of the UV-spectrum of the reaction solution in the cuvette thermostatted to the temperature wanted. The rate constants were calculated by the method of Guggenheim from the absorbances at the absorption maximum of the starting material. No curvature in the Guggenheim plots was observed, suggesting that the progress of a single reaction was followed.

Product analyses. Progress of the hydrolysis reactions was also followed by TLC on Silica gel 60 using mixtures of chloroform and methanol as eluent. With these experiments the initial substrate concentration was of the order of 5×10^{-2} mol dm^{-3}.

Product mixtures at one and three half-lives were prepared by hydrolyzing the substrates as 5×10^{-2} mol dm^{-3} solutions in 0.1 mol dm^{-3} aqueous sodium hydroxide. The unreacted starting material was removed by extraction with methylene dichloride and the aqueous phase was neutralized with aqueous hydrogen chloride. Evaporation to dryness under reduced pressure afforded the product mixture that was then fractionated by preparative TLC on Silica gel 60. The products were eluted from the silica gel by methanol. About 200 mg of the mixture was applicated on one plate. UV-spectra were recorded on Unicam SP 1700 spectrophotometer, ^1H NMR spectra on Jeol JNM-PMX 60 and ^{13}C NMR spectra on Jeol FX60 spectrometers. Internal standards (DSS or TMS) were employed.

The NMR spectra of the intermediates were obtained by comparing the spectra of the product mixtures at various time intervals with the spectra of the products isolated.

UV- and NMR-spectroscopic characterization of the methylene dichloride phases evaporated to dryness indicated that only the starting material was extracted from the aqueous solution under basic conditions.

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Letters

On the Mechanism of Chlorpromazine Cation Radical Decay in Aqueous Solution

R. L. McCREERY and J. S. MAYAUSKY

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, U.S.A.

Hammerich and Parker[1] have criticized a mechanism we proposed[2] for the hydrolysis of chlorpromazine cation radical ($CPZ^{\cdot+}$) in aqueous buffers to form 50% yields of chlorpromazine sulfoxide (CPZO) and neutral precursor (CPZ). The rate law for the process has the form (1),

$$\frac{d[CPZ^{\cdot+}]}{dt} = \frac{-A_1[B^-][CPZ^{\cdot+}]^2}{([CPZ]+A_2)[H^+]} \quad (1)$$

where B^- is a buffer anion which acts as a catalyst (*e.g.*, $H_2PO_4^-$ or citrate anion) and A_1 and A_2 are constants. Hammerich and Parker were critical of our proposed mechanism [eqns. (2)–(4)] and offered an alternative [(5)–(8)] which has the same observed rate law [eqn. (1)] if certain assumptions are met.

$$CPZ^{\cdot+} + B^- + H_2O \overset{K_2}{\rightleftarrows} [CPZB(OH)]^{\cdot-} + H^+ \quad (2)$$

$$[CPZB(OH)]^{\cdot-} + CPZ^{\cdot+} \underset{k_{-2}}{\overset{k_3}{\rightleftarrows}} [CPZBOH]^{\cdot} + CPZ \quad (3)$$

$$[CPZBOH]^{\cdot} \overset{k_4}{\rightarrow} HB + CPZO \quad (4)$$

$$CPZ^{\cdot+} + B^- \overset{K_5}{\rightleftarrows} CPZB^{\cdot} \quad (5)$$

$$CPZB^{\cdot} + CPZ^{\cdot+} \underset{k_{-6}}{\overset{k_6}{\rightleftarrows}} CPZ + CPZB^+ \quad (6)$$

$$CPZB^+ + H_2O \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} (CPZBOH) + H^+ \quad (7)$$

$$(CPZBOH) \overset{k_8}{\rightarrow} HB + CPZO \quad (8)$$

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Hammerich and Parker maintain that their mechanism [(5)–(8)] is simpler than ours [(2)–(4)] and is more consistent with other half regeneration mechanisms observed for other systems in nonaqueous solvents.

Apparently Hammerich and Parker were not aware of a paper we published in 1979[3] which further discussed the role of water and protons in the reaction. In that work, it was shown that the reaction of $CPZ^{\cdot+}$ with acetate ion is pH independent once the variation of free acetate ion concentration with pH is considered. Furthermore, water need not be involved until the final step in the process. We have also shown more recently[4] that the pH dependence of phosphate, acetate and several other nucleophiles is completely explained by the variation in concentration of various nucleophilic species ($H_2PO_4^-$, HPO_4^{-2}, *etc.*). In 1979, the following mechanism was concluded[3] for acetate attack of $CPZ^{\cdot+}$.

$$CPZ^{\cdot+} + RCO_2^- \overset{K_9}{\rightleftarrows} (CPZRCO_2)^{\cdot} \quad (9)$$

$$(CPZRCO_2)^{\cdot} + CPZ^{\cdot+} \overset{K_{10}}{\rightleftarrows} CPZ + (CPZRCO_2)^+ \quad (10)$$

$$H_2O + (CPZRCO_2)^+ \overset{k_{11}}{\rightarrow} CPZO + H^+ + RCO_2H \quad (11)$$

Note that there is no pH dependence of the observed rate, since the proton is involved after the rate determining step. It was also pointed out in the same paper that the phosphate/$CPZ^{\cdot+}$ reaction has the same mechanism, except for proton loss from phosphate in the first step. In subsequent work,[4,5] we showed in detail that the $[H^+]$ dependence indicated in eqn. (1) for the phosphate case stems solely from its effect on the distribution of HPO_4^{-2} and $H_2PO_4^-$ concentrations. Note that eqns. (9)–(11) (Ref. 3, Scheme II) are the same as proposed by Hammerich and Parker[1] for 10 methyl phenothiazine cation radical reacting with acetate ion in acetonitrile, except for the involvement of water. We are pleased to see these results confirmed for a different cation radical and solvent.

Hammerich and Parker are correct in their criticism of the intermediate produced in reaction

(2), particularly in light of our subsequent publications. While reactions (2)–(4) are consistent with the rate law available at the time, the anion radical $(CPZBOH)^{\cdot -}$ is a fairly unlikely intermediate. Furthermore, our subsequent work proved that reactions (9)–(11) apply to $CPZ^{\cdot +}$ reactions with acetate, phosphate and citrate, with the pH dependence of the rate stemming from variations in the concentrations of various nucleophilic species with $[H^+]$. We should note that bifunctional nucleophiles (of the type $R(CO_2^-)_2$) can undergo a different series of reactions[5] in parallel with eqns. (9)–(11), but this sequence is not relevant to the present disucssion.

While the mechanism in reactions (9)–(11) is similar to those reported by others[6,7] for different systems, it would have been extremely speculative to assume that $CPZ^{\cdot +}$ reacted the same way as thianthrene and diphenylanthracene without proof. We studied a different heterocyclic system, a different solvent, different nucleophiles, and both water and protons were involved. N-substituted phenothiazine cation radical reaction products and mechanism are highly dependent on radical structure,[3,8] nucleophile structure[9] and solution conditions, as was the case with the systems examined in Hammerich and Parker's work. While it is true that many systems, including $CPZ^{\cdot +}$, follow a half regeneration mechanism (for certain combinations of radical and nucleophiles) each chemical system has its own qualities, and generalizations are dangerous. The mechanism which Hammerich and Parker criticized was modified by us a year later, but the major points remain. The original conclusions about the reactivity of $CPZ^{\cdot +}$ (as opposed to a dication), the involvement of the buffer as a catalyst, and the detailed kinetic analysis were new to that work, and formed the basis of several subsequent studies.

In conclusion, the mechanism proposed by Hammerich and Parker (reactions (5)–(8)) is not possible given results from our laboratory both published and in press. When the nucleophilic attack of CPZ cation radical is carried out in aqueous solution, the pH and buffer effects are explained by a careful determination of the species involved in the reaction. Most importantly, each radical/nucleophile combination has its own qualities, and the many interesting aspects of the work from both laboratories should not be excessively generalized.

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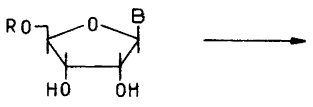
5'-*O*-Trityl Group Promoted Directive Effect in the Preparation of 2'-*O*-Methylribonucleosides

JARMO HEIKKILÄ,[a] SVEN BJÖRKMAN,[a] BO ÖBERG[b] and JYOTI CHATTOPADHYAYA[a,*]

[a]Department of Microbiology, Box 581, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden and [b]Antiviral Research and Developement Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

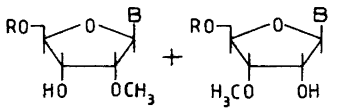
It has become apparent in the last decade that 2'-*O*-methylribonucleosides make a significant contribution to the structure and function of rRNA and tRNA.[1] It has also come to light in the last few years that a lack of a ribose-2'-*O*-methyl group is often responsible, in certain cases of rRNA, for the lack of formation of functional ribosomes.[1] A survey of literature[2-4] reveals several methods of partial methylation of ribonucleosides followed by extensive ion-exchange chromatographic separation and purification which eventually lead to poor overall yields of 2'-*O*-methylribonucleosides. Garegg *et al.*[5] have recently circumvented these tedious ion-exchange chromatographic separation procedures by carrying out a partial methylation on 5'-*O*-*t*-butyldiphenylsilylguanosine with the help of diazomethane in dimethylformamide at 50 °C, which is catalyzed by tin(II) chloride, followed by a facile separation of 2'- and 3'-monomethylated products by chromatography on silica gel using a chloroform–methanol mixture in the mobile phase. In this way, they obtained 5'-*O*-*t*-butyldiphenylsilyl-2'-*O*- and -3'-*O*-methylguanosine, respectively, in 22% and 40% yields. It is worthwhile emphasizing that it is the lipophilic silyl group at 5'-position that allowed these workers to resolve the 2'- and 3'-monomethyl ethers upon silica gel chromatography. We were obviously interested by their results and replaced the 5'-*O*-silyl group with a less expensive and more easily accessible trityl (triphenylmethyl-) group and repeated their experiment at 50 °C for partial methylation on 2-*N*-*t*-butylbenzoyl-5'-*O*-tritylguanosine (*1*). We isolated a glass, after a silical gel chromatography using $CHCl_3$-methanol mixture, in 48% yield. A ^1H NMR analysis of the glass showed the presence of 2'- and 3'-ethers, (*8a*) and (*10a*), respectively, in 71 and 29%. Thus the 2'-*O*-methylether of 2-*N*-*t*-butylbenzoyl-5'-*O*-tritylguanosine (*8a*) was obtained from this mixture in 29.9% yields. A more interesting observation was that when the partial methylation was carried out on (*1*) with CH_2N_2 in dimethylformamide at 0 °C and a catalytic amount of tin(II) chloride, we obtained only 2'-*O*-methyl ether (*8a*) in 43% yield. There was no detectable amount of 3'-*O*-methylether (*10a*) formed under the above condition as monitored by ^1H NMR spectroscopy. However, when we performed these partial methylations on 2-*N*-*t*-butylbenzoyl quanosine (*2*) at 0 °C and 50 °C, under the above condition we observed the formation of 2' and 3'-*O*-methyl 2-*N*-*t*-butylbenzoylquanosine, (*8b*) and (*10b*), in an almost identical ratio (55:45). The above experiments, along with Garegg and co-worker's observation, clearly lead us to attribute this hitherto unobserved phenomenon, to the trityl group at the 5'-position which is most probably exerting a directive influence on the methylation of the 2'-hydroxyl

*To whom correspondence should be addressed.



1, B=*11*, R=Tr;
2, B=*11*, R=H;
3, B=*12*, R=Tr;
4, B=*12*, R=H;

5, B=*13*, R=Tr
6, B=*13*, R=H
7, B=*14*, R=Tr
9, B=*14*, R=H



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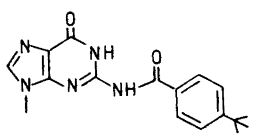
10b, B=*11*, R=H



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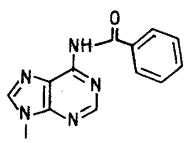

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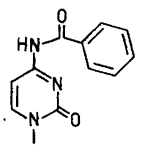

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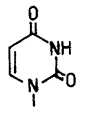

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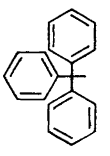

Tr (Trityl)

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Table 1. Yield and data of 2′- and 3′-methylribonucleosides.

Substrates	2′- and 3′-monomethyl ethers at 50 °C (%)[a]				2′- and 3′-methyl ethers at 0 °C (%)[a]				^{1}H NMR (δ)[b]				R_f values[c]	
	^{1}H NMR ratios		Isolated yields (%)		^{1}H NMR ratios		Isolated yields (%)		H-1′		O-CH_3			
	2′-O-CH_3	3′-O-CH_3	2′+3′	2′	2′-O-CH_3	3′-O-CH_3	2′+3′	2′	2′-	3′-	2′-	3′-	2′-	3′-
5′-O-Trityl-2-N-t-butylbenzoylguanosine (*1*)	71	29	48	29.9	100	0	43	43	5.91, 5.76		3.45, 3.41		0.45	0.44
2-N-t-Butylbenzoylguanosine (*2*)	56	44	36	–	55	45	–	–	6.01, 5.91		3.48, 3.46		0.63	
5′-O-Trityl-6-N-benzoyladenosine (*3*)	60	40	54.2	30.3	65	35	57.1	36.5	6.18, 6.0		3.52, 3.45		0.50	0.45
6-N-Benzoyladenosine (*4*)	45	55	65	–	50	50	–	–	5.95, 5.80		3,65, 3.37		0.70	
5′-O-Trityl-4-N-benzoylcytidine (*5*)	85	15	58.1	49.0	90	10	50.9	46.7	6.04, 5.95		3.76, 3.48		0.41	0.23
4-N-Benzoylcytidine (*6*)	63	37	58	–	60	40	–	–	5.94, 5.79		3.68, 3.47		0.73	
5′-O-Trityluridine (*7*)	62	38	51.6	27.6	74	26	57.6	41.2	5.96, 5.90		3.64, 3.44		0.53	0.42
Uridine (*9*)	45	55	–	–	51	49	–	–	5.87, 5.75		3.76, 3.64		0.42	

[a] These are based on 1 mmol scale experiment. [b] Solvent: $CDCl_3$ for tritylated compound and *ca.* 5% CD_3OD in $CDCl_3$ for other compounds. [c] Merck pre-coated silica gel F_{260} plates. Tritylated compounds: acetone – water – methylene chloride (30:0.5:69.5, v/v/v). Non-tritylated compounds: methanol – chloroform (20:80, v/v).

group by shielding the 3′-hydroxyl position. As far as our knowledge of the literature goes, this is the first direct evidence in ribonucleoside chemistry when a remotely located group kinetically controls the preferential formation of a product by interacting through space. Similar observations are also borne out in the case of other ribonucleoside derivatives, (*3*) to (*9*) (Table 1), when the yields of formations of 2′-*O*-methyl ethers are compared with the yield of 3′-*O*-methyl ethers, in the presence or absence of a trityl group at 5′ position, at 0 and at 50 °C.

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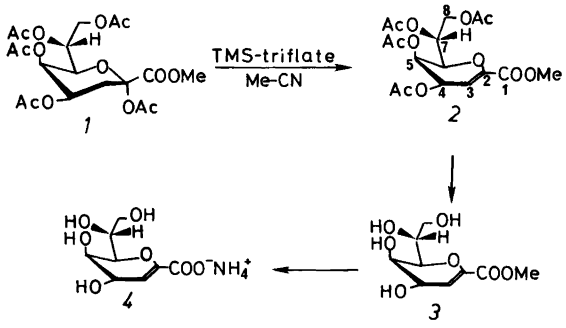
Short Communications

Synthesis of α,β-Unsaturated Analogues of KDO and *N*-Acetyl-neuraminic Acid by Trimethylsilyl Triflate-catalyzed Elimination Reactions

ALF CLAESSON and KRISTINA LUTHMAN

Department of Organic Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden



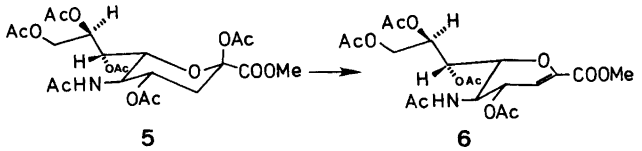
3-Deoxy-D-*manno*-2-octulosonic acid (KDO) is a unique constituent of the lipopolysaccharides of gram-negative bacteria. Very recently its chemistry and biological significance has been reviewed.[1] In a program aimed at synthesizing inhibitors of KDO metabolism we have discovered and report here a short synthesis of an unsaturated analogue (*4*), which might exhibit interesting biological properties. The synthesis, which brings a new facet to the already vivid chemistry of trimethylsilyl (TMS) sulfonates (review[2]), is simpler than one reported in a preliminary form.[3] We have also applied this method to the synthesis of the fully acetylated methyl ester of 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid. This acid and analogues are neuraminidase inhibitors of pharmaceutical interest.[5]

Treatment of the pentaacetate-methyl ester (*1*)[4] of KDO with catalytic amounts (0.1 mol equivalents) of TMS-trifluoromethanesulfonate gave after a few hours at room temperature the elimination product (*2*) in 91% yield. The solvent was acetonitrile. TLC data indicate that the reaction also goes to completion in nitromethane. Other solvents were not tested. A small amount of cyanotrimethylsilane was added as a scavenger of traces of acid in the catalyst. The omission of this reagent did not, however, seem to adversely affect the yield of the product (TLC data only). Deprotection of the unsaturated compound *2* by standard methods completed the synthesis of *4*.

The elimination of acetic acid from the neuraminic acid derivative *5* proceeded equally well by using 0.2 equivalents of TMS-triflate. The unsaturated compound *6* was isolated in 90% yield. The deprotection of this compound has already been described.[5]

The efficacy of TMS-triflate in catalytic amounts probably means that it is regenerated through the reaction of trifluoromethanesulfonic acid with the other reaction product *i.e.* TMS-acetate. The strong sulfonic acid might also in itself function as an efficient elimination catalyst.

Experimental. Melting points were determined in open capillary tubes and are uncorrected. NMR spectra were recorded on Jeol FX 90 Q or Jeol FX-200 spectrometers. TMS was used as internal standard in $CDCl_3$ and CD_3OD, and *t*-BuOH (^{13}C NMR δ 32.2, ^1H NMR δ 1.23) in D_2O. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Microanalyses were carried out at the Microanalytical Laboratory, Royal Agricultural College, Uppsala.



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Methyl 4,5,7,8-tetra-O-acetyl-2,6-anhydro-2,3-dideoxy-D-manno-2-octenoate (2). To a solution of KDO – pentaacetate – methyl ester (*1*)[4] (545 mg; 1.18 mmol) in 2 ml of dry acetonitrile was added 10 μl of cyanotrimethylsilane followed by trimethylsilyl trifluoromethanesulfonate (20 μl; 0.11 mmol). The mixture was left at room temperature for 4 h and then 0.5 g K_2CO_3 was added. After evaporation of solvent the residue was chromatographed on silica gel (60 ml) using ether – pentane, 3:1, as eluent. Pooling of the central fractions gave 350 mg of pure product, m.p. 132 – 133 °C (lit.[3] 129 – 132 °C), $[\alpha]_D^{23}$ −17.4° (*c* 1.42, $CHCl_3$) (lit.[3] $[\alpha]_D^{20}$ −14.8°). Altogether 429 mg (91 %) of crystalline product was obtained. Anal. $C_{17}H_{22}O_{11}$: C,H. ^{1}H NMR ($CDCl_3$): δ 5.89 (dd,H3), 5.71 (ddd,H4), 5.48 (ddd,H5), 5.19 – 5.36 and 4.13 – 4.72 (two m,H6-H8), 3.82 (s,OCH_3), 2.03 – 2.08 (Ac). ^{13}C NMR ($CDCl_3$): δ 169.1 – 170.1 (Ac), 161.2 (Cl), 144.3 (C2), 107.3 (C3), 73.2 (C6), 67.2 (C4), 64.6 (C7), 61.8 (C8), 60.6 (C5), 52.4 (OCH_3), 20.6 (Ac).

Methyl 2,6-anhydro-2,3-dideoxy-D-manno-2-octenoate (3). Compound *2* (400 mg) was treated with NaOMe (from 40 mg Na) in 5 ml MeOH for 1 h. Neutralization with MeOH-washed ion-exchange resin (H^+), filtration and evaporation gave 200 mg (~100 %) of crystalline residue. Recrystallization from MeOH-ether gave a product with m.p. 162 – 164 °C, $[\alpha]_D^{23}$ −31.9° (*c* 0.90, MeOH). Anal. $C_9H_{14}O_7$: C,H. ^{1}H NMR (CD_3OD): δ 5.45 (dd,H3), 4.08 (ddd,H4), 3.75 (ddd,H5), 3.6 – 3.2 (m,H6 – H8), 3.38 (s,OCH_3). ^{13}C NMR (CD_3OD): δ 164.0 (Cl), 145.0 (C2), 113.8 (C3), 78.9 (C6), 70.9 (C4), 66.7 (C7), 64.7 (C5), 64.5 (C8), 52.9 (OCH_3).

Ammonium 2,6-anhydro-2,3-dideoxy-D-manno-2-octenoate (4). Compound *3* (200 mg) was dissolved in 3 ml of water and 5 M NaOH was added dropwise until the mixture stayed alkaline (pH ~10) for 10 min. The reaction mixture was passed through an NH_4^+-saturated ion-exchange resin and the water was evaporated to almost dryness. The residue was treated with methanol, ethanol, and 2-propanol until no more crystals precipitated on placing the mixture in the refrigerator and then in the freezer. Yield = 76 %. M.p. 178 – 180 °C. $[\alpha]_D^{23}$ −15.8° (*c* 1.1 H_2O – MeOH 1:1). Anal. $C_8H_{15}NO_7$: C,H,N. ^{1}H NMR (D_2O): δ 5.56 (dd,H3), 4.57 (ddd,H4), 4.13 (ddd,H5), 3.97 – 3.74 (m,H6 – H8). ^{13}C NMR (D_2O): δ 171.7 (C1), 151.2 (C2), 110.1 (C3), 79.0 (C6), 71.7 (C4), 67.7 (C7), 65.5 (C5), 65.0 (C8).

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,6-anhydro-2,3,5-trideoxy-D-glycero-D-galacto-2-nonenoate (6). Fully acetylated neuraminic acid methyl ester (5) (70 mg, 0.13 mmol) in 2 ml of acetonitrile was treated with 5 μl (0.027 mmol) TMS-triflate for 5 h. After addition of K_2CO_3 (0.1 g) and evaporation, the residue was filtered through a silica gel column with ethyl acetate as eluent. Yield: 56 mg (90 %). The ^{13}C and ^{1}H NMR data were in full agreement with those reported in the literature.[6]

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Chlorination of Carboxylic Acid Derivatives. X. Chlorine Substitution on the Alcohol Chain of Aliphatic C_2-C_8 Alkyl Chloro-, Dichloro- and Trichloroacetates

ILPO O. O. KORHONEN

Department of Chemistry, University of Jyväskylä, Kyllikinkatu 1–3, SF-40100 Jyväskylä 10, Finland

Many investigations have been published on the chlorination of aliphatic alkyl acetates,[1] but few on the chlorination of alkyl chloroacetates. Waddle and Adkins have studied the chlorination of butyl trichloroacetates[2] and Gayler and Waddle the chlorination of propyl trichloroacetates.[3] The monochloro products of these esters were saponified and analyzed as the corresponding alcohols.

The present paper reports the isomer distributions of the monochloro products formed in liquid phase chlorinations of aliphatic C_2-C_8 alkyl

Table 1. The relative quantities[a] of monochloro isomers formed in the chlorinations of aliphatic C_2-C_8 *n*-alkyl acetates (A), chloroacetates (C), dichloroacetates (D) and trichloroacetates (T).

Chain length	Substrate	Isomeric monochlorinated esters							
		1-Cl	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl
C_2	A	170	100						
	C	175	100						
	D	156	100						
	T	134	100						
C_3	A	46	116	100					
	C	32	107	100					
	D	23	105	100					
	T	19	104	100					
C_4	A	29	94	179	100				
	C	17	69	163	100				
	D	11	61	151	100				
	T	9	60	150	100				
C_5	A	27	68	161	179	100			
	C	15	54	159	195	100			
	D	12	50	141	183	100			
	T	8	40	107	153	100			
C_6	A	21	62	132	173	182	100		
	C	15	48	126	188	193	100		
	D	9	41	104	161	174	100		
	T	7	38	89	148	166	100		
C_7	A	22	63	128	154	171	176	100	
	C	15	47	113	143	173	178	100	
	D	9	40	101	140	171	175	100	
	T	7	35	83	125	155	158	100	
C_8	A	22	65	127	151	166	176	182	100
	C	15	44	113	141	159	175	179	100
	D	9	40	97	129	145	163	164	100
	T	7	32	82	118	132	145	152	100

[a] Relative to the ω-chloro isomers (= 100); the values of acetic acid derivatives (A) are taken from an earlier paper.[1] Values are averages of two independent experiments and agree to within ±4%.

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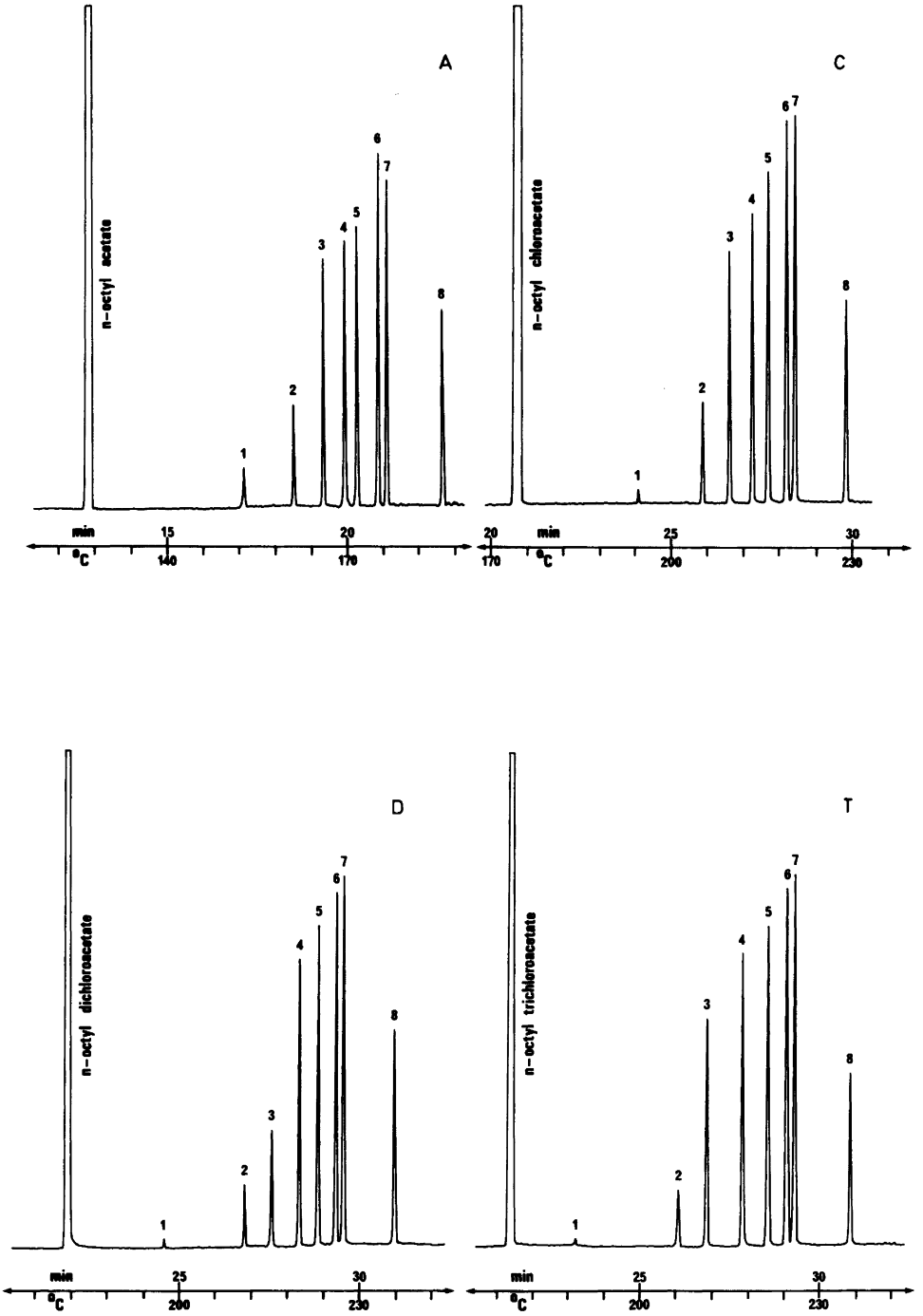

Fig. 1. Chromatograms of monochlorinated *n*-octyl acetates (A), chloroacetates (C), dichloroacetates (D) and trichloroacetates (T), analyzed on OV-351 quartz capillary column. Temperature programme from 50 °C at 6 °C/min. The peak number indicates the position of the Cl-substituent.

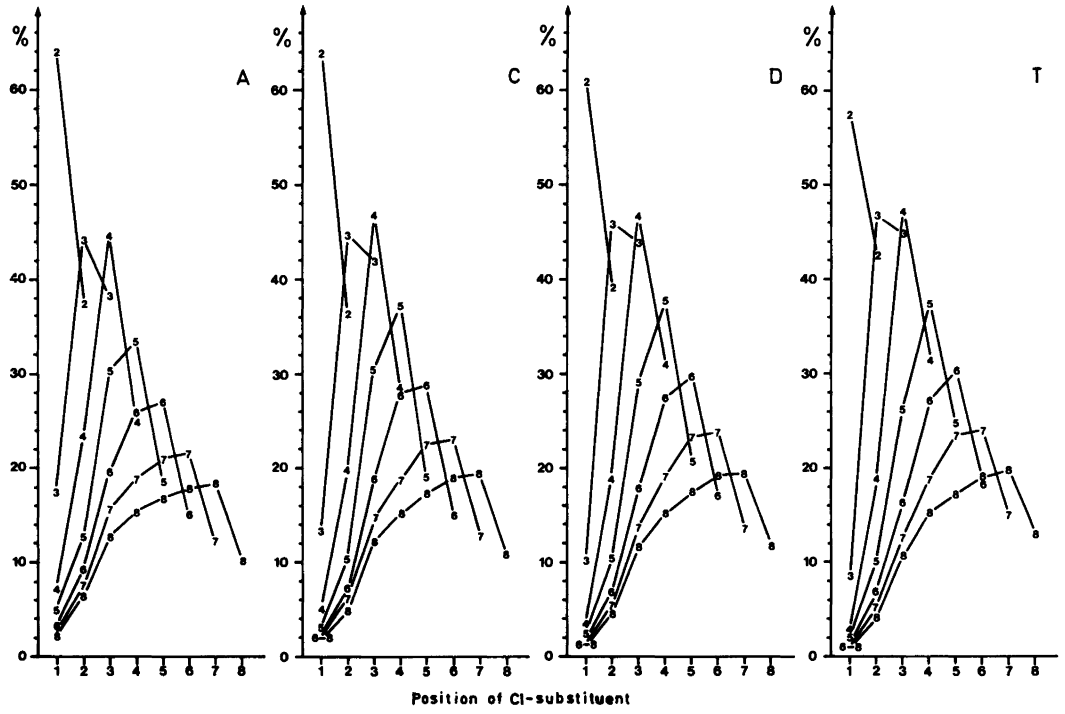

Fig. 2. Isomer distribution of monochlorinated C_2-C_8 *n*-alkyl acetates (A), chloroacetates (C), dichloroacetates (D) and trichloroacetates (T) based on GLC analyses. The numbers denote the alcohol chain length.

esters of chloroacetic, dichloroacetic and trichloroacetic acids. The results are compared with those of earlier chlorinations of acetic acid derivatives given in Ref. 1.

Results. The monochlorinated products were determined by gas-liquid chromatography (GLC) and gas-liquid chromatography-mass spectrometry (GLC-MS). Fig. 1 presents the chromatograms of monochlorinated octyl acetates,[1] and chloro-, dichloro- and trichloroacetates. As can be seen, complete separation of the isomers was achieved on a polar column under suitable operating conditions. The products were eluted in direct order from 1-chloro to ω-chloro compounds, as were the corresponding alkyl acetates.[1] On polar OV-351 phase the retention times of the corresponding chloroalkyl esters increased in the order chloroacetate < trichloroacetate < dichloroacetate, except for 1-chloroalkyl esters where the trichloro isomers were eluted before the monochloro isomers (Fig. 1). A quite different elution order was observed on a non-polar SE-30 column.[4]

Fig. 2 illustrates the isomer distributions of monochlorinated products. The values for chloroalkyl acetates are taken from an earlier paper.[1] The relative quantities are presented in Tables 1 and 2. As can be seen, the main products were always the $(\omega-1)$-chloro isomers. The deactivation at the 1-position increases with increase in the degree of chlorination of the adjacent acetoxy group[5] (Table 2). The effect is smaller at positions further away. In the case of butyl acetate, for example, the substitution in the 2-position is 3.2 times as great as in the 1-position. The corresponding ratios in butyl chloro-, dichloro- and trichloroacetates are 4.1, 5.5 and 6.7, respectively.

The 1-chloroalkyl esters of chlorinated acetic acids are somewhat unstable. Hydrogen chloride is clearly lost during storage leading to the formation of unsaturated compounds, particularly in the case of 1-chloroalkyl trichloroacetates. Thus, if hydrogen chloride is lost during the chlorination[1,6] or GLC analysis, the true proportions of monochlorinated products will be greater than those given in this paper.

Experimental. Aliphatic C_2-C_8 *n*-alkyl chloro-, dichloro- and trichloroacetates were prepared in our laboratory as described earlier.[7]

Table 2. The relative quantities[a] of monochloro isomers formed in the chlorinations of aliphatic C_2-C_8 *n*-alkyl chloroacetates (C), dichloroacetates (D) and trichloroacetates (T).

Chain length	Substrate	Isomeric monochlorinated esters							
		1-Cl	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl
C_2	C	101	98						
	D	97	105						
	T	91	115						
C_3	C	76	101	110					
	D	58	104	115					
	T	48	106	117					
C_4	C	70	85	105	115				
	D	49	80	105	124				
	T	39	80	105	126				
C_5	C	58	82	101	111	102			
	D	48	82	96	112	110			
	T	40	80	87	111	130			
C_6	C	72	77	95	109	106	100		
	D	47	74	90	106	109	114		
	T	38	74	83	105	112	123		
C_7	C	74	79	94	98	107	107	106	
	D	44	70	87	100	111	110	111	
	T	37	69	80	99	111	111	123	
C_8	C	73	73	95	99	102	106	105	107
	D	50	71	90	99	102	108	105	117
	T	41	64	84	100	102	106	108	129

[a] Relative (= 100) to the corresponding isomers formed in the chlorination of *n*-alkyl acetates (see Table 1).

The chlorinations were carried out with chlorine in the liquid phase at room temperature.[1] The amounts of higher chlorinated products were only a few per cent and no chlorine substitution in the acid chain in chloro- and dichloroacetates could be observed.

GLC analyses were run on a Perkin-Elmer Model Sigma 3 instrument equipped with a fused silica OV-351 quartz capillary column (25 m × 0.32 mm I.D.), supplied by Orion Analytica (Espoo, Finland). The operating conditions used are given in Ref. 4.

GLC-MS data were recorded as described earlier.[1] The mass spectra will be published later.

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Synthesis of the 1,2:3,4-Di-*O*-isopropylidene Derivatives of D-*xylo*- and D-*lyxo*-3-Hexulose

SVEIN MORGENLIE

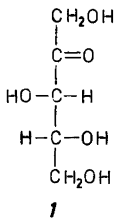
Agricultural University, Department of Chemistry, N-1432 Ås-NLH, Norway

In connection with analysis of product mixtures from aldol condensations, it was necessary to know the gas chromatographic behaviour and mass spectral data of the di-*O*-isopropylidene derivatives of the 3-hexuloses with the *xylo* and *lyxo* configurations. The preparation of 3-hexuloses by chromium trioxide oxidation in acetic acid of ethylidene or benzylidene derivatives of hexitols has been described.[1,2] Other, less general methods have also been reported for the preparation of such compounds,[3,4] but the need was felt for a more simple and rapid small-scale synthesis of the two required 3-hexuloses. It is known that the first step in the ribulose-monophosphate cycle of formaldehyde fixation in some methylotropic bacteria is the condensation of formaldehyde and D-*erythro*-pentulose 5-phosphate (D-ribulose 5-phosphate) to give D-*arabino*-3-hexulose 6-phosphate.[5-8]

The present paper reports on a method, elaborated with this formaldehyde fixation as a model, for the preparation of a mixture of the D-*xylo*- and D-*lyxo*-3-hexuloses and their separation as di-*O*-isopropylidene derivatives.

D-*threo*-Pentulose (*1*), easily prepared by isomerisation of D-*xylose* in hot pyridine,[9] and also commercially available, was treated with formaldehyde in sodium hydroxide solution at room temperature to give, in addition to unchanged starting material, approximately equal amounts of D-*xylo*- (*2*) and D-*lyxo*-3-hexulose (*3*). Traces of xylose were also observed in the product mixture. The optimal reaction time was about 30 min, prolonged reaction caused diminished yields of *2* and *3*, and several secondary products were formed. Treatment of the product mixture with acetone – sulfuric acid gave 2,3-*O*-isopropylidene-β-D-*threo*-pentulofuranose (*4*) from *1* and di-*O*-isopropylidene derivatives from *2* and *3*. The di-*O*-isopropylidene derivatives were easily separated from *4* by partitioning between water and chloroform. A procedure involving partial hydrolysis to mono-*O*-isopropylidene derivatives and a second partitioning between chloroform and water followed by re-isopropylidenation of the compounds from the water phase, gave a mixture which except for traces of the di-*O*-isopropylidene derivative of xylose contained almost exclusively 1,2:3,4-di-*O*-isopropylidene-β-D-*xylo*-3-hexulofuranose (*5*) and 1,2:3,4-di-*O*-isopropylidene-β-D-*lyxo*-3-hexulofuranose (*6*). Compounds *5* and *6* were readily separated by column chromatography on aluminum oxide, due to a surprisingly high mobility of the former, and they were obtained in a combined yield of about 25%.

The electron impact mass spectra of *5* and *6* are almost identical, and they show that the compounds are 1,2:3,4-di-*O*-isopropylidene derivatives. Primary fragmentation between C-2 and C-3 gives



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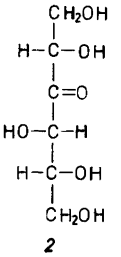

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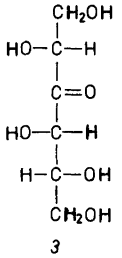

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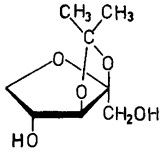

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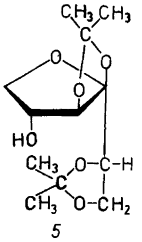

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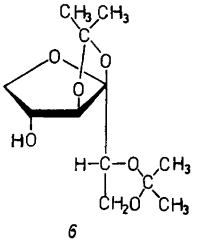

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rise to fragments with *m/z* 101 and 159, with a greater relative abundance of the latter than that usually observed for the corresponding *m/z* 159 fragment in the spectra of 1,2:5,6- or 2,3:5,6-di-*O*-isopropylidene aldoses.[10] This is presumably due to the possibility of charge stabilisation by lone-pair electrons from O-3 and O-6 in the fragment from the 3-hexulose derivatives, whereas lone-pair electrons only from O-4 may participate in the aldose derivative fragments. The *m/z* 200 and 185 peaks in the spectra of *5* and *6* are obviously analogues of the *m/z* 130 and 115 peaks in the spectra of 2-ketoses carrying a 2,3-*O*-isopropylidene group.[10,11] The fact that the two 3-hexuloses both form 1,2:3,4-di-*O*-isopropylidene derivatives confirms the expected *xylo* and *lyxo* configurations, since it has been shown that those with *ribo* and *arabino* configuration give 1,2:4,5- or 2,3:4,5-di-*O*-isopropylidene acetals under similar conditions.[2]

The compounds *4*, *5* and *6* are readily separated by gas chromatography, and the condensation reaction thus may be monitored by isopropylidenation of aliquots withdrawn after different reaction times or under varying reaction conditions. Good separation of *5* and *6* is also obtained on TLC on silica gel, and again a high mobility is observed for the *xylo* isomer (*5*). A reason for the low polarity of compound *5* may be suggested on the basis of molecular model inspections; intramolecular hydrogen bonding between OH-5 and O-2 appears sterically possible in a favourable conformation with *anti*-periplanar arrangement of C-1 and C-4 around the C-2/C-3 bond in *5*, whereas for the *lyxo* isomer (*6*), such hydrogen bonding is presumably less pronounced, since it would require an energetically less favourable *syn*-clinal orientation of C-1 and C-4 around the C-2/C-3 bond (Fig. 1).

In the infrared spectra of *5* and *6*, recorded in tetrachloromethane, a relatively sharp OH stretching absorption band at 3460 cm^{-1} is observed, which is unchanged on dilution and thus is characteristic of intramolecular hydrogen bonding. This band is of considerably greater intensity in the spectrum of *5* than in that of *6*, supporting the suggested difference in the intramolecular hydrogen bond formation.

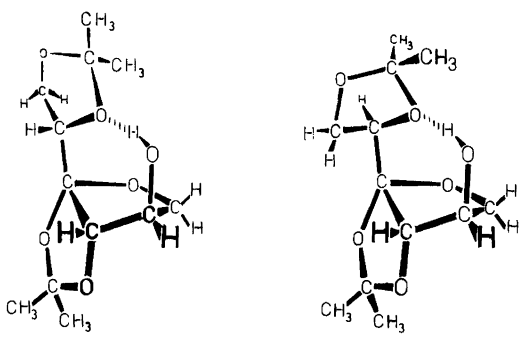

Fig. 1. Conformations of *5* (left) and *6* (right) allowing intramolecular hydrogen bonding.

Experimental. TLC was performed on silica gel G plates in chloroform – methanol 50:1, detection was effected with diphenylamine – aniline – phosphoric acid.[12] For GLC was used a Perkin-Elmer F 11 gas chromatograph, equipped with a flame ionisation detector and a glass column (2 m × 1.5 mm i.d.) filled with 3% OV-225 on 100/120 Supelcoport. The temperature was programmed at 4°/min from 90 to 210 °C. Mass spectra were recorded with a Micromass 12 F mass spectrometer, operating at 70 eV, and IR spectra with a Perkin-Elmer infrared spectrophotometer 597.

Base-catalysed condensation of D-threo-*pentulose and formaldehyde.* To D-*threo*-pentulose[9] (*1*, 10 mg) in water (2 ml) were added 3.5% formaldehyde solution (0.3 ml) and 0.2 M sodium hydroxide (2.2 ml). The solution was kept at room temperature, and aliquots (0.5 ml) were withdrawn at intervals, neutralised with Dowex 50 W (H^+) ion exchange resin and concentrated to dryness under reduced pressure. The residues were treated with 2% sulfuric acid in acetone (1 ml) for 90 min, the acetone solutions were neutralised with solid sodium hydrogencarbonate and subjected to GLC. Three major components, *4*, *5* and *6*, were observed, with retention times 10.10, 8.05 and 10.45 min, respectively.

*Preparation of 1,2:3,4-di-*O*-isopropylidene-β-*D-xylo-*(5) and* -lyxo-*(6)-3-hexulofuranose.* To D-*threo*-pentulose (*1*, 100 mg) in water (20 ml) were added 3.5% formaldehyde solution (3 ml) and 0.2 M sodium hydroxide (23 ml). The solution was kept at room temperature for 30 min and then neutralised with Dowex 50 W (H^+) ion exchange resin. After filtration of the solution, the solvent was removed under diminished pressure, and the residue was treated with 2% sulfuric acid in acetone (10 ml) for 90 min. The solution was neutralised with solid sodium hydrogencarbonate and, following filtration, the solvent was removed. The residue was dissolved in chloroform (10 ml), the chloroform solution extracted with water (10 ml) and the chloroform removed at reduced pressure. The residue was dissolved in 70% aqueous acetic acid (10 ml) and the solution kept at 55 °C for 90 min. After removal of the solvents under reduced pressure, the residue was dissolved in water (10 ml), the water solution was extracted with chloroform (5 ml) and the chloroform extract re-extracted with water (5 ml). From the combined water solutions the solvent was removed under reduced pressure, and the residue was stirred with 0.2% sulfuric acid in acetone (10 ml) for 1 h. The acetone solution was

neutralised with solid sodium hydrogencarbonate and then it was filtered. TLC showed that the solution contained traces of a compound (R_F 0.56), indistinguishable from 1,2:3,5-di-*O*-isopropylidene-α-D-xylofuranose, and two major components (R_F 0.52 and 0.29), giving a brown-to-red colour with the spray reagent. After removal of the solvent, the residue was dissolved in chloroform (1 ml) and subjected to chromatography on a column (20 cm × 1.4 cm i.d.) filled with neutral aluminum oxide, activity grade II. Hexane-chloroform 5:1 (v/v) eluted the compound with R_F (TLC) 0.52, crystallisation from hexane gave *5* (19 mg, 11 %), m.p. 103 – 105 °C, $[\alpha]_D^{20}$ +12° (*c* 2, chloroform) (Lit.[2] m.p. 104 – 105 °C, $[\alpha]_D$ −16.6° for the L-enantiomer). MS [IP 70 eV; *m/z* (% rel. int.)]: 245 (31), 200 (20), 187 (9), 185 (14), 159 (40), 127 (21), 101 (41), 85 (12), 71 (13), 59 (89), 43 (100). The compound with R_F (TLC) 0.29 was eluted with chloroform, evaporation of the solvent gave crystalline *6* (23 mg, 13 %), m.p. 119 – 122 °C, $[\alpha]_D^{20}$ +10° (*c* 2, chloroform) (Lit.[2] m.p. 122 – 123 °C, $[\alpha]_D$ −14.7° for the L-enantiomer). MS [IP 70 eV; *m/z* (% rel. int.)]: 245 (26), 200 (12), 187 (7), 185 (18), 159 (49), 127 (24), 101 (49), 85 (12), 71 (17), 59 (90), 43 (100).

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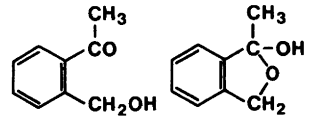
Some New Orthoderivatives of Benzene

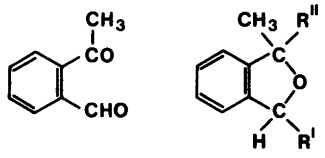
ENDRE BERNER

Universitetes Kjemiske Institutt, Blindern, Oslo 3, Norway

It has been suggested [1] that the self-condensation of 2-acetylbenzoic acid starts with the formation of a carbonium ion derived from the cyclic form of the acid. It would be interesting to see if another compound with the same constellation of a carbonyl and a hydroxyl group such as 2-acetylbenzylalcohol (*1*) behaves similarly. An attempt to prepare this hitherto unknown alcohol by partial reduction of 2-acetylbenzaldehyde *2* failed. The aldehyde *2* could be prepared by permanganate oxidation of 2-acetyl-*cis*-cinnamic acid. On longer standing the crystals of *2* liquefied, gradually taking up oxygen and turning into 2-acetylbenzoic acid.



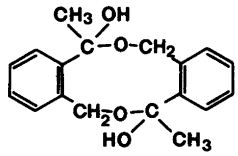
1a 1b



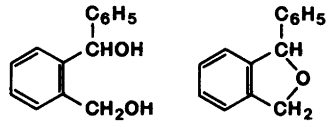
2 3

a: $R' = R'' = OCH_3$
b: $R' = H$, $R'' = OCH_3$
c: $R' = R'' = H$
d: $R' = CH_3$, $R'' = H$

The useful method for preparation of *1* started with reduction of the ketalized methylester of 2-acetylbenzoic acid with $LiAlH_4$. A direct deketalization of the reduction product by adding HCl to a solution in methanol was unsuccesful. It was necessary to protect the hydroxyl group by forming a *p*-nitrobenzoate. Deketalizing this gave the nitrobenzoate of *1a* which on saponification under strict exclusion of methanol or ethanol led to the pure acetylbenzylalcohol as a colourless liquid from which a semicarbazone was prepared. The ^1H NMR spectrum of 2-acetylbenzylalcohol shows two singlets (CH_3) near each other about δ 1.7 and two AB-quartets partly overlapping at about δ 5.0. An explanation for this would be that a part of the 2-acetylbenzylalcohol is present as a cyclic dimer (two diastereoisomers) as indicated in *4*. The other lines in the spectrum are then due to the monomer cyclic form *1b*, namely two singlets (CH_3) near each other at δ 2.4 and a quartet (CH_2) at δ 4.0.



4



5 6

Reduction of *1* led to a crystalline diol, 1-(2-hydroxymethylphenyl)ethanol (B), which on dehydration gave 1,3-dihydro-1-methylisobenzofuran (*3c*). Freshly propared, this was a very mobile and quite volatile liquid which readily underwent autoxidation.

For comparison, 1,3-dihydro-1,3-dimethylisobenzofuran *3d* was prepared.[3] Also this compound was a volatile liquid which behaved in the same way as *3c*.

The 1,3-dihydroisobenzofuran itself[4] reacted so rapidly with oxygen that it, after being left 2 days in an open vessel, gave a positive reaction on peroxide. The crystalline peroxide obtained after prolonged exposure to air had m.p. 130 °C and showed an NMR spectrum in agreement with the formula proposed before.[2]

An attempt to prepare *o*-benzoylbenzyl alcohol following the same procedure as for the methyl derivative failed. Instead of the expected product 3-phenylphthalide and 1-(2-hydroxymethylphenyl)-1-phenylmethanol (*5*) were isolated, of which the latter one was transformed into 1,3-dihydro-1-phenylisobenzofuran (*6*) as described.[5] The NMR spectrum of *6* is quite remarkable, obviously due to coupling of the protons in the CH and CH_2 groups with the neighbouring aromatic protons: 1 H, triplet at δ 6.1, 2 H triplet at δ 5.1 and 9 arom. H. An earlier observation[5] that the crystalline 1,3-dihydro-1-phenylisobenzofuran deteriorated on standing is obviously due to the fact that also this derivative of

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1,3-dihydroisobenzofuran reacts with oxygen in the air and gives a positive test for peroxide. It was also found that pure 1,3-dihydroisobenzofuran itself deteriorated on standing.

Experimental. 2-Acetyl-cis-cinnamic acid (A) was in the present case prepared by oxidizing 1-methyl-2-naphthol with ozone. The naphthol (3.16 g, 20 mmol) prepared according to literature [6] was dissolved in 100 ml methylene chloride and at 20 °C a stream of ozone led through until 20 mmol had been absorbed. Triphenylphosphine (5.2 g 20 mmol) dissolved in methylene chloride was added. The *A* formed was extracted with sodium bicarbonate and finally precipitated by adding acid. Yield 2.90 g (76%) m.p. 142 °C.

2-Acetylbenzaldehyde (2). 10 mmol *A* (1.90 g), 0.52 g sodium carbonate and 1.5 g magnesium sulfate were dissolved in 120 ml water and 50 ml benzene placed on top of the solution. Keeping the temperature between 0 and 5 °C and stirring vigorously, 2.5 g potassium permanganate dissolved in 100 ml water was slowly added. After two more portions of benzene had been used for extraction, 0.6 g of *2* was obtained as a liquid which spontaneously crystallized. Recrystallized from pentane; needles, m.p. 44 °C. Anal. $C_9H_8O_2$: C,H. NMR in $CDCl_3$ δ 2.63 (3H), 9.0 (1 H), 4 arom. H 7.7.

Autoxidation of 2. Crystalline *2* (0.88 g) left on an open dish for 3 weeks had become liquid and the solution of it in ether was shaken with sodium bicarbonate. From the ether was obtained 0.27 g unchanged *2* and from the aqueous solution 2-acetylbenzoic acid, m.p. 114 °C was isolated. The phenylhydrazone of *2* prepared in the usual way after being recrystallized from ethanol m.p. 213 °C. Anal. $C_{15}H_{14}ON_2$: N.

Dimethylketal of methylester of 2-acetylbenzoic acid (C). To a mixture of the methylester of 2-acetylbenzoic acid (19 g), methanol (40 ml) methylorthoformate (25 ml) and 5 drops conc. HCl were added and the solution boiled for 5 h. After adding 4 drops of HCl the boiling was continued for 2 h. After removing the volatile material in a vacuum at 50 °C the remaining liquid (24.8 g) showed the NMR expected for C: δ 1.59 (3H), 3.09 (6H), 3.79 (3H) and 4 arom. H.

Reduction of C. The liquid from above (24.8 g), dissolved in ether, was added slowly to a suspension of 3.1 g $LiAlH_4$ in ether. Excess of $LiAlH_4$ was destroyed by adding ethyl acetate. Finally, water was added and from the ethereal solution the dimethylketal of *1a* was obtained as a colourless liquid (20.6 g). NMR in $CDCl_3$: δ 1.58 (3H), 3.26 (6H) (partly overlapping OH), 4.67 (AB q) and 4 arom. H. After distillation in vacuum (145 °C at 12 mmHg) the NMR was unchanged.

The p-nitrobenzoates. To a solution of the dimethylketal of *1a* in 8 ml pyridine, *p*-nitrobenzoylchloride (1.2 g) was added. The temperature rose a little and crystals began to separate. After adding water, solid material (2.2 g) was filtered off. On boiling with ethanol most of it remained unsolved, obviously polymerized material. From the solution was obtained 0.7 g *p*-nitrobenzoate of the dimethylketal, which when recrystallized from ethanol had m.p. 113 °C. Anal: $C_{16}H_{19}O_6N$: C.H. ^{1}H NMR ($CDCl_3$) δ 1.59 (3H), 3.16 (6H), 5.63 (2H) and 8 arom. H. On recrystallization from ethanol containing HCl deketalization took place, and the *p*-nitrobenzoate of *1a* was obtained. M.p. 132 °C. Anal. $C_{16}H_{13}O_5N$: C,H,N. NMR ($CDCl_3$): δ 2.62 (3H), 5.75 (2H), and 8 arom. H.

Saponification of the *p*-nitrobenzoate in the presence of methanol led to a colourless liquid which, judging from the NMR spectrum, consisted of 50% of the cyclic ketal *3b* and 25% of the open and cyclic froms of 2-acetylbenzylalcohol (*1a* and *1b*). NMR ($CDCl_3$) for the cyclic ketal δ 1.71 (3H), 3.00 (3H), 5.11 (2H).

The reaction of 2-acetylbenzylalcohol (*1*) with methanol was demonstrated in an experiment which showed that the reaction product *3b* is very volatile. NMR ($CDCl_3$): δ 1.69 (3H), 2.98 (3H), 5.06) (2H) and 4 arom. H at about 7.5.

Preparation of B. When either *1* or 2-acetylbenzoic acid was reduced with $LiAlH_4$ the final product was a colourless liquid which distilled at 180 °C, 15 mmHg. After a few days, crystallization of *B* set in. M.p. 71 °C. Anal. $C_9H_{12}O_2$: C.H. NMR ($CDCl_3$): δ 1.46 (3H), 3.88 (2H), 4.57 (2H ABq), 5.01 (1 Hq), 4 arom. H at about 7.3. The dinitrobenzoate had m.p. 131 °C. (Found C 60.80, H 3.72; $C_{23}H_{18}O_8N_2$ calc. C 61.33, H 4.03, N 6.22).

Dehydration of B. To a few g of *B* an equal quantity of 85% phosphoric acid was added and the mixture heated slightly until an oily substance separated. Dissolved in ether and shaken thoroughly first with water and then with bicarbonate *3c* was obtained as a very mobile liquid which distilled at 85 °C, 10 mmHg. (Found C 80.87, H 7.45; calc. $C_9H_{10}O$: C 80.56, H 7.51. NMR ($CDCl_3$): δ 1.47 (3H), 5.07 (2H ABq), 4 arom. H at 7.2. Leaving *3c* in an open vessel it gradually became viscouse and gave a positive reaction on peroxide. After 2 weeks: C 69.69, H 6.17; calc. for a peroxide $C_{18}H_{18}O_4$: C 72.47, H 6.08.

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