described for uranium(IV) borohydride.[2] The data for the monomethyl compound are recorded in Table I.

TABLE I

VAPOR PRESSURES OF THE MONOMETHYL DERIVATIVE OF URANIUM(IV) BOROHYDRIDE

The calculated pressures were obtained from the equation: $\log P = -3.160/T + 10.690$

Temp., °C.	25.1	31.4	38.1	45.4	50.7	58.0	65.6
P, mm., obsd.	1.06	2.13	3.37	5.56	8.52	14.6	25.8
P, mm., calcd.	1.24	2.05	3.44	5.87	8.53	14.0	23.4

In Table II the data for the tetramethyl derivative are recorded.

TABLE II

VAPOR PRESSURES OF THE TETRAMETHYL DERIVATIVE OF URANIUM(IV) BOROHYDRIDE

The calculated pressures were obtained from the equation: $\log P = -2{,}960/T + 8.815$

Temp., °C.	40.1	45.6	53.2	60.3	65.3	70.6	73.7
P, mm., obsd.	0.24	0.34	0.51	0.84	1.03	1.66	1.93
P, mm., calcd.	0.23	0.34	0.55	0.86	1.17	1.60	1.91

Triethylboron and Uranium Borohydride.—Uranium borohydride, 9.6 mmoles, and 5.0 mmoles of triethylboron were heated in an evacuated reaction tube at 60° for two hours. The contents of the tube were then distilled through a series of U-tubes at −20, −80 and −196°. The −20° tube showed a tinge of green within a few minutes and within 20 min. there was green color in the −80° tube. Since very little material distilled from the reaction vessel at room temperature, the latter was heated to 70°. A ring of colorless, oily liquid was noted in the reaction vessel, just above the heating bath. The liquid might have been a compound analogous to the tetramethyl derivative but insufficient material was obtained to attempt its purification and identification.

The material collected at −20° was distilled through a second series of U-tubes at 0, −20 and −80° for 18 hours. About 100 mg. passed through the −20° trap into the −80° tube. The condensate in the latter was, however, obviously still a mixture of green and of lighter colored material. Neither the bulk of the material, trapped at 0°, nor that collected at −20° was very volatile and could not be handled effectively in the vacuum equipment. The difficulty of purification was enhanced by the fact that the volatility of the ethyldiboranes, formed in the reaction, does not differ greatly from that of the uranium compounds.

Analyses (which need not be reported in detail) of the various fractions obtained, showed that the uranium-to-boron ratio in all fractions was approximately 1:4, but the carbon content did not correspond to any of the possible ethyluranium borohydrides.

Uranium borohydride was also treated with triisopropylboron and tri-*t*-butylboron. However, the results were similar—no pure compound could be isolated from the complex reaction mixtures.

Acknowledgment.—The assistance of J. J. Katz, D. M. Ritter and H. Russell, Jr., with individual experiments and preparations, is gratefully acknowledged.

CHICAGO, ILL.

NOTES

A Suggested Reaction Mechanism for the Copolymerization of Ethylene and Carbon Monoxide

BY W. G. BARB

RECEIVED JULY 11, 1952

Brubaker, Coffmann and Hoehn[1] have recently discovered that polyketones can be prepared by copolymerizing carbon monoxide and ethylene in cyclohexane solution, using di-(*t*-butyl) peroxide as catalyst. Their paper gives, amongst other data, information about the effect of the monomer ratio and of the total pressure on the composition of the resulting copolymer and the yield obtained in a given time.

There is an obvious formal similarity between this reaction and the formation of polysulfones from olefins and sulfur dioxide. The kinetics of the latter have recently been investigated[2,3] and it has been shown that sulfur dioxide units are incorporatedin to the polymer chain by reaction of a 1:1 complex of sulfur dioxide and the olefin; this contrasts with copolymerizations of two vinyl compounds, where it is generally accepted that the original monomeric units react (see. *e.g.*, review by Mayo and Walling[4]). It is therefore of interest to examine whether the similarity between polysulfone and polyketone formation extends to the reaction mechanisms. One feature of the data of Brubaker, *et al.*,[1] immediately suggests that the usual copolymer composition equation[4] which holds for vinyl copolymerizations, and which only involves the *ratio* of the monomer concentrations, is not directly applicable in this system; namely, the composition of the copolymer obtained at a given monomer ratio varies greatly with the total pressure, *i.e.*, with the absolute monomer concentration.

It is therefore suggested that the reaction occurs by the usual type of free-radical polymerization processes but that, by analogy with polysulfone formation,[2] the reactants are the olefin M and a 1:1 complex C of the olefin and carbon monoxide; and that the concentration [C] of the complex is given by

$$[\mathrm{C}] = K[\mathrm{M}][\mathrm{CO}]$$

where [M] and [CO] are the total concentrations of olefin and carbon monoxide (*i.e.*, the equilibrium constant K is assumed to be small).

From the four possible propagation reactions (R = remainder of polymer radical), *viz.*

$$\begin{array}{ll} \mathrm{R{-}C\cdot + C \longrightarrow R{-}C\cdot} & k_{\mathrm{CC}} \\ \mathrm{R{-}C\cdot + M \longrightarrow R{-}M\cdot} & k_{\mathrm{CM}} \\ \mathrm{R{-}M\cdot + C \longrightarrow R{-}C\cdot} & k_{\mathrm{MC}} \\ \mathrm{R{-}M\cdot + M \longrightarrow R{-}M\cdot} & k_{\mathrm{MM}} \end{array}$$

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(1) M. M. Brubaker, D. D. Coffmann and H. H. Hoehn, THIS JOURNAL, **74**, 1509 (1952).

(2) W. G. Barb, *Proc. Roy. Soc.* (*London*), **A212**, 66, 177 (1952).

(3) F. S. Dainton and K. J. Ivin, *ibid.*, **A212**, 96, 207 (1952).

(4) F. R. Mayo and C. Walling, *Chem. Revs.*, **46**, 191 (1950).

we obtain, applying the copolymer composition equation,

$$\rho = \frac{\text{rate of reaction of olefin}}{\text{rate of reaction of complex}} = \frac{[\text{M}]}{[\text{C}]}\,\frac{r_{\text{M}}[\text{M}] + [\text{C}]}{r_{\text{C}}[\text{C}] + [\text{M}]} \qquad (1)$$

where r = reactivity ratio, *i.e.*, $r_{\text{M}} = k_{\text{MM}}/k_{\text{MC}}$ and $r_{\text{C}} = k_{\text{CC}}/k_{\text{CM}}$.

If we assume further that the complex C reacts so as to give a terminal M· group rather than a terminal CO·, and that the penultimate unit of the radical does not affect the reactivity ratio, then

$$k_{\text{CC}}/k_{\text{CM}} = k_{\text{MC}}/k_{\text{MM}}.\ i.e.,\ r_{\text{C}} = 1/r_{\text{M}}$$

Equation (1) thus becomes, very simply

$$\rho = r_{\text{M}}[\text{M}]/[\text{C}] \qquad (2)$$

and

$$n = \text{ratio of M to CO in copolymer} = 1 + \rho = 1 + r_{\text{M}}[\text{M}]/[\text{C}] = 1 + r_{\text{M}}/K[\text{CO}] \qquad (3)$$

i.e., n is now a function of the absolute carbon monoxide concentration. (We note that equation (2) corresponds to an "ideal" copolymerization as defined by Wall,[5] since the relative reactivity of the two reacting monomers (C and M) is the same toward all radicals in the system.)

To analyze the data of Brubaker, *et al.*,[1] in terms of equation (3) the monomer concentrations in solution must be related to pressures in the gas phase. This was done as follows.

(a).—The Ostwald absorption coefficients α_{CO} and $\alpha_{\text{C}_2\text{H}_4}$ were assumed constant up to partial pressures of about 200 atmospheres (a single experiment reported for a higher pressure was omitted from this analysis). This assumption regarding α seems to hold to within about 20% for a similar range of pressures in the case of several gases above their critical temperature, *e.g.*, CH_4 and N_2 in various organic solvents. (See data listed by Seidell.[6,7]) The mean values of [CO] and [C_2H_4] can therefore be taken proportional to $\bar{p}_{\text{CO}}/D$ and $\bar{p}_{\text{C}_2\text{H}_4}/D$, respectively, where $\bar{p}$ = mean partial pressure during the experiment and D = volume of solution per volume of solvent. Equation (3) thus becomes

$$(n - 1) \propto D/\bar{p}_{\text{CO}} \qquad (4)$$

(b).—$\bar{p}_{\text{CO}}$ and $\bar{p}_{\text{C}_2\text{H}_4}$ were calculated from the feed composition, the yield and composition of the polymer and the volume of the solution and the gas phase, assuming values of α as in (c) and ideality of the gas phase. The maximum difference between $\bar{p}_{\text{CO}}$ and the corresponding inital value p_{CO} was 35%, and that between $\bar{p}_{\text{C}_2\text{H}_4}$ and $p_{\text{C}_2\text{H}_4}$ rather less.

(c).—To calculate D, the actual values of α were assumed to be: $\alpha_{\text{CO}} = 0.3$, $\alpha_{\text{C}_2\text{H}_4} = 1.0$, for cyclohexane at 135°. (These figures are suggested by data for other organic solvents at various temperatures[6,7]; for both gases, α is very similar in different organic liquids.) Hence the weights W_{CO} and $W_{\text{C}_2\text{H}_4}$, in grams dissolved per ml. of cyclohexane, were calculated and inserted in the relation

$$D = W_{\text{CO}}/0.5 + W_{\text{C}_2\text{H}_4}/0.3$$

The "densities" 0.5 and 0.3 are extrapolated from data below the critical temperature, by assuming a decrease in density of 0.1 per 100°. In this calculation no allowance is made for the considerable dilution by the reaction product. However it is clear that if the solubilities of CO and C_2H_4 in the polyketone are similar to those in cyclohexane the effect on the copolymer composition should be slight.

The calculation of D and $\bar{p}_{\text{CO}}$ is not very sensitive to the numerical values chosen for the various constants. Thus (i) D never differs greatly from unity, the maximum value for any experiment included in the present analysis being 1.55, and (ii) $\bar{p}$ is little affected by the exact values of α since the greater part of both monomers is present in the gas phase.

Figure 1 shows the data of Brubaker, *et al.*,[1] relating to the dependence of n on the monomer concentration ratio and on the total pressure at a given feed composition. There is reasonable accord with equation (4); however, this cannot be regarded as more than supporting the plausibility of the suggested mechanism. At the same time, if CO were assumed to react in its molecular form, the variation of n with total pressure could only arise from (a) somewhat different *mean* compositions of the reaction mixture due to different conversions or (b) an effect of pressure on the solubility ratio of CO to C_2H_4. Of these, (a) can be shown to produce a converse trend in n to that observed, and would also require all polymers prepared at the same feed to have compositions to the same side of the feed composition, which is not the case (Table I, ref. (1)); (b) on the other hand would require the unjustifiable assumption that the solubility *ratio* was approximately proportional to the total pressure.

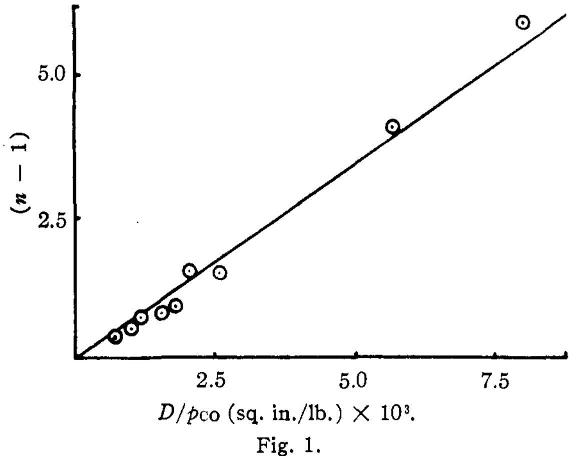

Fig. 1.

An analysis of reaction rate data is not possible without additional information, *e.g.*, on the dependence of the rate of initiation I on monomer concentration. However it is clear that since the usual copolymer composition equation does not explain the stoichiometry data if C_2H_4 and CO are taken to be the reacting entities, such a reaction scheme also cannot account simultaneously for $-d[\text{CO}]/dt$ and $-d[\text{C}_2\text{H}_4]/dt$. It may be noted that the mechanism proposed in this Note leads to very simple rate expressions since all radicals in the

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[5] F. T. Wall, This Journal, 66, 2050 (1944).

[6] A. Seidell, "Solubilities of Inorganic and Metal Organic Compounds," 3rd Ed., D. Van Nostrand Co., Inc., New York, N. Y., 1940.

[7] A. Seidell, "Solubilities of Organic Compounds," 3rd Ed., D. Van Nostrand Co., Inc., New York, N. Y., 1941.

system are similar and there is only one termination reaction: *e.g.*, it is found that

$$-d[CO]/dt \propto I^{1/2}[CO][M] \qquad (5)$$

It should be added that the inhomogeneity of the polyketone product[1] is not necessarily due to a multiplicity of mechanisms, but very probably arises from (i) the change in p_{CO} and $p_{C_2H_4}$ during reaction, if the polymer composition is not the same as the feed composition, and (ii) the increase in molecular weight caused by a decrease in catalyst concentration during the reaction.

Since this Note was originally submitted, some further data and a proposed interpretation assuming CO and C_2H_4 to be reacting entities have been published by Coffman, *et al.*[8] Of these new data, the temperature-dependence which the authors report would follow either from their mechanism or that suggested here. Again, the expected composition of the azeotropic mixture under conditions where Coffman, *et al.*, calculate 46% would be very similar, *viz.*, 44.5%, on the scheme in this Note; the mean experimental value, 45.2%, does not decide between these alternatives. On the other hand, the writer's assumption of a CO/C_2H_4 complex does seem to have the advantage of explaining, at least qualitatively, the reported dependence of the azeotropic composition on the total pressure[8]; this would merely be a special case of the variation of polymer composition with total pressure, discussed above in connection with the data of Brubaker, *et al.*[1] Finally, it is to be pointed out that much of the work of Coffman, *et al.*, has been carried out under heterogeneous conditions, in the absence of a solvent, and that this may constitute a kinetically more complicated system than obtains in solution.

I should like to thank Dr. C. H. Bamford for a discussion of this paper.

[8] D. D. Coffman, P. S. Pinkney, F. T. Wall, W. H. Wood and H. S. Young, THIS JOURNAL, **74**, 3391 (1952).

COURTAULDS LTD. RESEARCH LABORATORY
MAIDENHEAD, BERKSHIRE, ENGLAND

The Carbethoxylation Products of *p*-Aminoacetophenone and *p*-Dimethylaminoacetophenone[1]

BY H. SMITH BROADBENT AND CHAO-YUAN CHU

RECEIVED AUGUST 29, 1952

During the course of a program of synthesis of β-keto esters, the method of carbethoxylating the α-carbon atom of ketones by replacement of active hydrogen using sodium amide and ethyl carbonate[2] was applied to *p*-aminoacetophenone (I) in the hope that the α-carbon might be carbethoxylated as well as the nitrogen atom. In spite of the use of very large excesses of reactants and elevated temperatures, however, the only obtainable product was ethyl *p*-acetylphenylurethan (II) resulting from attack on the nitrogen alone, nor was it possible to effect further carbethoxylation of II by the same procedure subsequent to its isolation. The structure of II was established by elementary analysis, cryoscopic molecular weight determination, alkaline hydrolysis to I, comparison with an authentic sample obtained from I and ethyl chlorocarbonate,[3] and nitration to ethyl 2-nitro-4-acetylphenylurethan[3] previously synthesized. It was further characterized by the preparation of its 2,4-dinitrophenylhydrazone, hitherto unknown.

p-Dimethylaminoacetophenone (III) was successfully carbethoxylated by the same procedure to yield the new ethyl *p*-dimethylaminobenzoylacetate (IV) in low yields. The structure of IV was established by elementary analysis, molecular weight determination, hydrolysis to III and by conversion to 1-(2,4-dinitrophenyl)-3-(*p*-dimethylaminophenyl)-5-pyrazolone and 3-(*p*-dimethylaminophenyl)-5-isoxazolone, both new compounds.

It is interesting to note that both II and IV are very resistant to acid hydrolysis, but they are easily cleaved by 5% alcoholic potassium hydroxide.

Experimental

Ethyl *p*-Acetylphenylurethan (II).—Four hundred ml. of liquid ammonia was placed in a 1-l. three-neck flask fitted with a reflux condenser and Hershberg stirrer followed by 1 g. of anhydrous ferric chloride and 13.8 g. (0.6 atom) of clean sodium. As soon as the reaction forming sodamide was complete, a slurry of 27 g. (0.2 mole) of I in 600 ml. dry ether was added all at once. During two hours stirring, the evaporating ammonia was replaced by an equal volume of dry ether. Then 71 g. (0.6 mole) of diethyl carbonate was added and the whole suspension was refluxed for two hours with vigorous stirring. Finally the reaction mixture was slowly poured with stirring into 50 ml. of acetic acid and 500 g. of ice. The product was filtered dry and recrystallized from ethanol yielding 28 g. (68%) of pale yellow crystals melting at 157–158°. Even when the molar ratio of sodamide and ethyl carbonate to II was raised to six, no other product was obtained—only slight increase in yield.

II is insoluble in hot and cold water and ligroin, sparingly soluble in cold benzene and alcohol, quite soluble in acetone, dioxane, hot alcohol and hot benzene. It is soluble in concentrated sulfuric acid producing an orange-red color, but it is recovered unchanged upon dilution.

Anal. Calcd. for $C_{11}H_{13}O_3N$: N, 6.76; mol. wt., 207.2. Found: N, 6.97, 6.82; mol. wt. (Rast method), 206, 202, 201; (cryoscopic, in dioxane solution), 207.3, 205.8, 208.1.

Ethyl-*p*-acetylphenylurethan-2,4-dinitrophenylhydrazone was prepared in the usual manner. It occurs as orange-red crystals melting at 232–234°.

Anal. Calcd. for $C_{17}H_{17}O_6N$: N, 18.08. Found: N, 18.30, 18.19.

Ethyl *p*-Dimethylaminobenzoylacetate (IV).—A suspension of 0.26 mole of sodamide in 300 ml. of liquid ammonia was prepared in the same manner described above. After the ammonia had evaporated while being simultaneously replaced with dry ether, 16 g. (0.1 mole) of *p*-dimethylaminoacetophenone[4] dissolved in 300 ml. of ether was added. The mixture was then refluxed two hours. Finally 30 g. (0.254 mole) of diethyl carbonate was added, and stirring and refluxing were continued for four hours. After cooling, 500 ml. of water was cautiously added. The ether phase was washed, dried with anhydrous sodium sulfate and evaporated to dryness. After recrystallization from ethanol–water 2.5 g. (11%) of pale yellow needles melting at 64° was obtained.

IV is very slightly soluble in cold water and ligroin. It is quite soluble in 5% aqueous hydrochloric acid, warm 5% aqueous sodium hydroxide, ether, dioxane, acetone, hot alcohol and hot benzene.

Anal. Calcd. for $C_{13}H_{17}O_3N$: N, 5.95; mol. wt., 235.3. Found: N, 6.08, 6.17; mol. wt. (cryoscopic, in dioxane), 234.0, 233.6.

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[1] Abstracted from a thesis presented by Chao-Yuan Chu in partial fulfillment of the requirements for a degree of Master of Science at Brigham Young University.

[2] R. Levine and C. R. Hauser, THIS JOURNAL, **66**, 1768 (1944).

[3] Chr. W. Raadsveld, *Rec. trav. chim.*, **54**, 813 (1935).

[4] Prepared by treatment of *p*-aminoacetophenone with dimethyl sulfate: Weil, *Monatsh.*, **29**, 905 (1908).

1-(2,4-Dinitrophenyl)-3-(*p*-dimethylaminophenyl)-5-pyrazolone was prepared by treating 0.5 g. of IV with 1 g. of 2,4-dinitrophenylhydrazine and 2 ml. of concentrated hydrochloric acid in 15 ml. of ethanol. An orange-red precipitate immediately formed changing quickly to a dark purple-red. It was recrystallized from methanol yielding 0.4 g. (52%), m.p. 243–244°.

Anal. Calcd. for $C_{17}H_{15}O_5N_5$: N, 18.96. Found: N, 18.62.

3-(*p*-Dimethylaminophenyl)-5-isoxazolone was prepared by refluxing 0.7 g. of IV and 0.5 g. of hydroxylamine hydrochloride in 10 ml. of ethanol for one hour. On cooling and scratching the inner wall of the flask with a glass rod, 0.3 g. (50%) of white needles was obtained, m.p. 164°.

Anal. Calcd. for $C_{11}H_{12}O_2N_2$: N, 13.72. Found: N. 13.52.

BRIGHAM YOUNG UNIVERSITY
PROVO, UTAH

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trans-Dichlorobis-(N- or C-alkylethylenediamine)-cobalt(III) Chlorides[1]

BY FRED BASOLO

RECEIVED AUGUST 4, 1952

During the course of some investigations on the mechanism of substitution reactions in complex ions[2,3] it became of interest to compare the rates of reaction for an analogous series of compounds. Since *trans*-dichlorobis-(ethylenediamine)-cobalt(III) chloride[4] is one of the more readily available complexes suitable for this purpose, it was decided to attempt the synthesis of similar substituted ethylenediamine compounds. The preparation of some C-substituted ethylenediamine complexes[5,6] of this type have been described but no report was found for the synthesis of N-alkylethylenediamine derivatives. However, Keller and Edwards[7] have recently described the preparation of tris-(N-alkylethylenediaminecobalt(III) salts.

An account is given in this paper of the method of synthesis and some properties of *trans*-dichlorobis-(substituted-ethylenediamine)-cobalt(III) chlorides. The diamines employed and the symbolization used here are indicated below.

Diamine	Symbol
$CH_3NHCH_2CH_2NH_2$	N-Meen
$CH_3CH_2NHCH_2CH_2NH_2$	N-Eten
$CH_3CH_2CH_2NHCH_2CH_2NH_2$	N-*n*-Pren
$NH_2C(CH_3)_2CH_2NH_2$	Iso-bn
$NH_2C(CH_3)_2C(CH_3)_2NH_2$	Tetra-Meen

Attempts to prepare corresponding compounds with N-isopropylethylenediamine and N-*n*-butylethylenediamine were not successful. Addition of N-isopropylethylenediamine to an aqueous solution of cobalt(II) chloride resulted in the separation of a gelatinous precipitate of cobalt(II) hydroxide. Although the reaction with N-*n*-butylethylenediamine appears to proceed as with the lower alkylethylenediamines, it was not possible to isolate a crystalline product from the final green concentrate.

These green substituted ethylenediamine salts resemble the corresponding *trans*-dichlorobis-(ethylenediamine)-cobalt(III) chloride in color as shown by their similar absorption spectra (Fig. 1). The salts are all soluble in methanol and extremely soluble in water. Aqueous solutions of the N-alkylethylenediamine complexes turn red or aquate at about the same rate as does *trans*-$[Coen_2Cl_2]Cl$ while the isobutylenediamine and tetramethylethylenediamine derivatives aquate much more rapidly.[8]

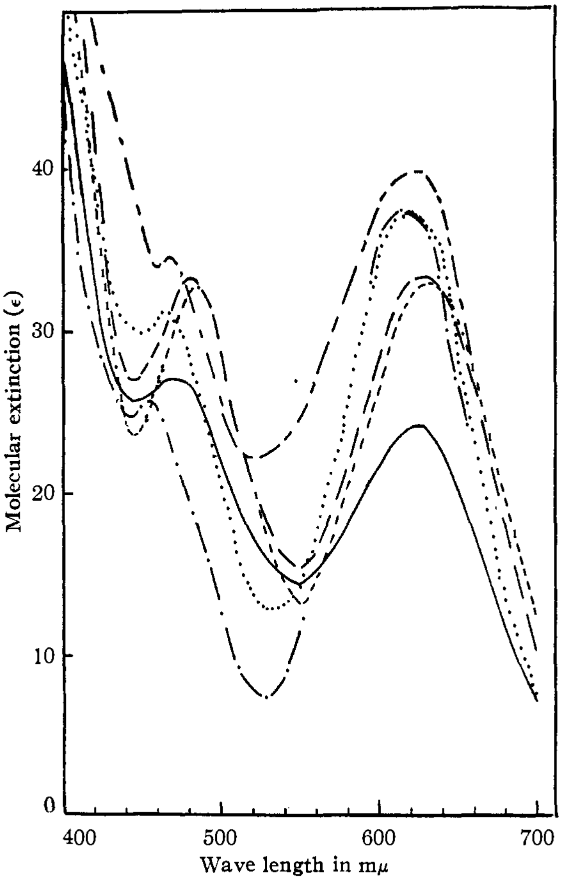

Fig. 1.—Absorption spectra of *trans*-$[Co(AA)_2Cl_2]Cl$ complexes in methanol solution: en (0.0071*M*) -·-; N-Meen (0.0068*M*), —: N-Eten (0.0053*M*), — —: N-*n*-Pern (00050*M*), - - - -; *iso*-bn (0.0057*M*), ...; tetra-Meen (0.0058*M*), — - —.

These green salts crystallize from acid solution as hydrogen chloride addition compounds but lose this adduct less readily than does the corresponding *trans*-$[Coen_2Cl_2]Cl \cdot HCl$. In fact even after heating the solid at 110° overnight, the complexes still contain as much as a half a mole of hydrogen chloride. No attempt was made to liberate all of the hydrogen chloride by either prolonged heating or use of a higher temperature. Finally, in no case was the *trans* isomer converted to the *cis* complex. The usual procedure[4] of

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[1] This investigation was supported by a grant from the United States Atomic Energy Commission under Contract AT(11-1)-89-Project No. 2.

[2] F. Basolo, J. G. Bergmann and R. G. Pearson, *J. Phys. Chem.*, **56**, 22 (1952).

[3] R. G. Pearson, C. R. Boston and F. Basolo, THIS JOURNAL, **74**, 2943 (1952).

[4] S. M. Jorgensen, *J. prakt. Chem.*, **39**, 16 (1889); **41**, 448 (1890).

[5] A. Werner and A. Fröhlich, *Ber.*, **40**, 2225 (1907).

[6] I. Lifschitz, J. G. Bos and K. M. Dijkema, *Z. anorg. allgem. Chem.*, **242**, 97 (1939).

[7] R. N. Keller and L. J. Edwards, THIS JOURNAL, **74**, 215 (1952).

[8] Quantitative studies on these rates of aquation will soon be reported in THIS JOURNAL.

evaporating an aqueous solution of the *trans* complex was followed but in every instance a green glass-like residue was obtained. It may be argued that the *cis* isomer is not obtained due to the use of *trans* salts which have not been completely freed of hydrogen chloride. However, even when the residue was redissolved and the evaporation procedure repeated several times in an attempt to liberate the slight excess of hydrogen chloride, there still was no sign of any purple *cis* compound.

Experimental

Substituted Ethylenediamines.—The anhydrous N-alkylethylenediamines were obtained from Mr. R. Kent Murmann.[9] The dihydrochlorides of isobutylenediamine and tetramethylethylenediamine were furnished by Dr. Yun-Ti Chen and R. Kent Murmann, respectively. These hydrochlorides were converted to the free amine by the treatment of concentrated aqueous solutions of the salt with an excess of solid sodium hydroxide. The mixture was in turn extracted with ether and finally the ether removed with the aid of a water aspirator. The residual-free amine was used without further purification.

***trans*-Dichlorobis-(N-alkylethylenediamine)-cobalt(III) Chlorides.**—The procedure described here for the preparation of *trans*-dichlorobis-(N-methylethylenediamine)-cobalt(III) chloride was likewise employed in the synthesis of corresponding ethyl and *n*-propyl derivatives. A solution of 7.5 g. of N-methylethylenediamine in 70 cc. of water was added to 50 cc. of a solution containing 16 g. of cobalt(II) chloride hexahydrate. A stream of air previously washed with water was slowly bubbled through the dark brown opaque reaction mixture for eight hours. At the end of this time 35 cc. of concentrated hydrochloric acid was added to the mixture and the acid solution was concentrated on a steam-bath. The green viscous concentrate was dissolved in 20 cc. of methanol and absolute ethanol was added just short of causing the separation of a green oil (approximately 75 cc.). The solution was allowed to stand overnight during which time a green crystalline product separated. This salt was collected on a filter washed with absolute ethanol followed by ether and dried overnight at 110°. A yield of 7 g. or 40% based on the diamine was obtained. *Anal.* Calcd. for $[Co(N\text{-}Meen)_2Cl_2]Cl\cdot 0.1HCl$: Cl, 37.1. Found: Cl, 37.3.

In the same manner a yield of 28% of *trans*-dichlorobis-(N-ethylethylenediamine)-cobalt(III) chloride was isolated. *Anal.* Calcd. for $[Co(N\text{-}Eten)_2Cl_2]Cl\cdot 0.6HCl$: Cl, 35.2. Found: Cl, 35.4.

The yield of *trans*-dichlorobis-(N-*n*-propylethylenediamine)-cobalt(III) chloride was 36%.

Anal. Calcd. for $[Co(N\text{-}n\text{-}Pren)_2Cl_2]Cl\cdot 0.5HCl$: Cl, 32.2. Found: Cl, 32.2.

***trans*-Dichlorobis-(isobutylenediamine)-cobalt(III) Chloride.**—A solution of 5 g. of isobutylenediamine in 15 cc. of water was added to that of 5 g. of cobalt(II) chloride in 20 cc. This mixture was then treated with 5 cc. of 30% hydrogen peroxide. A vigorous exothermic reaction occurred accompanied by the voluminous evolution of oxygen. After standing for 1 hr. at 60° the reaction mixture was made acid by the addition of 15 cc. of concentrated hydrochloric acid. This solution was concentrated on a steam-bath until crystals began to separate at which time the concentrate was removed from the bath and allowed to stand at room temperature overnight. The crystalline product was finally collected on a filter, washed with absolute ethanol and ether, then dried overnight at 110°. The product weighed 2 g. or was obtained in only a 19% yield. *Anal.* Calcd. for $[Co(iso\text{-}bn)_2Cl_2]Cl\cdot 0.5HCl$: Cl, 33.7. Found: Cl, 33.9.

***trans*-Dichlorobis-(tetramethylethylenediamine)-cobalt(III) Chloride.**—A small amount of crystalline material separated upon the addition of 5 g. of tetramethylethylenediamine in 10 cc. of water to a 20-cc. solution containing 5 g. of cobalt(II) chloride. However, this substance is completely dissolved during the gradual addition of 5 cc. of 30% hydrogen peroxide to the mixture. Unlike the procedure followed above this reaction mixture did not yield the desired *trans* complex when treated directly with concentrated hydrochloric acid. Instead it was found necessary to first allow the solution to concentrate to dryness at 60°. This residue was then extracted with 10 cc. of concentrated hydrochloric acid and the less soluble green salt collected on a filter. This crystalline material was dissolved in a minimum amount of methanol and then an excess of ether was added to the solution. After standing in an ice–salt-bath for several hours, the green product was collected, washed with ether and dried overnight at 110°. The dry salt weighed 3.5 g. or was obtained in a 39% yield. *Anal.* Calcd. for $[Co(tetra\text{-}Meen)_2]Cl_2\cdot 0.6HCl$: Cl, 30.5. Found: Cl, 30.6.

Spectral Measurements.—All measurements were made with a Beckman model DU spectrophotometer in silica cells having a 1-cm. light path. Extinction coefficients were calculated from the familiar equation

$$\epsilon = 1/cd \log_{10} (I_0/I)$$

Methanol was used as a solvent and measurements were made at room temperature.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

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(9) F. Basolo and R. K. Murmann, THIS JOURNAL, **74**, 2373 (1952).

The Behavior of Tetraborane toward Trimethylamine and Ethanol

BY ANTON B. BURG AND F. G. A. STONE

RECEIVED AUGUST 21, 1952

In the search for more knowledge of the chemistry of tetraborane (B_4H_{10})—especially with reference to structural implications—we have found that it reacts according to the equation $B_4H_{10} + (3 + x)\ (CH_3)_3N \rightarrow 3(CH_3)_3NBH_3 + BH\cdot x(CH_3)_3N$ when it is added to trimethylamine (excess) dissolved in tetrahydrofuran at −78°. A similar, but less stoichiometric, result was obtained when the solvent was omitted, or when a deficiency of trimethylamine was used. The value of *x* ranged from 0.54 to 0.37; this trimethylamine was retained *in vacuo* at room temperature, in the white or yellowish polymeric solid. Non-volatile B–H material was obtained also when tetraborane was allowed to react with an amount of ethanol too small for complete solvolysis, forming also hydrogen, diethoxyborine and triethyl borate.

From these results, it appears that tetraborane behaves like diborane, except that it furnishes three BH_3 groups for reaction, instead of two, and leaves polymeric material as a by-product. It is difficult to conceive of a B_4H_{10} structure in which three essentially intact BH_3 groups are attached to a BH unit by conventional bonds; hence the easy delivery of three BH_3 groups may well be due to a very labile structure which shifts easily to make them available for reaction. In fact, it is possible to write a structure based upon the principles recognized in B_5H_9,[1,2] such that three BH_3 groups would result solely from the breaking of B–H–B bridge bonds; and such a structure is consistent with preliminary results of the electron-diffraction study of tetraborane.[3]

In none of our experiments was it possible to find any evidence of compounds involving a covalent B–B bond of unit order. It appears that any unique B–B bond in tetraborane must be extremely

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(1) K. Hedberg, M. E. Jones and V. Schomaker, THIS JOURNAL, **73**, 3538 (1951).

(2) W. J. Dulmage and W. N. Lipscomb, *ibid.*, **73**, 3539 (1951).

(3) Private communication from K. Hedberg and V. Schomaker at the California Institute of Technology.

labile, or of order less than unity, or both. Also, since tetraborane furnishes three BH_3 groups per molecule, for reaction with trimethylamine, it would appear that the experimental facts concerning its ammonia chemistry[4] require an even more thorough reinterpretation than would be obvious from the intervening work on ammoniated diborane.[5]

Experimental Part

Preparation of Tetraborane.—Pure tetraborane was prepared by the action of hydrogen upon the unstable pentaborane B_5H_{11}, during ten minutes at 100°,[6] followed by careful fractionation and a check of the vapor tension of the product at 0°.[7] The B_5H_{11} was easily obtained by flow-heating of diborane at 120°,[6] using the apparatus indicated by Fig. 1. This demountable attachment to the vacuum system operated much in the manner of the original fixed apparatus, but was far more convenient.

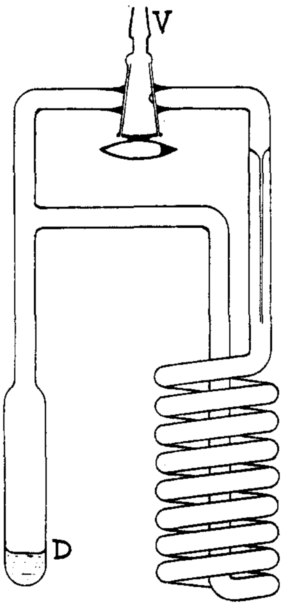

Fig. 1.—Diborane from D at −78° goes through the coil at 120° and is trapped at −196° in the vacuum system beyond the joint V. For further runs, the stopcock (Apiezon L grease) is turned the other way and the diborane is distilled back to D, leaving the higher boranes in the main system.

Reaction of Tetraborane with Trimethylamine.—A 41.1-cc. (gas at S.C.) sample of B_4H_{10} was added in five equal portions over a period of 5 hours, to 185 cc. (gas at S.C.) of $(CH_3)_3N$ dissolved in 1.7572 g. of tetrahydrofuran. The mixture was magnetically stirred between additions of B_4H_{10}, and subsequently for a period of 72 hours. During the first 48 hours, the temperature was −78°; thereafter it was raised gradually to −30°, with evolution of 1.26 cc. of hydrogen. The solvent was then removed and separated by fractional condensation (through −45° and trapped at −78°; weight 1.7616 g.), and the other components were brought together again in the reaction tube; during 15 hours at 0°, the recoverable $(CH_3)_3N$ fell from 48.7 to 46.8 cc.

The volatile solid was identified as $(CH_3)_3NBH_3$ by its melting point (94.5–95°) and vapor tensions (77.2 mm. at 96.9° or 48.3 mm. at 86.8°—1% above literature values)[8] as well as by a hydrolytic analysis (31.6 cc. gas at S.C. yielded 90.8 cc. of H_2 and 32.9 cc. of B calcd. as gas atoms). Its weight was equivalent to 122.0 cc. of standard gas, so that the ratio B_4H_{10}:$(CH_3)_3N$ (used):$(CH_3)_3NBH_3$ was 1:3.37:2.98. Hence the residual solid had the composition $BH\cdot 0.37(CH_3)_3N$.

Preliminary experiments had shown that this solid was not entirely hydrolyzed by aqueous HCl alone; hence in this case it was first heated with ethanol in a sealed tube (110°), yielding 51.4 cc. of hydrogen, and then during 15 hours with dilute HCl at 110° it gave a further 3.16 cc. of hydrogen. Since the hydrogen was expected to amount to 82.2 cc., and since the boric acid titration gave only 32.3 cc. of B-gas instead of 41.1, it was evident that the hydrolysis is very difficult to complete. After the indicated treatment, the odor of B–H solids persisted.

In a second experiment very like the one just described, the ratio B_4H_{10}:$(CH_3)_3N$ (used):$(CH_3)_3NBH_3$ was found to be 1:3.54:2.98.

In two experiments in which no solvent was used, such ratios were 1:2.98:2.49 and 1:2.86:2.54, respectively. The non-volatile white or yellowish solids failed to react with either trimethylamine or diborane, the latter having been used in an attempt to regain tetraborane from any possible amine-complex of a B–B bonded material.

Reaction of Tetraborane with Ethanol.—The action of a deficient proportion of ethanol upon tetraborane at −78° was undertaken in the hope of obtaining a volatile B–B linked compound. No such substance could be obtained in any of a considerable number of experiments; instead, about 13% of the boron from the unrecovered B_4H_{10} appeared in a colorless polymer, evidently containing ethoxy groups, while the rest was recovered as di- and triethoxyborines and a trace of diborane. In a typical experiment, 97.0 cc. of ethanol and 22.4 cc. of B_4H_{10} (gases at S.C.) were mixed at −78°, yielding 92.1 cc. of H_2 during 60 hours at that temperature. Fractional condensation then gave 0.55 cc. of B_2H_6 and 11.8 cc. of B_4H_{10} (confirmed by hydrolysis to yield boric acid and hydrogen in amounts corresponding to the stated gas volumes); a further 0.4 cc. of B_4H_{10} was demonstrated as excess $B(OH)_3$ and H_2 from the hydrolysis of the $(C_2H_5O)_2BH$ fraction; it had been shown by work with a known mixture, that a trace of B_4H_{10} is very difficult to remove from $(C_2H_5O)_2BH$ by distillation methods alone. The $(C_2H_5O)_2BH$ amounted to 13.6 cc., and the $(C_2H_5O)_3B$ to 19.8 cc. (gases at S.C.). A fraction of $(C_2H_5O)_2BH$, which was purified after recovering from the $(C_2H_5O)_3B$ fraction, showed a vapor tension of 36.5 mm. at 0° (literature 37.5).[9] The glassy solid, remaining in the original reaction tube, was hydrolyzed to form 5.5 cc. of H_2 and $B(OH)_3$ equivalent to 5.4 cc. of B-gas. Thus it appeared that 10.2 cc. of B_4H_{10} had reacted to form 92.1 cc. of H_2, 0.55 cc. of B_2H_6, 13.6 cc. of $(RO)_2BH$, 19.8 cc. of $(RO)_3B$ and 5.4 cc. of BH material. This result would account for 98% of the B and 102% of the H_2 which would correspond to the hydrolysis of 10.2 cc. of B_4H_{10}.

Acknowledgment.—The generous support of this work by the Office of Naval Research is gratefully acknowledged.

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[4] A. Stock, E. Wiberg and H. Martini, *Ber.*, **63B**, 2931 (1930).

[5] H. I. Schlesinger and A. B. Burg, THIS JOURNAL, **60**, 290 (1938).

[6] A. B. Burg and H. I. Schlesinger, *ibid.*, **55**, 4009 (1933).

[7] A. Stock and E. Kusz, *Ber.*, **56B**, 795 (1923).

[8] A. B. Burg and H. I. Schlesinger, THIS JOURNAL, **59**, 785 (1937).

[9] H. C. Brown, H. I. Schlesinger and A. B. Burg, *ibid.*, **61**, 676 (1939).

UNIVERSITY OF SOUTHERN CALIFORNIA
LOS ANGELES 7, CALIFORNIA

Interactions of the Aminobenzoate Ions and Serum Albumin[1]

BY R. K. BURKHARD

RECEIVED AUGUST 6, 1952

It has been shown that the anions derived from methyl red and two of its isomers exhibit differences in the extents of their interactions with bovine and human serum albumins. The anion derived from "*meta*-methyl red" (4′-dimethylaminoazobenzene-3-carboxylic acid) was found to react with these two proteins to a greater extent than the anions derived from either of its isomers, methyl red or "*para*-methyl red" (4′-dimethylaminoazobenzene-2-carboxylic acid and 4′-dimethylaminoazobenzene-4-carboxylic acid, respectively).[2] Preliminary

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[1] Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] I. M. Klotz, R. K. Burkhard and J. M. Urquhart, *J. Phys. Chem.*, **56**, 77 (1952).

investigations into these phenomena revealed that of these three isomeric dyes the *m*-isomer was the strongest acid.[3] Thus it appeared that there might be a relationship between protein binding ability and acid strength. Accordingly, this study was undertaken to determine whether the correlation between protein binding ability and acid strength as observed in the case of the anions of the methyl reds could be carried over to the aminobenzoate ions.

Experimental

The systems chosen for study involved bovine serum albumin (BSA) at 0.2% concentration, methyl orange (MO) and the aminobenzoate ions. Considerable difficulty was encountered in attempts to obtain data by equilibrium dialysis of the aminobenzoate ions themselves with the protein, and accordingly the spectral displacement technique and competitive equilibrium dialysis technique as developed by Klotz and his co-workers were used.[4,5] The spectral studies were performed at room temperature and *p*H 7.3. The competitive equilibrium dialysis studies were performed at 0° and *p*H 6.8. Phosphate buffers were used to maintain the *p*H values. In all cases the extent of interaction was measured by studying the effect that the aminobenzoate ions had on the interaction between methyl orange and bovine serum albumin. The molar ratios of aminobenzoate to methyl orange ranged from 62/1 to 925/1. All spectral studies and analyses were performed on the Beckman model DU spectrophotometer. The protein samples were obtained from the Armour Company.

Results and Discussion

A study of protein interactions involving the anions of anthranilic acid and *p*-aminobenzoic acid has been made by Klotz.[4] It was shown at that time that the anthranilate ion was more effective than the *p*-aminobenzoate ion in displacing methyl orange from protein surfaces. The results obtained in this study which pertain to the *o*- and *p*-isomers agree entirely with this earlier work. The *m*-isomer which was not studied at that time yielded very interesting results showing that the *m*-isomer had no greater effect on the displacement of methyl orange than did the *p*-isomer.

TABLE I

CHANGES IN THE MOLAR EXTINCTION COEFFICIENT OF METHYL ORANGE WHEN IN THE PRESENCE OF VARIOUS ADDITIVES (*p*H 7.3, ROOM TEMP.)

Concentration of methyl orange (MO) 1.58 × 10^{-5} mole/l.; concentration of bovine serum albumin (BSA) and the aminobenzoic acids 0.2%; ratio of aminobenzoic acids to MO 925/1.

System	$\Delta\epsilon$ at 4700 Å.
MO	0
MO, BSA	−4200
MO, BSA, anthranilic acid	−1000
MO, BSA, *m*-aminobenzoic acid	−1800
MO, BSA, *p*-aminobenzoic acid	−1800

TABLE II

BINDING OF METHYL ORANGE AND BOVINE SERUM ALBUMIN IN THE PRESENCE OF THE AMINOBENZOIC ACIDS (*p*H 6.8, 0°)

Concentration of methyl orange (MO) 0.942 × 10^{-5} mole/l.; concentration of bovine serum albumin (BSA) 0.2%; concentration of the aminobenzoic acids 5.8 × 10^{-4} mole/l.; ratio of aminobenzoic acids to MO 62/1.

System	Mole of MO bound per mole protein
MO, BSA, anthranilic acid	0.16
MO, BSA, *m*-aminobenzoic acid	.33
MO, BSA. *p*-aminobenzoic acid	.33

It has been pointed out by Klotz that the presence of groups capable of hydrogen bonding such as are found in the anthranilic acid molecule enhance protein binding.[4] The ions derived from *m*- and *p*-aminobenzoic acids, however, cannot engage in such bonding and hence acid strength and molecular configuration should be the paramount considerations in their interactions with proteins. The fact that these two isomers show no difference in their ability to displace methyl orange from protein surfaces strongly suggests that the interactions of these two isomeric ions are similar. If this be true then one would suspect that the results observed with the methyl reds might be due to factors other than acid strength. Biochemical implications of these findings are now being investigated.

DEPARTMENT OF CHEMISTRY
KANSAS STATE COLLEGE
MANHATTAN, KANSAS

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[3] R. K. Burkhard, Ph.D. Thesis, Northwestern University, 1950.

[4] I. M. Klotz, THIS JOURNAL, **68**, 2299 (1946).

[5] I. M. Klotz, F. M. Walker and R. B. Pivan, *ibid.*, **68**, 1486 (1946).

Simultaneous Vicinal Dichlorination, a Correction[1]

BY E. CAMPAIGNE AND JOSEPH R. LEAL[2]

RECEIVED JULY 11, 1952

We have been unable to confirm the phenomenon of "simultaneous vicinal dichlorination" previously reported from this Laboratory.[3] Using conditions which were as nearly as possible a duplication of those used in the earlier experiments, complex mixtures of halogenated products were obtained which could not be completely separated. It is possible that small amounts of vicinal chloro derivatives may have escaped detection in these mixtures. The situation is apparently further complicated by the existence of molecular compounds of two or more of the halogenated hydrocarbons which have relatively sharp melting points near those reported for pure compounds.

A study of the use of iodine trichloride as an aromatic halogenating agent and its reaction with benzene under various conditions has been made. When a carbon tetrachloride solution of acetanilide, for example, was refluxed with iodine trichloride, a 17% yield of *p*-chloroacetanilide[4] was found in the aqeuous washings of the reaction mixture. The crystalline material remaining after the removal of the solvent, however, was not completely resolved into its components, even after repeated fractional crystallizations from various solvents.

Benzene, on the other hand, gave reaction mixtures from which moderate yields of chlorobenzene

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[1] Contribution No. 568 from the Chemical Laboratories of Indiana University.

[2] Taken from a portion of the thesis submitted by Joseph R. Leal in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[3] E. Campaigne and W. Thompson, THIS JOURNAL, **72**, 629 (1950).

[4] E. Crepaz, *Atti Reale ist. Veneto sci.*, **94**, 555 (1934–1935) [*C. A.*, **31**, 6209 (1937)] obtained N-dichloroiodoacetanilide by treatment of acetanilide in cold chloroform with potassium chloroiodate (III), (KICl$_4$).

and iodobenzene, and small yields of *p*-dichlorobenzene and hexachlorobenzene could be separated by ordinary laboratory techniques. The reaction is a complex one requiring rigid control of conditions for duplication of results.[5] Variations in the ratio of iodine trichloride to benzene,[3] the degree of illumination and the state of the reagent influenced the yields and distribution of the products. These results are summarized in Table I.

TABLE I

HALOGENATION OF BENZENE BY IODINE TRICHLORIDE

Expt.	Molar ratio[c]	Illumination	Re-agent	Product, yield,[g] % C_4H_5-Cl	C_6H_5I	*p*-$C_6H_4Cl_2$	C_6Cl_6
1[a]	1/11	300-watt lamp	ICl_3[d]	54.2	33.8	4.10	2.10
2[a]	1/11	Diffuse light	ICl_3[d]	17.8	42.2		12.6
3[a]	1/11	Diffuse light	ICl_3[e]	30.2	38.4	8.10	
4[b]	1/1	300-watt lamp	ICl_3[f]	14.3	6.4	13.6	
5[a]	1/4	300-watt lamp	$KICl_4$	66.6	Trace		
6[a]	1/4	300-watt lamp	$HICl_4$	29.3	16.2		

[a] No solvent. [b] CCl_4 solvent. [c] ICl_3/C_6H_6. [d] Commercially prepared sample which had partially deteriorated and was recovered by treatment with chlorine. [e] Commercially prepared sample from sealed bottle. No chlorine treatment given. [f] Laboratory preparation from ICl and Cl_2. [g] Based on 1 g. atom of Cl per mole of ICl_3.

The reaction with anisole yielded a complex mixture of products which could not be readily separated into its components.

Experimental[6]

Iodine Trichloride.—Into approximately 250 ml. of liquid chlorine, contained in a one-liter flask immersed in an acetone–Dry Ice mixture and fitted with a dropping funnel and Hershberg stirrer, was added dropwise 163 g. (1 mole) of iodine monochloride with vigorous stirring. When all of the iodine monochloride had been added, the excess chlorine was allowed to evaporate from the flask. The essentially quantitative yield of dry orange iodine trichloride was used without further purification.

Commercially prepared iodine trichloride[7] was also used. After a bottle had been unsealed, however, the remaining iodine trichloride decomposed rapidly. Treatment of the decomposed material with 10–15 ml. of liquid chlorine restored it to its original orange color. Iodine trichloride apparently contains chlorine in excess of that required by the empirical formula.[8]

Potassium Chloroiodate(III).—Iodine trichloride was added to a cold aqueous solution of potassium chloride.[9] The fine orange crystals that separated were filtered off under suction, pressed between filter paper and used without further drying.

Chloroiodous Acid.—The acid was prepared from iodine trichloride and concentrated hydrochloric acid,[10] but was not crystallized for use in the reaction.

Reaction with Acetanilide.—To a solution of 13.7 g. (0.1 mole) of acetanilide in 500 ml. of carbon tetrachloride was added slowly 46 g. (0.2 mole) of orange iodine trichloride. The mixture was then refluxed for 30 minutes, cooled and washed successively with aqueous sodium bisulfite solution and water. The combined washings contained 4.5 g. of suspended material which was filtered off. Upon recrystallization from water, 3 g. (17% yield) of colorless crystals, m.p. 177–179° were obtained which did not depress the melting point of an authentic sample of *p*-chloroacetanilide.

Upon removal of the solvent present in the decolorized and dried reaction mixture, elemental iodine was liberated, as evidenced by the purple color of the distillate, and 8 g. of product separated from the residual solution. Further distillation allowed the separation of 2.5 g. more of product. Repeated fractional crystallizations from water, 50% acetic acid, carbon tetrachloride, petroleum ether and dilute ethanol finally gave two fractions of m.p. 128–136° and 152–157°, respectively. These fractions were considered to represent impure substances and were not further investigated.

Reaction with Benzene.—In experiment 1, 233 g. (1 mole) of yellow iodine trichloride was added slowly with vigorous stirring to a two-liter flask equipped with stirrer, reflux condenser and addition tube and containing 858 g. (11 moles) of anhydrous thiophene-free benzene. A 300-watt lamp approximately 30 inches away illuminated the reaction vessel during the addition of the reagent and during the subsequent one-hour refluxing period. After refluxing, the dark-colored mixture was cooled, decolorized with aqueous sodium bisulfite solution, washed with water, dried and rectified through a heated distillation column packed with glass helices. During the distillation, elemental iodine was liberated; consequently, after the unreacted benzene had been removed, the reaction mixture was again decolorized, washed and dried. Subsequent distillation of the mixture, now weighing 200 g., gave 4.5 g. of forerun, b.p. 80–128° and 61 g. (54.2%) of chlorobenzene, b.p. 128–129°, identified by conversion to a bromochlorobenzene, which did not depress the melting point of an authentic sample of *p*-bromochlorobenzene,[11] m.p. 65–66°, and a dinitrochlorobenzene, m.p. 49°, which did not depress the melting point of 2,4-dinitrochlorobenzene.[12] Further distillation under reduced pressure gave 11 g. of material boiling over the range 39–65° at 20 mm. and 5 g. of crystals (A) which had condensed in the column and still-head. Recrystallization of (A) yielded 3 g. (4.1%) of *p*-dichlorobenzene, m.p. 53–55°, which did not depress the melting point of an authentic sample of *p*-dichlorobenzene. After removal of the *p*-dichlorobenzene and resumption of the distillation, free iodine was again liberated, coloring the 79 g. of distillate (B) collected at 65–70° at 20 mm. Refractionation of (B) yielded 69 g. (33.8%) of iodobenzene, b.p. 69–70° at 20 mm., brominated to a crystalline compound, m.p. 164–165°, which did not depress the melting point of 2,4,5-tribromoiodobenzene, m.p. 164–165°.[13] It was also converted to 2,4-dinitroiodobenzene, m.p. 86–88°, as shown by mixed melting point with an authentic sample.[14] Passage of chlorine into a portion of (B) in chloroform gave an almost quantitative yield of phenyliodo dichloride.[13]

The residue, which amounted to 18 g. after removal of 2 g. of crystals (C), m.p. 225°, gave 12 g. more of distillate collected over the range 76–96° at 8 mm. Recrystallization of (C) gave 1 g. (2.11%) of hexachlorobenzene, m.p. 226–227°, which did not depress the melting point of an authentic sample of hexachlorobenzene.

In experiments 2 and 3, the reaction vessel was shielded from direct light rays during the addition of the iodine trichloride as well as the refluxing period. Benzene was dropped into a mixture of carbon tetrachloride and iodine trichloride in experiment 4, while in experiments 5 and 6, the reagents involved were added to an excess of benzene. Table I summarizes the results of these experiments.

DEPARTMENT OF CHEMISTRY
INDIANA UNIVERSITY
BLOOMINGTON, INDIANA

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(5) Other investigators have reported the products as: (a) mono-, di- and trichlorobenzenes, H. Müller, *J. Chem. Soc.*, **15**, 41 (1862); (b) hexachlorobenzene, V. Thomas and P. Depuis, *Compt. rend.*, **143**, 282 (1906); (c) chlorine substitution products of benzene and small amounts of iodobenzene, V. Arreguine, Jr., and E. D. Garcia, *Anales asoc. quím. argentina*, **9**, 121 (1921); (d) chlorobenzenes, chlorocyclo-olefins and benzene hexachloride, G. Calingaert, M. E. Griffing, E. R. Kerr, A. J. Kolka and H. D. Orloff, THIS JOURNAL, **73**, 5224 (1951).

(6) Melting points and boiling points are uncorrected.

(7) Obtained from the Fairmount Chemical Company.

(8) Iodine trichloride varied in color from bright yellow to red-brown. Chlorine/iodine determinations indicated a ratio of 3.03 for a partially decomposed red-brown sample and 4.04 for a bright yellow sample formed by treating the decomposed material with excess chlorine. Other lots of the orange reagent varied between $ICl_{1.2}$ and $ICl_{1.1}$.

(9) Fritz Ephraim, "Inorganic Chemistry," 5th English edition by P. C. L. Thorne and E. R. Roberts, Gurney and Jackson, London, 1948, p. 254.

(10) H. W. Cremer and D. R. Duncan, *J. Chem. Soc.*, 1857 (1931).

(11) W. Körner, *Gazz. chim. ital.*, **4**, 342 (1874).

(12) E. H. Huntress, "Organic Chlorine Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 1081.

(13) C. Willgerodt, *J. prakt. Chem.*, [2] **33**, 154 (1886).

(14) W. Körner, *Gazz. chim. ital.*, **4**, 323 (1874).

Metathetical Reactions of Silver Salts in Solution. I. The Synthesis of Acyl Trifluoroacetates[1]

By Arthur F. Ferris and William D. Emmons

Received September 13, 1952

Recently it has been shown that an equimolar mixture of trifluoroacetic anhydride and a carboxylic acid is an extremely active acylating medium.[2] This high order of reactivity has been ascribed to the *in situ* formation of an acyl trifluoroacetate. Although cryoscopic evidence for the existence of this unsymmetrical anhydride has been obtained, an attempt to isolate acetyl trifluoroacetate by distillation of a trifluoroacetic anhydride–acetic acid mixture was unsuccessful.[3] Anhydrides of this type have, however, been prepared in this laboratory by the metathetical reaction of silver trifluoroacetate in ether with the appropriate acid chloride. Mixed anhydrides obtained in this manner were benzoyl trifluoroacetate (80% yield), furoyl trifluoroacetate (64% yield), phenylacetyl trifluoroacetate (58% yield), *o*-methoxybenzoyl trifluoroacetate (44% yield) and lauroyl trifluoroacetate (59% yield). The structures of these anhydrides are assigned on the basis of elemental analysis, saponification equivalents, infrared spectra, and their unequivocal method of synthesis.

$$CF_3CO–O–COCF_3 + RCOOH \longrightarrow CF_3COOCOR + CF_3COOH$$

$$CF_3COOAg + RCOCl \longrightarrow CF_3COO–COR + AgCl$$

In order to obtain satisfactory yields of these compounds, it was necessary to flash distil them. Otherwise anhydride interchange took place to a

$$2\ RCOOCOCF_3 \longrightarrow RCOOCOR + CF_3COOCOCF_3$$

considerable extent, and the principal product isolated was the symmetrical carboxylic acid anhydride.[4] All of the acyl trifluoroacetates prepared here were chosen so that separation of the product from the two symmetrical anhydrides was facilitated by large differences in boiling points between each of the three components of the reaction mixture. Conditions for flash distillation were established so that the high molecular weight symmetrical anhydrides remained in the distillation flask.

It is possible that traces of silver trifluoroacetate may serve as a catalyst for this interchange reaction, since after an initial flash distillation the aroyl trifluoroacetates have been found sufficiently stable for conventional distillation. Usual distillation procedures applied to lauroyl and phenylacetyl trifluoroacetates caused significant losses of product, however. The effectiveness of flash distillation in purification of these anhydrides is illustrated by the fact that when phenylacetyl trifluoroacetate was distilled initially in the conventional manner only a 13% yield was obtained; the chief product in this case was, of course, phenylacetic anhydride. With some acid chlorides, the interchange reaction took place so readily that only the symmetrical anhydride could be isolated; thus, *o*- and *p*-chlorobenzoyl chlorides gave *o*- and *p*-chlorobenzoic anhydrides, respectively.

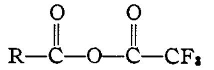
Investigation of these interesting compounds is currently underway. Acyl trifluoroacetates are extremely reactive and, indeed, drops of the anhydrides are hydrolyzed completely by standing in contact with moist air for even a few seconds. It has been suggested that these anhydrides react with most species by a mechanism involving prior ionization to form oxo-carbonium ions.[5] The availability of pure samples of acyl trifluoroacetates should permit this hypothesis to be tested unequivocally.

Experimental[6]

The procedure for the preparation of the five acyl trifluoroacetates was essentially identical except as indicated in Table I. and has been described specifically for benzoyl trifluoroacetate. The properties of the anhydrides are summarized in Table I. All boiling points are uncorrected.

TABLE I

ACYL TRIFLUOROACETATES,



$$R—\overset{\overset{\large O}{\|}}{C}—O—\overset{\overset{\large O}{\|}}{C}—CF_3$$

R-	Yield,[a] %	Flash distillation °C.[b]	Flash distillation Mm.	B.p.,[c] °C.	B.p.,[c] Mm.	$n^{20}D$	Frequency of carbonyl bands, cm.$^{-1}$	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Saponification equivalents Calcd.	Saponification equivalents Found[d]
C_6H_5–	80	120	1.0	57–58	1.7	1.4524	1845, 1773	49.55	50.13	2.31	2.53	109.1	107.3
o-$CH_3OC_6H_5$–	44	120	0.7	83–84	0.4	1.4717	1835, 1773	48.40	48.44	2.84	3.03	124.1	123.5
C_4H_3O–	64	115	1.0	42–43	.6	1.4310	1838, 1775	40.24	41.26	1.45	1.30	104.0	103.5
$C_6H_5CH_2$–	58	115	0.5	57–58	.3	1.4445	1860, 1792	51.73	51.99	3.04	3.17	116.1	114.2
$CH_3(CH_2)_{10}$–	59	145	0.3	90–91[e]	.05	1.4039	1859, 1787	56.74	57.12	7.82	7.85	148.2	144.9

[a] Flash distilled product. [b] External pot temperature. [c] In conventional distillation apparatus. [d] Averages of two or more determinations. [e] M.p. 12–14°.

Benzoyl Trifluoroacetate.—A solution of 28.1 g. of benzoyl chloride in 50 ml. of anhydrous ether was added to 42.8 g. of silver trifluoroacetate[7] dissolved in 200 ml. of ether. A vigorous exothermic reaction occurred, and silver chloride separated rapidly from solution. After 30 minutes the theoretical amount of silver chloride was collected on a filter, and the solvent was removed from the filtrate under reduced pressure. The light brown liquid residue was flash distilled at 1.0 mm. and an external pot temperature of 120°. The colorless distillate amounted to 33.9 g. (80%). This material was redistilled in conventional apparatus; b.p. 57–58° (1.7 mm.), yield 30.9 g. The refractive index had not changed significantly after the benzoyl trifluoroacetate had stood a week at room temperature. The infrared spectrum of the compound showed very sharp anhydride carbonyl bands at 1845 cm.$^{-1}$ and 1773 cm.$^{-1}$.

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(1) This work was carried out under Army Ordnance Contract W-01-021-ORD-334.

(2) E. J. Bourne, M. Stacey, J. C. Tatlow and J. M. Tedder, *J. Chem. Soc.*, 2976 (1949).

(3) P. W. Morgan, THIS JOURNAL, 73, 860 (1951).

(4) A. R. Emery and V. Gold, *J. Chem. Soc.*, 1448 (1950).

(5) E. J. Bourne, E. B. Randles, T. C. Tatlow and J. M. Tedder, *Nature*, 168, 942 (1951).

(6) We are indebted to Dr. Keith S. McCallum for infrared interpretations and to Mr. Al Kennedy and Miss Annie Smelley for microcombustions and saponification equivalents.

(7) R. N. Haszeldine, *J. Chem. Soc.*, 584 (1951).

Trifluoroacetic anhydride itself shows bands at 1880 cm.$^{-1}$ and 1815 cm.$^{-1}$. The absence of these higher frequency bands in the distilled product is further evidence for the structure and purity of benzoyl trifluoroacetate.

JOSIAH GORGAS LABORATORY, ROHM AND HAAS COMPANY
REDSTONE ARSENAL RESEARCH DIVISION
HUNTSVILLE, ALABAMA

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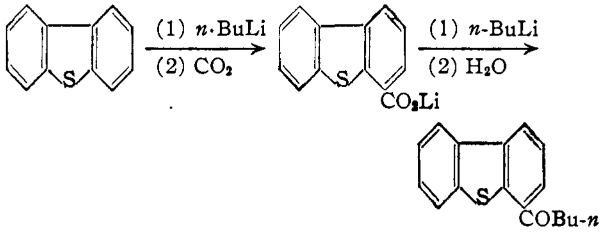
n-Butyl 4-Dibenzothienyl Ketone: A Carbonation By-product from the Metalation of Dibenzothiophene with *n*-Butyllithium

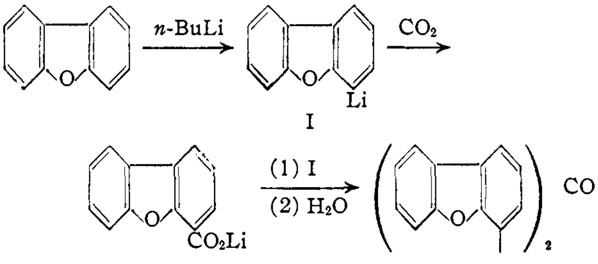
BY HENRY GILMAN AND DONALD L. ESMAY

RECEIVED SEPTEMBER 19, 1952

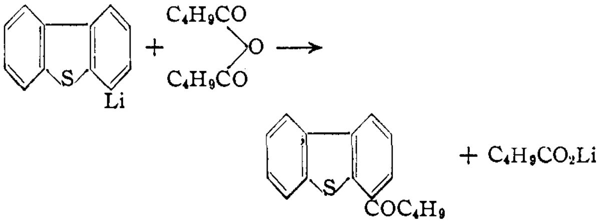
The reaction of organolithium compounds with carbon dioxide may yield, subsequent to hydrolysis and acidification, acids, ketones or tertiary alcohols.[1] The product obtained depends upon the type of RLi used and the reaction conditions employed. Pouring the solution of the organolithium derivative jetwise onto solid carbon dioxide (or a slush of ether and Dry Ice[2]) or spraying the organometallic compound into an atmosphere of gaseous carbon dioxide provides a high local concentration of carbon dioxide and thereby leads to high yields of the corresponding carboxylic acid salt. Even under these conditions, however, the secondary reaction which yields a ketone may be of some importance, and if the carbonation is carried out slowly, ketone formation often predominates. It is generally agreed that the reaction of an RLi compound with carbon dioxide probably gives first the acid salt, RCOOLi, which, in the presence of a local excess of the organolithium compound, may react further to give the dilithium salt of a dihydroxymethane, $R_2C(OLi)_2$. This view finds support in previous work[1] which showed that the preformed lithium salt of an acid, RCOOLi, reacted with an organolithium compound, R'Li, to give excellent yields of the ketone, RCOR' (R and R' may be the same, or different).

We are reporting the identification of *n*-butyl-4-dibenzothienyl ketone as one of the products obtained during the preparation of 4-dibenzothiophenecarboxylic acid by carbonation of the reaction mixture obtained from the metalation of dibenzothiophene with *n*-butyllithium in ether.[3] Since an excess of *n*-butyllithium was used for the metalation, the unsymmetrical ketone was not a wholly unexpected by-product, although its isolation was somewhat surprising in view of the fact that the symmetrical di-4-dibenzofuryl ketone was isolated during the analogous preparation of 4-dibenzofurancarboxylic acid.[4] In all probability, some di-4-dibenzothienyl ketone was also formed, even though none was isolated. No special search was made for the symmetrical ketone, however.





The structure of our compound was established by comparing its properties with those of an authentic specimen of *n*-butyl 4-dibenzothienyl ketone prepared by the reaction of 4-dibenzothienyllithium with *n*-valeric anhydride.[5] A mixed melting point of the two compounds was not depressed, and, in addition, their 2,4-dinitrophenylhydrazones possessed identical properties.



Experimental[6]

Dibenzothiophene (44.9 g., 0.24 mole) was metalated with *n*-butyllithium[7] (0.449 mole) by following the procedure previously reported from this Laboratory.[3] Carbonation was effected by pouring the organolithium solution jetwise onto solid carbon dioxide. Hydrolysis and filtration gave 3.7 g. (5.7%) of an ether- and alkali-insoluble material melting at 109-113°. Recrystallization from ethanol yielded 3.1 g. (4.7%) of pure product, m.p. 116-117°. In the only other run in which an attempt was made to isolate the by-product quantitatively, a 2.6% yield of pure material melting at 116-117° was obtained. A mixed m.p. of the two compounds showed no depression. Varying quantities of the ketone were obtained from other, similar preparations.

Anal. Calcd. for $C_{17}H_{16}OS$: S, 11.95. Found: S, 11.60, 11.74.

In order to establish that the compound was a ketone, the 2,4-dinitrophenylhydrazone was prepared.[8] The small orange needles melted at 270-271° after recrystallization from chloroform.

Anal. Calcd. for $C_{23}H_{20}O_4N_4S$: S, 7.15. Found: S, 6.92.

Two attempts to prepare the phenylhydrazone yielded an oil which could not be made to crystallize.

An authentic sample of *n*-butyl 4-dibenzothienyl ketone was prepared in low yield by treating an ether solution of *n*-valeric anhydride with an ether solution of 4-dibenzothienyllithium[3] at −70°.[5] The compound melted at 116-117°, and a mixed melting point with the carbonation by-product showed no depression. In addition, the 2,4-dinitrophenylhydrazone was prepared and found to agree in

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[1] H. Gilman and P. R. Van Ess, THIS JOURNAL, 55, 1258 (1933).

[2] H. Gilman and S. M. Spatz, *ibid.*, 63, 1553 (1941); H. Gilman and J. A. Beel, *ibid.*, 71, 2328 (1949).

[3] H. Gilman and A. L. Jacoby, *J. Org. Chem.*, 3, 108 (1938); H. Gilman and J. F. Nobis, THIS JOURNAL, 67, 1479 (1945).

[4] H. Gilman, M. W. Van Ess and D. M. Hayes, *ibid.*, 61, 643 (1939).

[5] M. S. Newman and A. S. Smith, *J. Org. Chem.*, 13, 592 (1948).

[6] All melting points are uncorrected.

[7] H. Gilman, J. A. Beel, C. G. Brannen, M. W. Bullock, G. E. Dunn and L. S. Miller, THIS JOURNAL, 71, 1499 (1949).

[8] R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1940, p. 143.

properties with those of the corresponding hydrazone of the by-product. A mixture of the two hydrazones showed no depression in the melting point.

Department of Chemistry
Iowa State College
Ames, Iowa

The Addition of Triphenylsilylpotassium to *trans*-Stilbene

By Henry Gilman and T. C. Wu

Received July 31, 1952

Incidental to a study of the properties of organosilicon-metallic compounds, we have observed that triphenylsilylpotassium adds to *trans*-stilbene to give β-triphenylsilyl-α,β-diphenylethylpotassium which on hydrolysis yields triphenyl-α,β-diphenylethylsilane [I].

$$(C_6H_5)_3SiK + C_6H_5CH{=}CHC_6H_5 \longrightarrow$$
$$(C_6H_5)_3SiCH(C_6H_5)CH(C_6H_5)K \xrightarrow{H_2O}$$
$$(C_6H_5)_3SiCH(C_6H_5)CH_2(C_6H_5) \quad \text{(I)}$$

The yield of pure [I] was 55% when a solution of 3.6 g. (0.02 mole) of *trans*-stilbene in 50 ml. of benzene was added to a triphenylsilylpotassium suspension prepared[1] by cleaving 5.2 g. (0.01 mole) of hexaphenyldisilane with sodium-potassium alloy in ether, after removing the excess alloy by amalgamation. During the addition of *trans*-stilbene to the triphenylsilylpotassium the mixture changed in color from brownish-tan to deep brownish-red. After stirring the reaction mixture for 30 minutes at room temperature hydrolysis was effected by the slow addition of water. The organic layer was dried over sodium sulfate and the solvents were removed by distillation. The residue was recrystallized three times from petroleum ether (b.p. 60–70°) to give 4.8 g. (55%) of colorless granular crystals melting at 135–136°. *Anal.* Calcd. for $C_{32}H_{28}Si$: Si, 6.37. Found: Si, 6.40, 6.41. In another experiment the triphenylsilylpotassium suspension was added to the benzene solution of *trans*-stilbene to give a 39% yield of pure [I]. A like yield was obtained under these latter conditions when the reaction time was 24 hours.

This reaction which indicates a high order of reactivity of organometallic compounds is being extended to other organosilicon-metallic[2] compounds and other unsaturated systems like those containing the azomethylene, azo and carbonyl groups.

(1) H. Gilman, T. C. Wu, H. A. Hartzfeld, G. A. Guter, A. G. Smith, J. J. Goodman and S. H. Eidt, This Journal, **74**, 561 (1952).

(2) It will be interesting to observe whether related high orders of reactivity will be shown by other types having the generalized formula R_nMM' where M is a metal or metalloid and where M' is an alkali or alkaline earth metal.

Department of Chemistry
Iowa State College
Ames, Iowa

Decaethylcyclopentasiloxane

By Dallas T. Hurd and Robert C. Osthoff

Received September 4, 1952

In the course of preparing a series of diethylsiloxanes, we have observed that the cyclic pentamer, *i.e.*, decaethylcyclopentasiloxane, is a solid. This was not anticipated; the minimum in the melting points of the known cyclic dimethylsiloxanes occurs with the pentamer and the observed melting points of the cyclic trimer and tetramer compounds are lower in the diethyl series than in the dimethyl series as shown in Table I.

Table I

Melting Points of Cyclic Diorganosiloxanes

No. of Si atoms	M.p., °C. Dimethyl series[1]	M.p., °C. Diethyl series
3	65	14
4	17.5	−50
5	−38	38
6	− 3	
7	−26	
8	31.5	

Attempts to obtain cyclic diethylsiloxanes beyond the pentamer compound were not successful (*v.i.*).

The cyclic pentamer of diethylsiloxane was studied in some detail. The compound is a soft transparent solid having the consistency of a very soft wax. It did not exhibit any liquid flow at room temperature and a sharp impression made in the soft solid with a needle showed no evidence of closure over a period of a week at normal room temperature. The material could, however, be extruded under slight pressure.

It is well known that molecules with near spherical symmetry can rotate (hindered) in the solid state and that this strongly affects the physical properties of such substances.[2] In particular, the heat and entropy of fusion are low, the melting point anomalously high and the phase between the melting and solid state transition points is clear (frequently isotropic) and of a waxy texture. It is believed that the physical properties of several of the siloxanes studied here are affected by the aforementioned molecular rotation in the solid state. For example, the freezing point of decaethylcyclopentasiloxane is very sensitive to impurities; this implies a high freezing point depression constant and hence a low heat of fusion. Decaethylcyclopentasiloxane is a clear solid near the melting point. Evidence of solid state transition points, although not specifically sought in this study, has been observed with various silicone compounds.[3] A definite first-order transition at −17.30° has been found in octamethylcyclotetrasiloxane (m.p. 17.538°), which has a low heat of fusion.[4]

Anal. Calcd. for decaethylcyclopentasiloxane: Si, 27.50; C, 47.00; H, 9.87; mol. wt., 511. Found: Si, 27.46; C, 46.8; H, 9.80; mol. wt. (from cryoscopic measurements in cyclohexane), 495.

The measured physical properties are summarized in Table II.

(1) E. G. Rochow, "Chemistry of the Silicones," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1951, p. 185.

(2) W. O. Baker and C. P. Smyth, *Ann. N. Y. Acad. Sci.*, XL, 447 (1940).

(3) Private communication from S. W. Kantor of this Laboratory, 1952.

(4) This investigation has been carried out by John D. Hoffman of this Laboratory and will be published in detail in the near future.

TABLE II

PHYSICAL PROPERTIES OF DECAETHYLCYCLOPENTASILOXANE

Melting point, °C.	38–39
Boiling point, °C.	167.5 at 1 mm.
n^{20}_{D} (solid)	1.4408
Density, g./ml.	0.968 at 20°
MR_{D} (obsd.)	139.3
(calcd.)	139.5[5]

The authors determined the dipole moment of this siloxane in benzene solution at 20°. Dielectric constant measurements were carried out at 1,000 cycles using a Schering bridge.[6,7] The observed data are listed in Table III.

TABLE III

DATA FOR THE EVALUATION OF THE DIPOLE MOMENT OF DECAETHYLCYCLOPENTASILOXANE AT 20° IN BENZENE SOLUTION

Mole fraction of solute C_2	Dielectric constant ϵ	Density d	Total molar polarization of solution $P_{1,2}$ cm.³	Total molar polarization of solute P_2, cm.³
0.0871	2.29 ± 0.02	0.8819	39.53	173.5
.0651	2.29	.8809	36.32	173.9
.0370	2.28	.8803	32.06	170.5
.0277	2.28	.8781	30.68	168.9
.0194	2.28	.8779	29.46	166.9
			$P_{2\infty}$ =	162

If 5% of the molar refraction is allowed for the atomic polarization, the dipole moment of the cyclic pentamer (calculated according to the method of Smyth[8]) is found to be 0.88 ± 0.25 *D*. It is interesting that this value is somewhat smaller than the dipole moment of the corresponding methyl compound, 1.35.[9]

Attempts were made to isolate the cyclic hexamer compound following the separation of the trimer, tetramer and pentamer compounds from the mixture of diethyl siloxanes obtained by the hydrolysis of diethyldichlorosilane, but when the distillation pot temperature was raised above that necessary for the separation of the pentamer, the higher diethylsiloxanes were cracked thermally and only the cyclic trimer and tetramer could be collected. Since the thermal rearrangement process in organopolysiloxanes is very sensitive to, and catalyzed by, traces of acid or base, it may be possible to isolate the cyclic hexamer and higher compounds if a rigorous purification of the mixed diethylsiloxanes is made prior to the actual distillation.

We are grateful to Dr. F. E. Luborsky of the Research Laboratory for his measurements of dielectric constants and to Dr. John D. Hoffman for his assistance and helpful suggestions.

GENERAL ELECTRIC RESEARCH LABORATORY
SCHENECTADY, NEW YORK

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(5) E. L. Warrick, THIS JOURNAL, **68**, 2455 (1946).

(6) R. M. Fuoss, *ibid.*, **59**, 1703 (1937).

(7) D. J. Mead and R. M. Fuoss, *ibid.*, **61**, 2048 (1939).

(8) C. P. Smyth, "Dielectric Constant and Molecular Structure," Chemical Catalog Co. (Reinhold Publ. Corp.), New York, N. Y., 1931.

(9) R. O. Sauer and D. J. Mead, THIS JOURNAL, **68**, 1794 (1946).

Podophyllotoxin from *Juniperus* Species; Savinin

BY JONATHAN L. HARTWELL, JAMES M. JOHNSON, DOROTHEA B. FITZGERALD AND MORRIS BELKIN

RECEIVED AUGUST 11, 1952

In the course of a survey of the conifers for possible damaging effect against Sarcoma 37 in mice, it was found[1] that aqueous suspensions of the dried needles of certain junipers caused hemorrhage and necrosis in this tumor. A search for the components responsible for this activity was instituted, using successive fractionation with different solvents and chromatography, and following the distribution of biological activity in the fractions by quantitative bioassay with mice bearing Sarcoma 37.[2] In this way crystalline podophyllotoxin (one of the tumor-damaging components of podophyllin) was isolated from all the junipers listed (Table I), and from one of these (savin) a new substance called savinin, inactive toward tumors, was also obtained.

The occurrence of podophyllotoxin reported here is interesting in being the first report of this substance in anything but a plant of the *Podophyllum* genus.

Experimental[3]

Fractionation of Savin.—Since savin was the product on which most of the present work was done, the fractionation finally adopted for this material is given. The other species of juniper were treated in similar fashion, and gave a similar distribution of activity. The successive fractions were extracted at room temperature with the different solvents by shaking a few minutes in stoppered flasks until further treatment failed to remove additional material, and the solvents were evaporated off at room temperature; in cases where the extract was gummy, further extraction was facilitated by breaking up the gum with a stirring rod or triturating it in a mortar with the new solvent.

Extraction of the dried ground needles with acetone yielded 17% of a soluble fraction containing essentially all the biological activity. Ligroin treatment of the acetone-soluble fraction removed an inactive soluble part, leaving 47% insoluble containing practically all the activity. Chromatography on activated alumina of an absolute ethanol solution of the ligroin-insoluble fraction, and successive elution with absolute ethanol, 70% ethanol and water gave correspondingly, three cuts from the filtrate. These represented 38, 31 and 11%, respectively, of the original fraction chromatographed, while an estimated 20% remained on the tower. Essentially all the biological activity appeared in the first cut. Treatment of the first cut with ethyl acetate removed 9% of an insoluble, inactive fraction. Extraction of the soluble part with xylene gave 92% of a soluble fraction containing most of the activity. Fractional evaporation of the xylene solution gave a succession of several crops of crystals called A, B, C, D, E and F in yields (based on the original savin) of 0.145, 0.015, 0.043, 0.090, 0.013 and 0.013%, respectively. Fractions A, B and C had similar melting points and high biological activity, while fractions D, E and F had similar melting points and low biological activity. The mother liquor, after separation of the crystals, still had biological activity.

Podophyllotoxin.—Fractions A, B and C were combined (0.20% yield) and recrystallized from benzene yielding colorless needles, m.p. 111° (softens 107°) and having a second m.p. of 185°, $[\alpha]^{21}_{\mathrm{D}}$ −123° (*c* 1.03, chloroform). This was identified as podophyllotoxin (m. p. of anhydrous form 183.4°, $[\alpha]^{20}_{\mathrm{D}}$ and anhydrous form −132°[4]). Acetyla-

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(1) D. B. Fitzgerald, M. Belkin, M. D. Felix and M. K. Carroll, to be published in *J. Natl. Cancer Inst.*, **13**, Feb. (1953).

(2) J. Leiter, V. Downing, J. L. Hartwell and M. J. Shear, *ibid.*, **10**, 1273 (1950).

(3) Melting points in this paper were taken on the Hershberg apparatus and are corrected values.

(4) J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **73**, 2909 (1951).

tion with acetic anhydride gave the acetyl derivative, m.p. 207–210°, no depression on mixed melting point with an authentic specimen of acetylpodophyllotoxin. Treatment with piperidine in boiling alcohol (footnote 4, procedure for epipicropodophyllin) gave picropodophyllin (m.p. 213–223°) in 75% yield; acetyl derivative, m.p. 211–214°, no mixed m.p. depression with an authentic specimen of acetylpicropodophyllin. The ultraviolet and infrared absorption spectra were identical with those of podophyllotoxin from podophyllin.[5]

Savinin.—Fractions D, E and F were combined (0.12% yield) and crystallized first from benzene then from absolute ethanol, yielding large, colorless, transparent prisms m.p. 146.4–148.4°, $[\alpha]^{21}\text{D}$ −87° (*c* 0.95, chloroform).

Anal. Calcd. for $C_{20}H_{14(16)}O_6$: C, 68.57 (68.16); H, 4.03 (4.57); mol. wt., 350.3 (352.3). Found: C, 68.63; H, 4.65; OCH_3, nil.; loss of weight on drying, nil.; mol. wt. (Signer, isopiestic), 360.

The Gaebel test[6] for the methylenedioxy group was positive. The ultraviolet absorption spectrum (Fig. 2) bore no resemblance to that of podophyllotoxin. In the infrared (Fig. 1), the absence of a band around 3450 cm.⁻¹ indicates the absence of a hydroxyl group, while bands at 1751 and 1651 cm.⁻¹ are strong evidence for the presence of an unsaturated lactone. Savinin was insoluble in cold and hot dilute aqueous sodium hydroxide and therefore has no phenolic or carboxylic acid function. Solubility in alcoholic alkali after short boiling, with formation of a water-soluble salt, indicated a lactone. Structural studies are in progress on this compound.

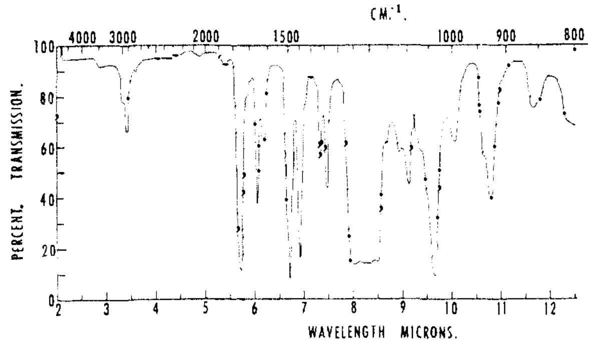

Fig. 1.—Infrared absorption spectrum of savinin in chloroform.

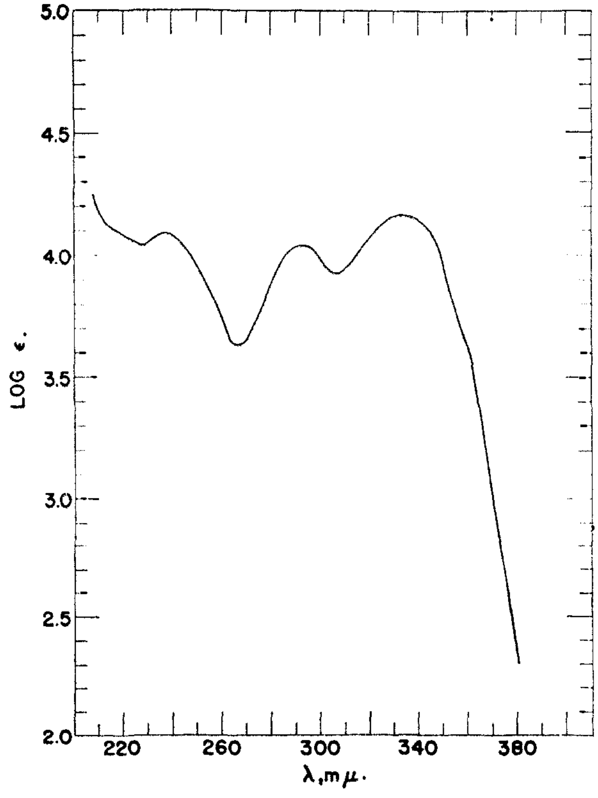

Fig. 2.—Ultraviolet absorption spectrum of savinin in 95% ethanol (based on mol. wt., 298.3).

TABLE I

PODOPHYLLOTOXIN IN DIFFERENT *Juniperus* SPECIES

Juniper	Source	Podophyllotoxin, %
Savin[a]	S. B. Penick and Co.	0.20
J. virginiana[b]	Bethesda, Maryland	.10
J. lucayana[c]	Florida	.10
J. scopulorum[d]	California	.17
J. sabina var. *tamariscifolia*, male plant[d,e]	California	.14

[a] The dried needles of an evergreen stated by the supplier to be *J. sabina*. [b] Identified by Mr. E. C. Leonard, Smithsonian Institution, Washington, D.C. [c] Provided through the courtesy of Mr. R. A. Bonninghausen, Florida Board of Forestry, Tallahassee, Fla. [d] Provided through the courtesy of Dr. J. W. Duffield, Institute of Forest Genetics, Placerville, California. [e] The female plant, collected from the same source, yielded a new active substance instead of podophyllotoxin. These results will be reported in a later communication.

Distribution of Biological Activity in Savin.—This is summarized in Table II. The xylene mother liquor is being investigated for other active components.[7]

TABLE II

DISTRIBUTION OF TUMOR-DAMAGING ACTIVITY IN SAVIN

Substance	Yield, %	MED (approx.),[a] μg./g.	Proportion of total activity,[b] %
Original savin	100	400	100
Podophyllotoxin	0.20	2	40
Savinin	0.10	>1000	0
Xylene mother liquor	2.3	25	37

[a] Minimum effective dose for a single subcutaneous injection in mice bearing implants of Sarcoma 37. [b] Yield × 400/MED. The failure of the last three items in this column to add up to 100% is due to accumulated losses in the fractionation and to large uncertainties in the values of MED.

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(5) J. L. Hartwell and W. E. Detty, THIS JOURNAL, **72**, 246 (1950); A. W. Schrecker and J. L. Hartwell, *ibid.*, **74**, 5672 (1952).

(6) G. O. Gaebel, *Arch. Pharm.*, **248**, 225 (1910).

(7) A series of crystalline waxes called "etholides," probably linear polyesters of ω-hydroxy fatty acids, have been isolated from *J. sabina* and other conifers [J. Bougault and L. Bourdier, *Compt. rend.*, **147**, 1311 (1908); *J. pharm. chim.*, **29**, 561 (1909)]. One of these, m.p. 67–69°, obtained by us from savin, had no activity against Sarcoma 37 in mice.

LABORATORY OF CHEMICAL PHARMACOLOGY
NATIONAL CANCER INSTITUTE
BETHESDA, MARYLAND

Biosynthesis of Carotene in Phycomyces

BY G. MACKINNEY, T. NAKAYAMA, C. O. CHICHESTER AND C. D. BUSS

RECEIVED SEPTEMBER 5, 1952

The effect of β-ionone on β-carotene production in *Phycomyces*[1] raises many questions as to the course of carotenoid biosynthesis. While it seems most probable that the β-ionone may be incorpo-

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(1) G. Mackinney, T. Nakayama, C. D. Buss and C. O. Chichester, THIS JOURNAL, **74**, 3456 (1952).

TABLE I

EFFECT OF TIME ON CULTURE DEVELOPMENT

Time in hr.	Sugar,[a] g./100 ml.		Nitrogen[b] % of original		Dry matter in g.		Pigment μg./g.		$D_{370}:D_{450}$	
	A[c]	B	A	B	A	B	A	B	A	B
0	2.62	2.62	100	100	...	...	...	..	...	...
42	2.30	..	46.7	53.0	0.268	0.208	93	822	0.336	0.266
50	1.80	2.06	31.4	48.1	.375	.238	185	1200	.181	.182
65	1.55	1.74	26.9	31.4	.439	.392	...	853	.166	.137
90	1.29	1.29	19.8	21.6	.462	.438	463	932	.140	.142
113	1.27	1.03	19.6	22.8	.388	.405	585	1170	.126	.123

[a] Alkaline ferricyanide–ceric sulfate. [b] Micro-Kjeldahl. [c] A, controls; B, ionone-treated.

rated directly into the carotene molecule, it is still an assumption. We observed in our previous report that the age of the culture was quite critical in determining the magnitude of the effect, so we have extended the duration of the experiments, determining dry weight production, utilization of sugar and nitrogen as well as production of pigment. The antioxidant role of diphenylamine has been investigated by Turian[2,3] and Goodwin.[4] It became therefore of interest to determine whether β-ionone could overcome the adverse effect of diphenylamine (DPA) on carotene production. Finally it seemed desirable to set down in detail the techniques whereby the effects have been observed.

Methods

Time of Treatments.—The β-ionone and DPA may be added at the start, when the medium is inoculated, or after a given period to permit reasonable growth. Both procedures have been used.[5]

Inoculation—1. **By Spores.**—For spores, the *Phycomyces* is cultured on 10% wort-3% agar for 3 to 4 weeks, on a flat 1-pint bottle, surface *ca.* 14 × 9 cm. The heavy growth is shaken gently with 75 ml. of water, and each petri dish is inoculated with 2.5 ml. of the resultant suspension by pipet. To ensure uniform growth on the supported filter paper, we have used the spore culture inoculum.

2. **By Vegetative Transfer.**—The vegetative transfer requires a more solid support. For qualitative demonstration, we have transferred portions of young white vegetative growth to the center of an agar plate. A spreading colony develops rapidly, covering the plate in about 36 hours. Recently such transfers have been made on a weak gelatin medium with good results. The medium can be dissolved in warm water and the mycelium harvested on a buchner funnel. There appears to be no reason why this procedure should not be made quantitative, though the growth is obviously not of uniform age throughout the giant colony.

Temperature.—For convenience, after the initial incubation, the plates are normally exposed on a table at room temperature, unstacked. However, we have grown cultures at 25° and then retarded subsequent growth by holding them at 15° to study pigment formation.

Method of Application.—The DPA is added in alcoholic solution, 0.1 ml. per plate to give a final concentration of 1 in 33,000, occasionally also of 1 in 50,000.[5]

The β-ionone has been added in three ways. A spray in alcoholic solution from an atomizer has been found unsatisfactory owing to erratic and non-uniform growth. If a drop of the ionone is placed on the surface of the filter paper, growth will occur around the drop, and will be highly colored, so that growth inhibition is apparently purely a surface effect. We now place the ionone at four points just off the filter paper support. The petri dish is *ca.* 9 cm. in diameter and 7 cm. filter paper is used. A hanging drop of ionone on the under side of the lid is equally effective for vegetative transfers, but is not so effective for spore cultures. The reason for this may be that in the former case, pigment formation and growth are proceeding concurrently, whereas in the latter, growth has already taken place. Absorption may therefore take place *via* the vapor as well as from solution.

Harvesting.—In the present series of experiments, the tared filter papers with the washed growth are lyophilized to constant weight. The loss in weight of control papers is corrected for.

Chromatography.—In our cultures, we have invariably found a heavy β-carotene band, phytofluene and a small but clearly defined lycopene band. We doubt that our strain produces α-carotene, as the spectrum of the first colored eluate is unequivocally that of β-carotene. There is also on occasion, a colorless zone preceding the phytofluene with a blue fluorescence. Its absorption does not interfere with the 368 mμ maximum for phytofluene.

Calculations.—Since the colored component of our strain is 98–99% β-carotene, we have approximated its concentration routinely from the optical density at 450 mμ of the crude extract in petroleum ether, using an absorption coefficient of 250 l. per g. cm.

While we have chromatographed many samples, it has not been practicable to do so in all cases. We have been interested in lycopene and phytofluene fluctuations only insofar as they may have affected β-carotene. We have routinely measured the optical densities of the crude extracts at several wave lengths and we have used the ratios $D_{500}:D_{450}$ and $D_{370}:D_{450}$ as measures of lycopene and phytofluene, respectively, relative to β-carotene. The expediency of the procedure is justified on the ground of speed and also of accuracy so long as the β-carotene continues to represent 98% or more of the colored components.

Sources of Ionone.—Novoviol, beta (Fritzsche Bros.) redistilled was used in our early work. We have since used Ionone (100%:99% β, 1% α. Dodge and Olcott) with similar results.

Experimental Results

The Effect of Time on Carotene Production.—During short periods, 5 to 23 hours, striking results were observed (1) when β-ionone was added to young cultures. We show in Table I results for cultures with and without β-ionone. added at the time of inoculation, for five time intervals. Sugar and nitrogen utilization are also given. The figures are based on five culture plates per sample. Growth and degree of pigmentation was very uniform and consistent within each set at the time of the first harvest, 42 hours. Previous experience had shown that a minimum of 35 to 40 hours was required for appreciable growth, and application of the ionone at the edge eliminated the erratic growth due to sprays. It is however difficult to maintain consistency in this type of experiment over long periods. Growth and sporulation as the cultures mature become more uneven. To avoid selection of plates for the periodic harvests, they were marked at random at the beginning.

From Table I, it is seen that for the untreated samples in the initial stages, the sugar and nitrogen are utilized somewhat more rapidly, and dry matter accumulates faster than for the treated samples. The pigment content for the latter averaged 995 μg. per g. dry matter, with a P.E. of 107, and the quantity of pigment per unit growth remains therefore constant from an early stage. This is not the case for the controls, where pigment production is secondary to growth

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(2) G. Turian, *Helv. Chim. Acta*, **33**, 13, 1988 (1950).

(3) G. Turian and F. Haxo, *J. Bact.*, **63**, 690 (1952).

(4) T. W. Goodwin, *J. Biochem.*, **49**, Proc. xxiii (1951).

(5) In this paper, results are reported only for DPA added at the higher concentration after an initial period of 35–40 hours, favorable to growth. The effect of the ionone had to be determined at the most unfavorable level of DPA attainable, on cultures with good initial growth.

in the early stages and levels off at approximately one-half the concentration for the treated samples, in 90 to 100 hours.

Diphenylamine and the Ionone Effect.—Several experiments have been run to determine whether β-ionone could overcome the adverse effect of DPA on carotene production. Results vary with culture age and degree of maturity. A 36-hour culture (6 plates each) was treated with ionone, ionone + DPA, DPA and a fourth set served as control. Twelve hours later, yields of carotene were 38.7, 5.9, 1.1 and 4.1 µg., respectively, per culture set. A 74-hour culture, similarly treated, showed no such effect, averaging 36 µg. carotene per set, though a slight increase, *ca.* 25% was noted on a weight basis. However since both DPA and ionone influence normal culture development, a direct effect of the ionone can only be postulated after DPA treatment if there is an increase in the absolute amount of pigment synthesized *per culture*.

A series of runs were then made in which cultures were incubated for 40 hours, after which different treatments were applied. To eliminate possible differences in the rates of absorption of ionone and DPA, one was applied at stated time intervals after the other. At this stage, much trouble was encountered with the temperature controls. Only the results of Table II are strictly comparable. Those of Table III are comparable horizontally. The physiological ages of the cultures for the latter, at the time of ionone or DPA treatment are not identical, and this is reflected in differences in for example the response to the ionone alone. The DPA treatment, followed by ionone 3 hours later varies also. It seems clear that on a weight basis, the β-ionone overcomes in part the DPA effect. It never equals the ionone-treated samples alone, and it may or more often may not be as effective as the controls. It appears in general to be most effective with young cultures. This of course has been characteristic of the ionone treatment unaffected by the complication of DPA action.

TABLE II

EFFECT OF DPA AND IONONE

Treatment	β-Carotene, µg./5 cultures	µg./g.
β-Ionone	78.5	296
Control	49.5	197
β-Ionone (DPA 3 hr. later)	43.2	238
β-Ionone (DPA 2 hr. later)	35.4	173
β-Ionone (DPA 1 hr. later)	25.5	175
β-Ionone (DPA 0 hr. later)	17.7	128
DPA (β-ionone 1 hr. later)	17.3	111
DPA (β-ionone 2 hr. later)	16.6	126
DPA (β-ionone 3 hr. later)	16.2	113
DPA	14.4	77.4

TABLE III

PIGMENT PRODUCTION AND DPA–IONONE EFFECTS WITH TIME

Time in hr.[c]	Control		Ionone		DPA Alone		DPA + Ionone at once		DPA + Ionone 3 hr. later	
	1[a]	2[b]	1	2	1	2	1	2	1	2
0	2.54	141	...	..	..	...	...	...	..	...
12	36.5	185	100.5	543	17.6	115	...	...	67	438
48	189	401	229	627	29.1	98	51	175	64	192
96	284	710	446	1230	19	58	105	292	95	271

[a] Micrograms carotene per 5 cultures. [b] Micrograms per gram dry mycelium. [c] After addition of ionone, or of DPA alone.

Miscellaneous

Temperature.—It had been thought from visual observation that a greater difference in carotenoid content would be found, between treated and control cultures, if held at 15° than when maintained at 25°. This however proved illusory. Regular 36-hour cultures were subdivided and held at 15° and 25° for an additional 24 hours, with and without ionone. Treated cultures contained 1565 and 2240 µg. of pigment per g., for 15° and 25°, respectively, compared with 119 and 144 for the controls. A second run confirmed a ratio of *ca.* 1.3 in the quantities of carotene produced at the two temperatures, regardless of treatment.

Vitamin A.—A slight yellowing of the culture was noted when vitamin A alcohol or acetate was added to an agar or gelatin medium. The yellowing, shown by chromatography to be due to β-carotene is however only observed when the vitamin A has been added to the medium prior to autoclaving. We steam distilled 0.5 g. of Crystalets (Chas. Pfizer and Co., Crystalline vitamin A acetate stabilized with gelatin and sugar) and obtained 250 ml. of distillate. This was ether-extracted, the extract evaporated and transferred to alcohol. Placed in the usual liquid medium, it enhanced carotene production, as shown by a marked yellowing of the culture during the next 12–24 hours.

Discussion

We feel that any extensive discussion of the above phenomenon must await the outcome of labeled ionone studies. Citral is completely ineffective in promoting carotene synthesis. Whether β-cyclocitral might show a response is not known.

The most attractive hypothesis, essentially a speculation at this stage, is that either β-ionone or a large fragment of it including the ring structure, is incorporated into the carotene molecule.[6] If the probability of this is conceded, it would follow that carotene production in the mold is limited by the rate at which it can effect ring closure of the appropriate intermediate. This is supplied by β-ionone and apparently by breakdown products of vitamin A.

(6) Evidence reported at the Pacific Slope Biochemical Conference, Berkeley, October 11, 1952, indicates that with carbonyl-labeled ionone, the carotene is inactive. Also, ionone has no effect on production of carotene in a nitrogen atmosphere. Two possibilities therefore exist—that a β-ionone fragment is incorporated or that the effect is indirect. It may be added that no effect was obtained with methylheptenone.

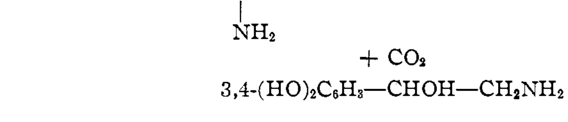
BERKELEY 4, CALIFORNIA

β-Arylserine Ethyl Esters[1]

BY WALTER H. HARTUNG, T. T. DITTRICH AND YEN-TSAI CHANG[2]

RECEIVED FEBRUARY 6, 1952

β-Arylserines are of interest because the 3,4-dihydroxy compound is the hypothetical precursor of the hormones elaborated by the suprarenal medula, *viz.*, norepinephrine and epinephrine; *e.g.*



$$3,4\text{-}(HO)_2C_6H_3\text{—CHOH—}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{—COOH} \longrightarrow 3,4\text{-}(HO)_2C_6H_3\text{—CHOH—}CH_2NH_2 + CO_2$$

Perhaps the non-catechol analogs may serve as models with which to examine the prospect of *in vivo* enzymatic decarboxylation to give rise to arylethanolamines, all of which would be expected to exhibit pressor properties.[3]

The following convenient reactions have been used to prepare the ethyl esters of phenyl- and *p*-chlorophenylserine.

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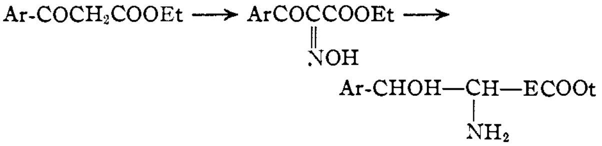
[1] Number 13 on Amino Acids. For No. 9 see W. E. Weaver and W. H. Hartung, *J. Org. Chem.*, **15**, 741 (1950).

[2] Fellow American Foundation for Pharmaceutical Education, 1948-1951.

[3] K. H. Beyer, *Advances in Chemistry*, Ser. No. 2, 37 (1950).



$$\text{Ar-COCH}_2\text{COOEt} \longrightarrow \text{ArCO}\underset{\substack{\| \\ \text{NOH}}}{\text{C}}\text{COOEt} \longrightarrow \text{Ar-CHOH—}\underset{\substack{| \\ \text{NH}_2}}{\text{CH}}\text{—ECOOt}$$

The products, capable of existing in two racemic forms, are obtained in the (±)-*erythro*-configuration.[4]

Experimental

Ethyl benzoyloximinoacetate, prepared by treating an ethereal solution of ethyl benzoylacetate with isopropyl nitrite according to the general procedure of Hartung and Munch[5] was obtained in 85–88.5% yields, formed white crystals m.p. 123–124° (uncor.) after two crystallizations from toluene.[6]

Anal. Calcd. for $C_{11}H_{11}O_4$: N, 6.33. Found: N, 6.35, 6.29.

Ethyl ester of *dl-erythro-β*-phenylserine, as obtained by catalytic hydrogenation is described by Chang and Hartung.[7]

Ethyl *p*-chlorobenzoyloximinoacetate was obtained from ethyl *p*-chlorobenzoylacetate and isopropyl nitrite[5] in yields 57–62.5%. Crystallized from toluene, the product melted 135–136° (uncor.).

Anal. Calcd. for $C_{11}H_{10}O_4NCl$: N, 5.49. Found: N, 5.45.

Ethyl ester of *dl-erythro-β-p*-chlorophenylserine was obtained by hydrogenating with Pd-on-C catalyst 7.0 g. of the oximino intermediate in 175 ml. ethanol in which was dissolved 20 g. of HCl. The H_2 uptake was 12% more than calculated, and it is possible that some of the chlorine was removed from the phenyl nucleus. Obtained 6.3 g. of the hydrochloride, 82.5%, m.p. 168–170°.

Anal. Calcd. for $C_{11}H_{14}O_3NCl{\cdot}HCl$: N, 5.22. Found: N, 5.19, 5.30.

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(4) The configuration is assigned on the basis of correlation studies by (a) K. N. F. Shaw and S. W. Fox, abstracts, p. 28N, Chicago Meeting, American Chemical Society, 1950; (b) G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949); (c) D. Billet, *Compt. rend.*, **230**, 1074 (1950); (d) K. Vogler, *Helv. Chim. Acta*, **33**, 2111 (1950).

(5) W. H. Hartung and J. C. Munch, This Journal, **51**, 2262 (1929).

(6) A. Bernton, *Arkiv. Kemi Mineral. Geol.*, **7**, No. 13, 1 (1919); *C. A.*, **14**, 2168 (1920), gives m.p. 121°.

(7) Y. T. Chang and W. H. Hartung, This Journal, **75**, 89 (1953).

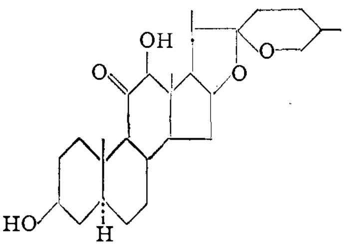
School of Pharmacy
University of North Carolina
Chapel Hill, N. C.

Steroidal Sapogenins. XXV.[1] Experiments in the Hecogenin Series (Part 4).[2] Degradation of 22a-5α-Spirostane-3β,12β-diol-11-one[3]

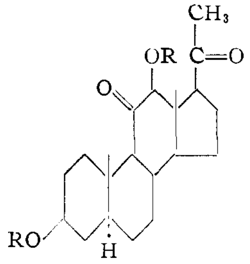
By H. Martinez, Howard J. Ringold, G. Rosenkranz and Carl Djerassi[4]

Received September 17, 1952

It has been reported previously[2] that Gallagher's[5] procedure for the shift of the C-12 carbonyl function to position 11 developed in the bile acid series is inapplicable to the sapogenins, since the penultimate step—displacement of the C-12 hydroxyl group of 22a-5α-spirostane-3β,12β-diol-11-one (I)[6] by phosphorus tribromide—fails due to side reactions with the spiroketal system. It was necessary, therefore, to develop an alternate path through bismuth oxide oxidation[2] to an 11,12-dione and subsequent removal of the C-12 carbonyl group. Simultaneously with this work,[2] we have also investigated the feasibility of Gallagher's method[5] in the pregnane series and the present note deals briefly with such attempts.



I



IIa, R = Ac
b, R = H

It was planned to degrade the ketol I to the corresponding allopregnane derivative II where troublesome interference with phosphorus tribromide was not anticipated, and to effect the removal of the 12-hydroxyl function at this stage with the formation of allopregnan-3β-ol-11,20-dione, a substance which has already been converted to cortisone.[7,8] In contrast to the unsatisfactory side chain degradation of 22a-5α-spirostan-3β-ol-12-one (hecogenin),[9] the corresponding reaction with the 11-isomer[10] proceeded rather readily and it is interesting to observe that a similar degradation of the 3,12-diol-11-one (I) recorded in the present paper gave equally satisfactory results. The intermediate $\Delta^{20(22)}$-5α-furostene-3β,12β,26-triol-11-one has already been described earlier,[6] but for optimum yields of Δ^{16}-allopregnene-3β,12β-diol-11-one diacetate it was neither necessary nor desirable to isolate this intermediate furostene derivative. The Δ^{16}-20-ketone diacetate was hydrogenated readily to the corresponding saturated analog (IIa) but all attempts to saponify this diacetate completely to the diol IIb—a necessary operation before selective acylation at C-3 and subsequent displacement at C-12 with phosphorus tribromide can be carried out—resulted in very poor yields. This disappointing result may well be due to two factors, the ready isomerization of the ketol system under basic conditions to a mixture of isomeric ketols[5] and possibly also partial isomerization at C-17 to the α-epimer, which may be favored by the 12β-hydroxy substituent. At the present time, therefore, the only successful conversion of hecogenin to cortisone still remains the one through 22a-5α-spirostan-3β-ol-11,12-dione.[2]

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(1) Paper XXIV, M. Velasco, J. Rivera, G. Rosenkranz, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **17**, December (1952).

(2) Part 3, C. Djerassi, H. J. Ringold and G. Rosenkranz, This Journal, **73**, 5513 (1951).

(3) Our sapogenin nomenclature (G. Rosenkranz and C. Djerassi, *Nature*, **166**, 104 (1950)) has been changed slightly in accordance with the recommendations of the Ciba Conference on Steroid Nomenclature (*cf. Chemistry and Industry*, June 23, 1951, SN1).

(4) Department of Chemistry, Wayne University, Detroit 1, Michigan.

(5) E. Borgstrom and T. F. Gallagher, *J. Biol. Chem.*, **177**, 951 (1949).

(6) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 303 (1951).

(7) J. M. Chemerda, E. M. Chamberlin, E. H. Wilson and M. Tishler, This Journal, **73**, 4053 (1951).

(8) G. Rosenkranz, J. Pataki and C. Djerassi, *ibid.*, **73**, 4055 (1951); **74**, 5615 (1952).

(9) R. B. Wagner, J. A. Moore and R. F. Forker, *ibid.*, **72**, 1856 (1950).

(10) C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, *ibid.*, **74**, 3634 (1952).

Incidental to the above experiments, there was also carried out the epoxidation of the initial degradation product Δ^{16}-allopregnene-3β,12β-diol-11-one diacetate with alkaline hydrogen peroxide. The resulting 16α,17α-oxide, isolated as the free diol, was transformed to the bromohydrin with hydrogen bromide in glacial acetic acid and immediately debrominated with palladized calcium carbonate catalyst[11] to yield allopregnane-3β,12β,17α-triol-11,20-dione.

Experimental[12]

Δ^{16}-Allopregnene-3β,12β-diol-11,20-dione Diacetate.—22a-5α-Spirostane-3β,12β-diol-11-one (I)[6] (4.9 g.) was converted into the furostene diacetate and directly oxidized with chromium trioxide and hydrolyzed with bicarbonate solution exactly as described for Δ^{7}-22a-5α-spirosten-3β-ol.[13] Chromatography through a short alumina column afforded 2.24 g. of colorless crystals with m.p. 213–215°, which upon further recrystallization from ether furnished the analytical sample with m.p. 214–216°, $[\alpha]^{20}\text{D}$ +22°, $\lambda_{\max}^{\text{EtOH}}$ 230 mμ (log ε 4.08),[14] $\lambda_{\max}^{\text{CS}_2}$ 1736 (acetate) and 1680 cm.$^{-1}$ (Δ^{16}-20-ketone).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.39; H, 8.11.

Allopregnane-3β,12β-diol-11,20-dione Diacetate (IIa).—The catalytic hydrogenation of the above Δ^{16}-20-ketone (0.63 g.) was carried out in ethyl acetate solution (45 cc.) at room temperature and atmospheric pressure employing 0.11 g. of 10% palladized charcoal catalyst. The hydrogen uptake corresponding to one mole ceased within one hour, whereupon the catalyst was filtered, the filtrate evaporated to dryness and the residue was recrystallized from ether–pentane; yield 0.52 g., m.p. 155–157°, $[\alpha]^{20}\text{D}$ +29°, $\lambda_{\max}^{\text{CS}_2}$ 1736 (acetate) and 1710 cm.$^{-1}$ (saturated 20-ketone).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.79; H, 8.03.

Attempts at partial saponification (limited amounts of bicarbonate at room temperature) furnished after chromatography some oily material and chiefly recovered diacetate. Potassium carbonate (room temperature or refluxing) afforded mixtures containing free diol, monoacetate and/or diacetate (free hydroxyl band as well as carbonyl bands at 1736 and 1706 cm.$^{-1}$). Saponification with boiling 5–10% methanolic potassium hydroxide yielded about 50% of solid with m.p. 147–155°, which after several recrystallizations from ether led to an analytical sample, m.p. 167–169°, $[\alpha]^{20}\text{D}$ +100°, which may be the desired diol IIb or an isomer (in ring C).

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.47; H, 8.96.

16α,17α-Oxidoallopregnane-3β,12β-diol-11,20-dione.—An ice-cold solution of 0.5 g. of Δ^{16}-allopregnene-3β,12β-diol-11,20-dione diacetate in 40 cc. of methanol was treated with 1.25 cc. of 30% hydrogen peroxide followed by the addition of a solution of 0.5 g. of sodium hydroxide in 2 cc. of water. After 89 hours in the refrigerator, the solution was diluted with chloroform, washed with water until neutral, dried and evaporated. Recrystallization from acetone–hexane furnished 0.24 g. of colorless crystals with m.p. 183–185°, $[\alpha]^{20}\text{D}$ +105°, $\lambda_{\max}^{\text{CS}_2}$ 1718 cm.$^{-1}$ and free hydroxyl band. The method of preparation and the physical constants do not preclude the possibility of epimerization of the ketol system in ring C.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.42; H, 8.44.

Allopregnane-3β,12β,17α-triol-11,20-dione.[15]—A solution of 2.49 g. of the above oxide in 25 cc. of glacial acetic acid was treated for 15 minutes at 18° with 5 cc. of a 32% solution of hydrogen bromide in glacial acetic acid and then diluted with much water. The bromohydrin was filtered, washed well with water, air-dried, and then hydrogenated directly with 9.0 g. of 2% palladized calcium carbonate in 75 cc. of 95% ethanol at room temperature and atmospheric pressure for 16 hours. After filtration of the catalyst and evaporation of the filtrate to dryness, the residue was allowed to stand at room temperature for two hours in 1% methanolic potassium hydroxide solution in order to saponify any 3-acetate formed during the oxide opening. Neutralization with acetic acid and concentration *in vacuo* followed by chilling afforded (two crops) 2.08 g. of crystals with m.p. 270–274°. The analytical sample was obtained from acetone and exhibited m.p. 278–282° (Fisher block), $[\alpha]^{20}\text{D}$ +52° (dioxane).

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.29; H, 8.82.

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[11] F. B. Colton, W. R. Nes, D. A. van Dorp, H. L. Mason and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

[12] Melting points are uncorrected. Unless indicated otherwise, rotations were determined in chloroform and ultraviolet absorption spectra in 95% ethanol solution. We are grateful to Srta. Paquita Revaque for these measurements and to Srta. Amparo Barba for the microanalyses.

[13] C. Djerassi, J. Romo and G. Rosenkranz, *J. Org. Chem.*, **16**, 754 (1951).

[14] It is interesting to note that the ultraviolet absorption maximum is at the rather low wave length of 230 mμ, also observed with a 12-keto (ref. 9) and 8(14)-unsaturated (O. Mancera, D. H. R. Barton, G. Rosenkranz and C. Djerassi, *J. Chem. Soc.*, 1025 (1952)) Δ^{16}-20-ketone, in contrast to the expected maximum (*ca.* 238 mμ) found with the corresponding 11-ketone (ref. 10) and 12α-acetoxy derivatives (C. Djerassi and C. R. Scholz, *J. Org. Chem.*, **14**, 660 (1949)).

[15] As pointed out above, the preparation of the oxide with alkaline hydrogen peroxide, though carried out at low temperature, may nevertheless involve isomerization of the ketol system (*cf.* ref. 5). Since the triol was obtained from the oxide, this reservation concerning the stereochemistry of the ketol system (in ring C) applies to both substances.

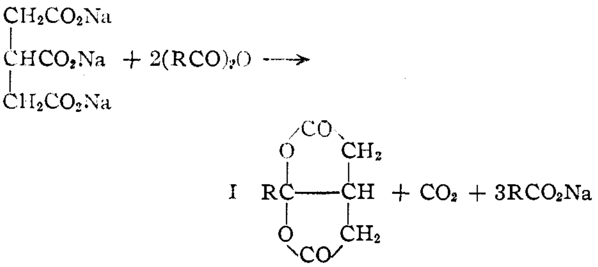
LAGUNA MAYRAN 413
MEXICO CITY 17, D.F.

Condensation of Trisodium Tricarballylate with Propionic Anhydride

BY NARIMAN B. MEHTA AND WILLIAM E. MCEWEN

RECEIVED SEPTEMBER 3, 1952

The condensation of trisodium tricarballylate with an acid anhydride to form a β-acylglutarobislactone (I) appears to be a fairly general reaction.[1,2] The method has been applied successfully to acetic anhydride,[3] *n*-butyric anhydride,[4] isobutyric anhydride,[4] benzoic anhydride[5] and phthalic anhydride.[6] The yields of I ranged from 5 to 43%. The position of condensation, at the site of the central carboxyl group of trisodium tricarballylate, was proved beyond question.



Trisodium camphoronate (II) has also been reported to form β-aceto-α,α,β-trimethylglutarobislactone (III) by reaction with acetic anhydride.[7]

The bislactones I were reported to undergo hydrolysis to β-acylglutaric acids IV on being boiled

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[1] R. Fittig, *Ber.*, **30**, 2145 (1897).

[2] R. Fittig, *Ann.*, **314**, 1 (1901).

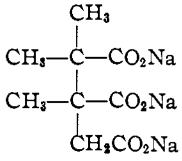
[3] R. Fittig and E. Roth, *ibid.*, **314**, 16 (1901).

[4] R. Fittig and T. Guthrie, *ibid.*, **314**, 40 (1901).

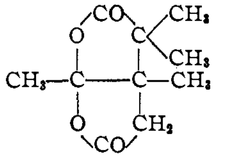
[5] R. Fittig and H. Salomon, *ibid.*, **314**, 58 (1901).

[6] R. Fittig and O. Gottsche, *ibid.*, **314**, 74 (1901).

[7] R. Fittig and H. Salomon, *ibid.*, **314**, 89 (1901).



II



III

with water. The bislactones I could also be reduced by means of sodium amalgam to γ-alkyl-γ-butyrolactone-β-acetic acids V, but yields were not specified.[2,8]

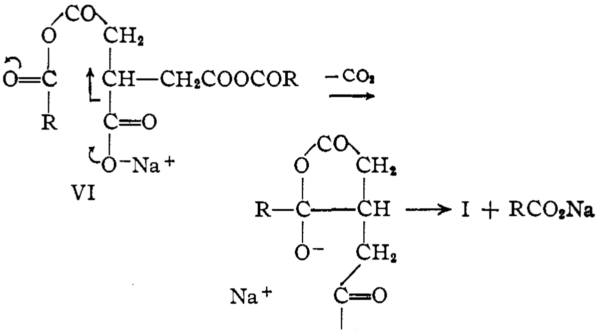
IV V

Since the condensation reaction of trisodium tricarballylate with propionic anhydride had never been reported, and since we desired the unknown γ-caprolactone-β-acetic acid (V, R = C_2H_5) as an intermediate for another project, we investigated the condensation reaction and subsequent reduction. β-Propionylglutarobislactone (I, R = C_2H_5) was obtained in 80% yield in the condensation step. To avoid the cumbersome sodium amalgam method of reduction, we reduced β-propionylglutarobislactone by means of sodium borohydride,[9] γ-caprolactone-β-acetic acid (V, R = C_2H_5) being obtained in 93% yield. Although two racemates for the acid are theoretically possible, only one pure substance was isolated from the reduction. This parallels the experience of the earlier workers.[1-8]

β-Propionylglutarobislactone was hydrolyzed to β-propionylglutaric acid (IV, R = C_2H_5), which was converted to ethyl β-propionylglutarate. A reduction of the ester with sodium borohydride afforded a good combined yield of γ-caprolactone-β-acetic acid and its ethyl ester. Ethyl γ-caprolactone-β-acetate could also be prepared from the corresponding acid either by direct esterification or *via* the acid chloride.

There has existed some doubt as to whether structure I is correct for the anhydrides of the β-acylglutaric acids IV.[8] An alternate structure to be considered is the keto anhydride. To settle this question, the infrared spectrum of β-propionylglutarobislactone (I, R = C_2H_5) was determined. It possesses a single absorption band in the carbonyl region, at 5.60 μ. This indicates with considerable certainty that structure I is correct.

With regard to the mechanism of the condensation reaction of trisodium tricarballylate with an acid anhydride, we suggest that a mixed anhydride is first formed, in which the two terminal carboxyl groups are converted to anhydride groups in preference to the more highly hindered central carboxyl group. Then, by way of the five-membered "ring" transition, VI, a molecule of carbon dioxide is lost and a new carbon-to-carbon bond is formed, giving VII.[10] Finally, VII undergoes further ring closure to I.



VI VII

The best available evidence indicates that the decarboxylative acylation which takes place in the Dakin–West reaction of α-amino acids and arylacetic acids involves acylation at the α-position of the acid (actually an intermediate azlactone or anhydride) prior to decarboxylation.[11] This mechanism obviously requires the α-amino acid or arylacetic acid to have at least one α-hydrogen. The same mechanism cannot apply to the reaction of trisodium camphoronate with acetic anhydride to give III. By analogy, it is probable that the reaction of trisodium tricarballylate with an acid anhydride to give I also does not involve this mechanism.

Acknowledgment.—This work was supported in part by a grant from the Office of Naval Research.

Experimental[12]

Trisodium Tricarballylate.—To 400 cc. of water containing 40.0 g. (1.0 mole) of sodium hydroxide was added 58.0 g. (0.33 mole) of tricarballylic acid.[13] The solution was evaporated to dryness on the steam-bath. The residue was ground to a fine powder and dried to constant weight in a vacuum oven at 45°, 80.0 g.

Reaction of Trisodium Tricarballylate with Propionic Anhydride.—To 80.0 g. of trisodium tricarballylate in a 250-cc. flask fitted with a thermometer well, a condenser and a calcium chloride drying tube was added 80.0 g. of freshly distilled propionic anhydride. The mixture was heated on an oil-bath at 130°, the reaction mixture itself being maintained at 120°. An immediate evolution of carbon dioxide was observed. At intervals of two hours, 30.0-g. portions of propionic anhydride were added until a total of 192.0 g. (1.47 moles) was used. The mixture turned light brown, and at the end of 50 hours, the evolution of carbon dioxide stopped. The mixture solidified on cooling. The solid mass was triturated with seven 200-cc. portions of low-boiling Skellysolve, followed by six 150-cc. portions of chloroform. On evaporation of the combined Skellysolve, chloroform extracts, some crystalline β-propionylglutarobislactone formed and was filtered. The filtrate was subjected to distillation at 10 mm. pressure, and distillate up to b.p. 62° was rejected. The residue in the flask

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[8] W. O. Emery, *Ann.*, **295**, 94 (1897).

[9] (a) A. M. Soldate, THIS JOURNAL, **69**, 987 (1947); (b) S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949); (c) H. Heymann and L. F. Fieser, *ibid.*, **73**, 5252 (1951); (d) R. P. Zelinski, B. W. Turnquest and E. C. Martin, *ibid.*, **73**, 5521 (1951).

[10] There are other recorded examples of decarboxylation reactions in which the fragment remaining after loss of carbon dioxide adds to a carbonyl carbon atom. (a) P. Dyson and D. Ll. Hammick, *J. Chem. Soc.*, 1724 (1937); (b) M. R. F. Ashworth, R. P. Daffern and D. Ll. Hammick, *ibid.*, 809 (1939); (c) B. R. Brown and D. Ll. Hammick *ibid.*, 173, 659 (1949); (d) M. S. Schechter, N. Green and F. B. LaForge, THIS JOURNAL, **71**, 1517 (1949); (e) G. Stork and H. Conroy, *ibid.*, **73**, 4743 (1951).

[11] (a) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 91 (1928); (b) G. H. Cleland and C. Niemann, THIS JOURNAL, **71**, 841 (1949); (c) J. A. King and F. H. McMillan, *ibid.*, **73**, 4911 (1951); (d) S. Searles and G. J. Cvejanovich, *ibid.*, **72**, 3200 (1950).

[12] Analyses by Weiler and Strauss, Oxford, England. Infrared spectra by Samuel P. Sadtler and Son, Inc., Philadelphia, Pa. All m.ps. are corrected.

[13] "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 523.

consisted of β-propionylglutarobislactone and some sodium propionate. The bislactone was extracted with chloroform, and on addition of ice-cold low boiling Skellysolve, the bislactone crystallized.

A total of 45.0 g. (0.27 mole) of bislactone was obtained, 80%. It was easily purified by dissolving it in a minimum of anhydrous acetone and adding a few drops of anhydrous ether. It crystallized as colorless needles, m.p. 62.0–62.5°.

Anal. Calcd. for $C_8H_{10}O_4$: C, 56.46; H, 5.92. Found: C, 56.78, 56.82; H, 6.18, 6.24.

The infrared absorption spectrum showed a single absorption maximum in the carbonyl region, at 5.60 μ, in nujol mull.

β-Propionylglutaric Acid.—To 150 cc. of water was added 19.3 g. of β-propionylglutarobislactone, and the solution was refluxed for 18 hours. Water was distilled *in vacuo*. The residual sirup solidified on trituration with low boiling Skellysolve, 19.0 g., m.p. 82–87°. Colorless crystals were obtained from anhydrous toluene, m.p. 87–88°.

Anal. Calcd. for $C_8H_{12}O_5$: C, 51.06; H, 6.43. Found: C, 51.20, 51.51; H, 6.37, 6.23.

Ethyl β-Propionylglutarate.—A mixture of 40.0 g. (0.21 mole) of β-propionylglutaric acid, 200 cc. of absolute ethanol, 100 cc. of benzene and 20 cc. of concentrated sulfuric acid was distilled through an 18″ electrically heated Vigreux column to which was attached an azeotropic distillation head. When only one liquid phase appeared in the condensed distillate and the temperature of the overhead vapor reached 68°, the distillation was stopped. Most of the remaining benzene and alcohol was distilled *in vacuo* and the residue neutralized with sodium bicarbonate. The mixture was extracted with ether and dried over anhydrous sodium sulfate. The ether was distilled and the crude ester fractionated. After a slight forerun there was obtained 41.0 g. (79%) of ethyl β-propionylglutarate, b.p. 121–122° (0.2 mm.).

Anal. Calcd. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 59.61; H, 8.27.

The infrared spectrum of the liquid showed a somewhat broad absorption band at 5.81 μ. There was a small shoulder at 5.60 μ, probably indicating the presence of a trace of β-propionylglutarobislactone as impurity.

A higher boiling fraction in the above distillation consisted of 1.5 g. of β-propionylglutarobislactone, b.p. 137° (0.2 mm.), m.p. 62.0–62.5°.

Reduction of β-Propionylglutarobislactone with Sodium Borohydride.—To a solution of 53.0 g. (0.312 mole) of the bislactone in 250 cc. of water containing 11 g. of potassium hydroxide was added 12.0 g. of sodium borohydride in small portions during three hours. The reaction was carried out in an erlenmeyer flask with stirring by a magnetic stirrer. The mixture was stirred for an additional six hours, then allowed to stand 12 hours more. The mixture was acidified with 6 *N* hydrochloric acid, then extracted with ether in a continuous extraction apparatus for 48 hours. γ-Caprolactone-β-acetic acid crystallized in the ether flask, 50.0 g. (93%). Colorless crystals were obtained from toluene, m.p. 98.0–98.4°.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03. Found: C, 56.03, 55.78; H, 7.15, 7.15.

Reduction of Ethyl β-Propionylglutarate with Sodium Borohydride.—To a solution of 55.0 g. (0.23 mole) of ethyl β-propionylglutarate in 180 cc. of ethanol was slowly added a solution of 5.4 g. of sodium borohydride in 180 cc. of ethanol and 37 cc. of 2 *N* sodium hydroxide solution. The reaction mixture was mechanically stirred during the addition of the sodium borohydride solution and for 15 minutes thereafter. A white complex which had formed was dissolved by addition of 55 cc. of water, and the solution was stirred for three hours, with occasional warming on the steam-bath. The solution was filtered and the alcohol distilled *in vacuo*. The aqueous alkaline solution was acidified and thoroughly extracted with ether. The ether solution was dried over anhydrous sodium sulfate.

Fractionation of the organic layer gave 14.5 g. (31%) of ethyl γ-caprolactone-β-acetate, b.p. 133–136° (0.08 mm.), $n^{30}\mathrm{D}$ 1.4525.

Anal. Calcd. for $C_{10}H_{16}O_4$: C, 59.88; H, 8.06. Found: C, 59.77, 59.75; H, 8.33, 8.53.

The infrared spectrum of ethyl γ-caprolactone-β-acetate showed carbonyl absorption bands at 5.63 and 5.80 μ.

A second major fraction from the distillation consisted of 15.0 g. (38%) of γ-caprolactone-β-acetic acid, b.p. 175–180° (0.03–0.05 mm.), m.p. 98.0–98.4°.

Ethyl γ-Caprolactone-β-acetate. A. *Via* **the Acid Chloride.**—To 20.0 g. of γ-caprolactone-β-acetic acid in a flask fitted with a stirrer and reflux condenser was added dropwise 14.0 g. of thionyl chloride. The mixture was refluxed on the steam-bath for an additional hour. The unreacted thionyl chloride was removed by distillation, then 25 cc. of absolute ethanol was added and the mixture refluxed on the steam-bath for one hour. Ethanol was distilled *in vacuo*, and the residue was fractionated. There was obtained 22.0 g. (94%) of ethyl γ-caprolactone-β-acetate, b.p. 135° (0.09 mm.).

B. **Azeotropic Esterification.**—The reaction was carried out in the same manner as that described for ethyl β-propionylglutarate. From 50.0 g. of γ-caprolactone-β-acetic acid there was obtained 48.0 g. (83%) of the ester.

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF KANSAS
LAWRENCE, KANSAS

N,N'-Dialkyloxamides[1]

BY LEONARD M. RICE, CHARLES H. GROGAN AND E. EMMET REID[2]

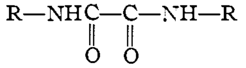
RECEIVED OCTOBER 9, 1952

In the course of another investigation we have prepared the series of symmetrical N,N'-dialkyloxamides through octadecyl, with the exception of heptadecyl. The oxamides were obtained in excellent yields by the reaction of 2 moles of a primary amine with one mole of ethyl oxalate. In all cases the compounds formed readily at room temperature and separated in a high state of purity. Several members of the series had been previously prepared in isolated cases.

The derivatives may be formed in an aqueous alcohol medium in contrast to acid chlorides and aryl isocyanates which are not water stable. Ethyl oxalate, besides being readily available, is not readily affected by water. In addition, this reagent may be used to separate mixtures of primary, secondary and tertiary amines, as the last two are unreactive with ethyl oxalate under the conditions employed.

TABLE I

N,N'-DIALKYLOXAMIDES,



$\mathrm{R{-}NH}\underset{\mathrm{O}}{\underset{\|}{\mathrm{C}}}{-}\underset{\mathrm{O}}{\underset{\|}{\mathrm{C}}}{-}\mathrm{NH{-}R}$

R	Formula	M.p., °C.	Nitrogen, % Calcd.	Nitrogen, % Found
Butyl	$C_{10}H_{20}N_2O_2$	153–154	13.99	14.08
Amyl	$C_{12}H_{24}N_2O_2$	141–142	12.27	11.82
Hexyl	$C_{14}H_{28}N_2O_2$	134–135	10.93	10.71
Heptyl	$C_{16}H_{32}N_2O_2$	132–132.5	9.85	9.45
Octyl	$C_{18}H_{36}N_2O_2$	124–125	8.97	8.93
Nonyl	$C_{20}H_{40}N_2O_2$	128–129	8.23	8.33
Decyl	$C_{22}H_{44}N_2O_2$	122–123	7.60	7.97
Hendecyl	$C_{24}H_{48}N_2O_2$	124–125	7.06	7.33
Dodecyl[a]	$C_{26}H_{52}N_2O_2$	123–124	6.59	6.67
Tridecyl	$C_{28}H_{56}N_2O_2$	120–121	6.19	6.29
Tetradecyl[b]	$C_{30}H_{60}N_2O_2$	117.5–119	5.82	5.98
Pentadecyl	$C_{32}H_{64}N_2O_2$	120	5.51	5.42
Hexadecyl[b]	$C_{34}H_{68}N_2O_2$	117–118	5.22	5.02
Octadecyl	$C_{38}H_{76}N_2O_2$	118–120	4.72	4.54

[a] Grunfeld, *Compt. rend.*, **194**, 893 (1932). [b] S. P. Massie, *Iowa State Coll. J. Sci.*, 21, 41 (1946).

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[1] Supported in part by a grant from the Geschickter Fund for Medical Research, Inc.

[2] Professor Emeritus, Johns Hopkins University, Baltimore, Md.

The compounds prepared are listed in Table I with the exception of the methyl, ethyl and propyl derivatives.[3]

Experimental

Preparation of N,N'-Dialkyloxamides.—The N,N'-dialkyloxamides were in general readily prepared by the reaction of ethyl oxalate with two moles of the desired primary amine in ethanol or aqueous ethanol. The reaction was exothermic. The flask was then allowed to stand until it cooled and the contents generally formed a solid cake due to precipitation of the oxamide. With the lower amines it was necessary to stopper the flask during the initial reaction to prevent loss of the amine. The reaction mixture was then warmed gently just to the boiling point and diluted with an equal volume of boiling alcohol. On cooling to room temperature, the oxamides were isolated in average yields of 80% or better and in a relatively high state of purity. Without the dilution nearly quantitative yields of somewhat less pure materials were obtained.

In the case of the higher amines, beginning approximately at decyl, it was found that repeating the warming procedure twice or more before dilution was advisable in order to complete the reaction. All of the dialkyloxamides readily recrystallized from 95% ethanol in which they were only sparingly soluble at room temperature but in which they were readily soluble at the boiling point.

Preparation of N,N'-Dipentadecyloxamide.—Into 25 cc. of 95% ethanol were placed 1.46 g. (0.01 mole) of ethyl oxalate and 4.55 g. (0.02 mole) of *n*-pentadecylamine. The reaction mixture was warmed to the boiling point and permitted to cool to room temperature twice and, on the third warming, it was diluted with an equal volume of boiling ethanol. After standing at room temperature for one hour, the crystalline oxamide was filtered, sucked dry on the funnel, and finally dried at 100° in an air oven. 4.2 g. or 82% of the oxamide was obtained, m.p. 116–117°. Two recrystallizations from 95% ethanol gave the compound with m.p. 119.5–119.8°.

Anal. Calcd. for $C_{32}H_{64}N_2O_2$: N, 5.51. Found: N, 5.42.

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[3] Dermer and Hutcheson, *Proc. Okla. Acad. Sci.*, **23**, 60 (1943).

GEORGETOWN UNIVERSITY MEDICAL CENTER
WASHINGTON 7, D. C.

Reactions of Trimethylene Sulfide with Chlorine and Bromine

BY JOHN M. STEWART AND CHARLES H. BURNSIDE

RECEIVED SEPTEMBER 2, 1952

A previous report[1] from this Laboratory has dealt with the cleavage of the three-membered sulfide ring in propylene sulfide by chlorine and bromine. Varying the amount of halogen used or the manner of addition of the reactants, or changing from anhydrous solutions to aqueous solutions led to the formation of a number of different types of products. It was of interest to see whether these reactions could be extended to the four-membered sulfide ring compound, trimethylene sulfide. The four-membered sulfide ring has been shown to be more stable to cleavage than the three-membered sulfide ring. For example, trimethylene sulfide formed a stable sulfone when treated with 30% hydrogen peroxide or potassium permanganate,[2,3] while propylene sulfide was cleaved by 30% hydrogen peroxide to yield 2-hydroxy-1-propanesulfonic acid and sulfuric acid.[1] Cleavage of the trimethylene sulfide ring has been reported to take place on treatment with methyl iodide[4] and ammonia.[2,5] Nitric and hydrochloric acids have been reported to cause polymerization.[3,5] Bost and Conn[3] found that trimethylene sulfide formed an addition compound with bromine which decomposes even at −15°. They did not identify the decomposition products.

Discussion

When chlorine was added to trimethylene sulfide in a chloroform solution in a ratio of one mole of chlorine to two moles of the sulfide, bis-(3-chloropropyl) disulfide (I) was formed as the principal product. Reaction of this compound with excess piperidine gave bis-(3-piperidinopropyl) disulfide (II) isolated as the dihydrochloride salt. Similarly, addition of bromine to a chloroform solution of trimethylene sulfide resulted in the formation of an addition compound which was, however, unstable to heat and could not be purified by distillation. Sulfur analysis on the crude product showed a rough agreement with that calculated for bis-(3-bromopropyl) disulfide, and reaction of this crude product with piperidine gave the same compound II previously obtained from I.

When the manner of addition of the reactants was reversed and trimethylene sulfide in a chloroform solution was added to a solution of chlorine in chloroform at temperatures of −40 to −60° with a one-to-one molecular ratio of reactants, one of the principal products was 3-chloro-1-propanesulfenyl chloride (III). This compound was stable enough to be purified by vacuum distillation and had the characteristic odor and deep color of the sulfenyl halides. It was necessary to add a small amount of hydroquinone as chlorination inhibitor in order to obtain even fair yields of III. Reaction of III with a molecular equivalent of trimethylene sulfide gave I. It appears likely, then, that III is an intermediate when chlorine is added to trimethylene sulfide in a one-to-two molecular ratio and that III then reacts further with the sulfide to form I. These results parallel those obtained in the study of the reactions of propylene sulfide with the same halogens[1] and a similar mechanism to that proposed in the discussion of that work may operate in this case.

The reaction of trimethylene sulfide and excess chlorine in a 75% acetic acid solution was found to give a good yield of a liquid product whose physical constants were in close agreement to those reported for 3-chloro-1-propanesulfonyl chloride by Helberger, *et al.*[6] The sulfonamide derivative of this material had a melting point identical to that reported for 3-chloro-1-propanesulfonamide by Kharasch, *et al.*[7]

The behavior of trimethylene sulfide and of propylene sulfide with chlorine and bromine under the same conditions is identical as to the types of products formed. The only differences appear to be a slower rate of reaction of the four-membered

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[1] J. M. Stewart and H. P. Cordts, THIS JOURNAL, **74**, 588 (1952).

[2] E. Griskevich-Trokhimovskii, *J. Russ. Phys. Chem. Soc.*, **48**, 880 (1916).

[3] R. W. Bost and M. W. Conn, *Ind. Eng. Chem.*, **25**, 526 (1933).

[4] G. M. Bennet and A. L. Hock, *J. Chem. Soc.*, 2496 (1927).

[5] D. S. Tarbell and D. P. Harnish, *Chem. Revs.*, **49**, 22 (1951).

[6] J. H. Helberger, G. Manecke and H. M. Fischer, *Ann.*, **562**, 23 (1949).

[7] M. S. Kharasch, E. M. May and F. R. Mayo, *J. Org. Chem.*, **3**, 157 (1938).

sulfide ring compound accompanied by a somewhat lower yield of the final products.

Experimental

Trimethylene sulfide was prepared by a modification of the procedure of Bennet and Hock[6] in an average yield of 45%, b.p. 90–93° (680–685 mm.), n^{20}_D 1.5070.

Bis-(3-chloropropyl) Disulfide (I).—A solution of 5 g. (0.0676 mole) of trimethylene sulfide in 25 ml. of chloroform was stirred and externally cooled by a cold water-bath while 2.4 g. (0.0338 mole) of chlorine was passed in as a vapor beneath the surface of the solution. Argon was then bubbled through the mixture for a short time and the solvent was stripped. On distillation and redistillation there was obtained 3.6 g. (48.7%) of a colorless oil, b.p. 113–115° (1 mm.), n^{20}_D 1.5450.

Anal.[8] Calcd. for $C_6H_{12}Cl_2S_2$: C, 32.87; H, 5.52; S, 29.25. Found: C, 33.03; H, 5.22; S, 29.57.

A solid derivative was prepared by refluxing the above product with excess piperidine in ethanol, followed by water-washing to remove unreacted piperidine and ethanol, drying the product, and then dissolving it in absolute ethanol and bubbling dry hydrogen chloride through the solution. Ether was added to precipitate the dihydrochloride of II. Recrystallization from an ethanol–ether mixture gave white crystals, melting with decomposition at 210–212°.

Anal. Calcd. for $C_{16}H_{34}Cl_2N_2S_2$: Cl, 18.21; S, 16.46. Found: Cl, 18.03; S, 16.30.

Bis-(3-bromopropyl) Disulfide.—The reaction between trimethylene sulfide and bromine was carried out as in the preparation of I described above except that a solution of bromine in chloroform was added dropwise. Attempts to distil the crude product resulted in decomposition. A sulfur analysis was made on the *crude* product.

Anal. Calcd. for $C_6H_{12}Br_2S_2$: S, 20.81. Found: S, 22.03.

Reaction of the crude product with piperidine followed by dry hydrogen chloride as described for I gave a dihydrochloride identical with that prepared from I, m.p. 210–212° with decomposition. A mixed melting point of the two dihydrochlorides showed no depression.

3-Chloro-1-propanesulfenyl Chloride (III).—A solution of 3.83 g. (0.054 mole) of chlorine in 10 ml. of chloroform to which a few crystals of hydroquinone had been added was chilled in a Dry Ice–acetone-bath. To this a solution of 4 g. (0.054 mole) of trimethylene sulfide in 40 ml. of chloroform, also precooled in a Dry Ice–acetone-bath, was added dropwise over a period of 10 minutes. The reaction mixture was allowed to warm to room temperature, argon was bubbled through it for a short time, and the solvent was stripped. Distillation gave about 3 ml. of orange liquid of extremely strong and irritating odor, b.p. 48–55° (3.5 mm.), and a mixture of higher-boiling lighter-colored liquids of no definite boiling range. The orange liquid was redistilled and yielded 2.3 g. (30%) of product, b.p. 51–53° (4 mm.), n^{20}_D 1.5190. This compound III was not analyzed directly, but was immediately combined with trimethylene sulfide (1.2 g.) in chloroform to form the previously prepared bis-(3-chloropropyl) disulfide in a yield of 1.9 g. (54%), b.p. 112–115° (1 mm.), n^{20}_D 1.5456. Reaction of this compound with piperidine followed by dry hydrogen chloride gave the same derivative, bis-(3-piperidinopropyl) disulfide dihydrochloride, previously prepared from I.

3-Chloro-1-propanesulfonyl Chloride.—To 80 ml. of a saturated, externally cooled solution of chlorine in 75% acetic acid was added dropwise with stirring 5.8 g. (0.0784 mole) of trimethylene sulfide, and chlorine was introduced below the surface at such a rate that an excess was always present. Addition of chlorine was continued for five minutes after all the sulfide had been added. Air was then blown through the reaction mixture to remove excess chlorine. The mixture was diluted with 400 ml. of water, extracted with ether and the combined extracts were dried over sodium sulfate. Distillation gave 10 g. (72%) of colorless liquid, b.p. 82–85° (1 mm.), n^{23}_D 1.4890. The reported physical constants[6] for 3-chloro-1-propanesulfonyl chloride are b.p. 117–118° (15 mm.), n^{23}_D 1.4900. The sulfonamide derivative was prepared and found to have a melting point of 62–63°. This is in agreement with the previously reported melting point of 63° for 3-chloro-1-propanesulfonamide.[7]

CHEMISTRY DEPARTMENT
MONTANA STATE UNIVERSITY
MISSOULA, MONTANA

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[8] Microanalyses for carbon and hydrogen were performed by the Clark Microanalytical Laboratory, Urbana, Ill. Chlorine and sulfur analyses were performed by the authors.

Alkyl Esters of Isodehydroacetic Acid

BY RICHARD H. WILEY, NEWTON R. SMITH AND JOSEPH A. BAUER

RECEIVED AUGUST 4, 1952

This note will serve to record the preparation and properties of some previously undescribed alkyl esters of isodehydroacetic acid. The esters were obtained by reaction of isodehydroacetic acid chloride with the alcohol. The acid chloride was prepared from the acid and thionyl chloride and the acid was obtained as previously described.[1] Since the acid chloride has not been previously characterized, details of its preparation and supporting analytical data are included. The methyl, *n*- and isopropyl, butyl, isoamyl and cetyl esters described in Table I have been obtained from the corresponding alcohols. The procedure used in their preparation is similar to that described for the *n*-propyl ester in the Experimental section. With redistilled, commercial 2-ethylhexyl alcohol a liquid product was obtained from which crystals separated on standing. This product did not give analytical values in accord with theory.

Since this procedure has not been previously used for the preparation of esters of isodehydroacetic acid, we have prepared the known methyl ester from the acid chloride and converted it to the known 3-bromo derivative. Both have melting points in agreement with those previously recorded for these compounds as prepared by other methods.

Acknowledgment.—The authors acknowledge grants from the Research Corporation and the Research Committee of the College of Arts and Sciences in support of this program.

Experimental[2]

Isodehydroacetic Acid Chloride.—Eighteen ml. (0.25 mole) of thionyl chloride was added to 25 g. (0.15 mole) of isodehydroacetic acid in a 125-ml. claisen flask. The mixture was heated under reflux for 10–20 minutes. The gaseous products and excess thionyl chloride were removed under vacuum. The residue was distilled to give 22–25.0 g. (79–90% of the theoretical amount) of isodehydroacetic acid chloride, b.p. 145–150° at 15 mm.; reported[3] b.p. 138–140° at 12 mm. The product solidifies on cooling, m.p. 52°.

Anal. Calcd. for $C_8H_7O_3Cl$: Cl, 19.00. Found: Cl, 18.88.

***n*-Propyl Isodehydroacetate.**—The acid chloride, prepared as above, was melted and portions (4.9–5.6 g.) were poured into 1 × 4 inch test-tubes. Such a sample of acid chloride (5.6 g., 0.03 mole) was melted under a reflux condenser on a water-bath and to it was added an excess (3.24 g., 0.054 mole) of dried *n*-propyl alcohol. The solution was refluxed 10–15 minutes and fractionated to give 5.0 g. (79% of the theoretical amount) of *n*-propyl isodehydroacetate, b.p. 166° at 8 mm.), $n^{23.5}_D$ 1.5093.

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[1] R. H. Wiley and N. R. Smith, THIS JOURNAL, 73, 3531 (1951).

[2] Analyses by Clark Microanalytical Laboratory and Micro-Tech Laboratory.

[3] H. M. Basel, *et al.*, U. S. Patent 2,364,304, December, 1944.

TABLE I

ALKYL ESTERS OF ISODEHYDROACETIC ACID

Alkyl	Yield, %	B.p., °C.	Mm.	n_D	t, °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Methyl	99	67[a]	....	....	....	...	...	...	...
n-Propyl	79	116	8	1.5093	23.5	62.85	62.64	6.67	6.80
i-Propyl	85	158	8	1.5050	24	62.85	62.74	6.67	6.80
n-Butyl	89	167	7	1.5063	23.5	64.22	64.27	7.15	7.37
i-Amyl	68	177	8	1.5032	23.8	65.47	65.20	7.56	7.62
Cetyl	71.5	55[a]	....	....	....	73.42	73.33	10.41	10.50

[a] Melting point.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.85; H, 6.67. Found: C, 62.64; H, 6.80.

Methyl isodehydroacetate was prepared from 1.0 g. (0.054 mole) of the acid chloride and 5 ml. of absolute methanol. Evaporation gave *ca.* 1.0 g. of the ester, m.p. 67°; reported m.p. 67–67.5°.[4] The product was converted to the 3-bromo derivative by reaction with an equivalent amount of bromine in carbon tetrachloride; m.p. 133–134°; reported m.p. 135°.[5]

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[4] R. Anschutz, P. Bendix and W. Kerp, *Ann.*, **259**, 156 (1890).

[5] E. Buchner and H. Schröder, *Ber.*, **35**, 790 (1908).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF LOUISVILLE
LOUISVILLE, KY.

Studies on Pituitary Adrenocorticotropin. III. Differentiation of Three Active Types on XE-97 Resin

BY W. F. WHITE AND W. L. FIERCE

RECEIVED SEPTEMBER 29, 1952

By the use of the cation exchange resin Amberlite XE-97,[1] three active ACTH types have been differentiated in hog pituitary extracts. Two active types have been separated from the highly purified fractions of acid- and pepsin-treated extracts described in a previous publication.[2] A third active type has been found in highly potent oxycellulose eluates made from hog pituitaries[3] and in other non-hydrolyzed preparations.

Figure 1 shows a typical fractionation of an acid- and pepsin-hydrolyzed sample which had previously been purified by means of a chromatopile. The XE-97 bed[4] was equilibrated with 0.1 *M* sodium carbonate–bicarbonate buffer at *p*H 8.5 before application of the sample as a solution in the same buffer. The portion passing directly through the column at *p*H 8.5 (fraction I) was physiologically inert. However, successive substitution of *p*H 9.25 and 11.25 buffers eluted two additional fractions (II and III, Fig. 1) which showed activities in the range of 100 to 150 u./mg. of peptide.[5] In other runs the range between *p*H 8.5 and 11.25 has been investigated in small increments without revealing additional components. The non-identity of fractions II and III has been proved by rerunning them individually through the same type of column, under which condition the integrities of the fractions were maintained.

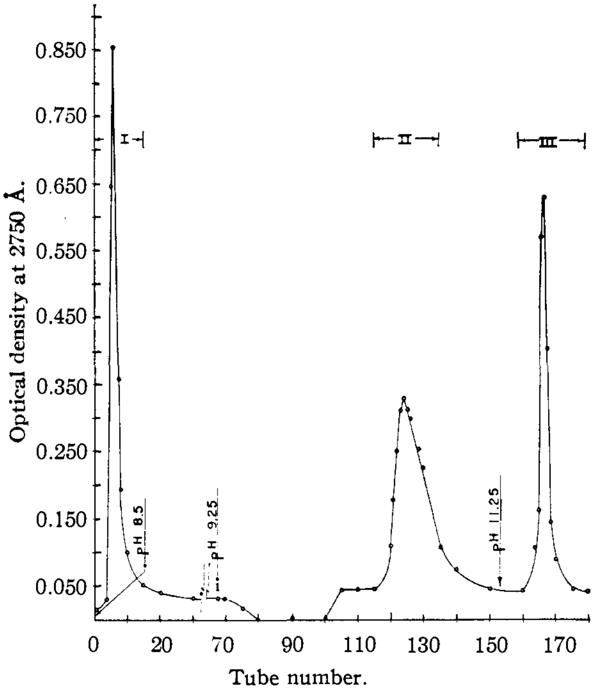

Fig. 1.—Chromatography of a highly purified acid- and pepsin-treated ACTH preparation on Amberlite XE-97 resin. The sample (10.5 mg. at 65 u./mg.) in 1 ml. *p*H 8.5 buffer was applied to a column 15 cm. high in a tube 0.9 cm. in diameter. Rate of flow was 0.5 ml./min. Volume collected per tube was 1 ml. The distribution of ultraviolet absorption in the fractions indicated was: I, 25%; II, 28%; III, 24%. The distribution of activity was: I, < 2%; II, 64%; III, 19%.

A much different result was obtained when highly active unhydrolyzed ACTH preparations were subjected to the XE-97 procedure. Here almost all of the ultraviolet absorbing material was eluted at *p*H 8.5 and the activity appeared to be associated with a minor component poorly separated from the major peak.[6] Very little ultraviolet absorbing material and no activity appeared either at *p*H 9.25 or *p*H 11.25. Increasing the height of the

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[1] Rohm & Haas Co., Washington Square, Philadelphia 5, Pa.

[2] W. F. White, W. L. Fierce and J. B. Lesh, *Proc. Soc. Exptl. Biol. Med.*, **78**, 616 (1951).

[3] E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).

[4] The resin was prepared as follows: The material as supplied by the manufacturer was stirred three or four times with water, each time allowing the suspension to settle four or five minutes and pouring off the fines. The washed resin was then cycled three times batchwise using *N* sodium hydroxide and *N* hydrochloric acid. After thorough washing with water, the resin was stored in the acid form either wet or dry until use.

[5] Fractions were assayed by the Munson modification of the adrenal ascorbic depletion method of M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinol.*, **42**, 379 (1948). This technique has provisional USP approval. The samples were administered intravenously. All activities are expressed as USP units.

[6] Dixon, *et al.*, *Nature*, **168**, 1044 (1951), noted a similar behavior with a crude unhydrolyzed preparation. However, their conditions of *p*H and buffer composition were different.

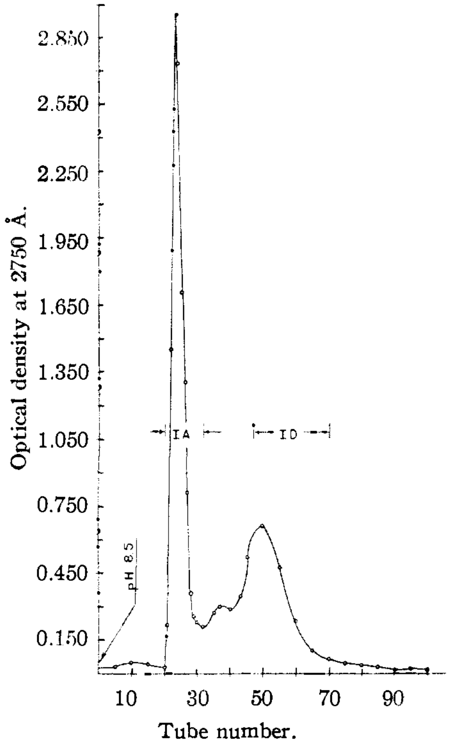

Fig. 2.—Chromatography of an oxycellulose eluate ACTH preparation on Amberlite XE-97 resin. The sample (430 mg. at 40 u./mg.) in 100 ml., *p*H 8.5 buffer was applied to a column 70 cm. high in a tube 5.4 cm. in diameter. Rate of flow was 3.5 ml./min. Volume collected per tube was 17 ml. The distribution of ultraviolet absorption was: IA, 54%; ID, 20%. The distribution of activity was: IA, < 2%; ID, 70%.

XE-97 column improved the resolution at *p*H 8.5. Figure 2 shows a run using a 70-cm. bed.

TABLE I

EFFECT OF VARIOUS HYDROLYTIC TREATMENTS ON THE PERFORMANCE OF AN OXYCELLULOSE ELUATE[a] ON XE-97 RESIN

	*p*H 8.5 (Type ID)		*p*H 9.25 (Type II)		*p*H 11.25 (Type III)	
Type of hydrolytic treatment	O.D.[b]	Activity[c]	O.D.	Activity	O.D.	Activity
None	31	70	5	<5	17	<5
Pepsin[d]: 2 hours	None	<5	21	100	17	<5
Pepsin: 24 hours	None	<5	44	80	12	<5
Pepsin: 4 hours followed by acid (1 hour at 100° in 0.01 *N* HCl)	None	<5	27	75	21	25

[a] The starting material in the experiments summarized in this table was a typical oxycellulose eluate made by the Astwood process and having a potency of about 25 μ/mg. [b] Optical densities are given as percentages of the total optical density recovered in all fractions. The difference between the totals given in the three fractions listed and 100 is the amount appearing before fraction ID. [c] Given as percentages of the amount of activity put on the column. Where the totals do not add up to 100, it is assumed that the remainder was destroyed in handling. [d] In these experiments, the amount of pepsin was 1% of the ACTH fraction and the digestion was done at 37° in 0.01 *N* HCl (*p*H 2.1–2.3). In other experiments, a considerable variation (0.6–4%) in the percentage of pepsin was without appreciable effect on the results.

In studying the relationships between the three types of ACTH, an oxycellulose eluate has been treated with pepsin and with pepsin and acid. Table I shows the fractionation of these materials on XE-97 resin. The percentages of ultraviolet absorption and of activity going to the various positions are shown in each case. As seen in the table, treatment with pepsin for as little as two hours converts all the type ID activity to type II, while even 24 hours does not produce an appreciable amount of type III. However, subsequent treatment with acid converts at least part of type II into type III.

In view of the fact that the variations in the conditions of pepsin treatment in the experiments of Table I and of other experiments (*cf.* footnote *d*, Table I) include those used by Brink, *et al.*,[7] it would appear likely that Type II activity predominated in the concentrate from which Corticotropin-B was isolated. Our experiments, using XE-97 resin on material processed by successive pepsin and acid treatment, appears to be the first in which two hydrolyzed types of ACTH are clearly differentiated. Further work directed toward the isolation in pure form of the three active types is under way.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. R. L. Peters.

(7) N. G. Brink, F. A. Kuehl, Jr., J. W. Richter, A. W. Bazemore, M. A. P. Meisinger, D. E. Ayer and K. Folkers, THIS JOURNAL, **74**, 2120 (1952).

THE ARMOUR LABORATORIES
CHICAGO, ILLINOIS

Rates of Solvolysis of Some Alkyl Fluorides and Chlorides[1]

BY C. GARDNER SWAIN AND CARLETON B. SCOTT

RECEIVED MAY 14, 1952

Table I shows that the RCl/RF rate ratio for hydrolysis in neutral or slightly acidic solutions varies from 10^6 for triphenylmethyl (trityl) halides to less than 10^2 for benzoyl halides. This reflects the tendency of C–X rupture to be more complete than O–C formation at the transition state of trityl halide hydrolysis, and the opposite tendency with benzoyl halides.[2] The change from Cl to F hinders the C–X break, but facilitates O–C formation by making the carbon more electron-deficient and positive.

The ratio is further reduced in basic solution (*cf.* Table II). Toward hydroxide ion, benzoyl fluoride actually reacts faster (by 40%) than benzoyl chloride.

Experimental

Reagents.—Benzoyl fluoride was prepared from 140 g. (1 mole) of benzoyl chloride in a polyethylene bottle, fitted with copper entrance and exit tubes in a 2-hole rubber stopper, by passing in anhydrous hydrogen fluoride until the exit gas gave no precipitate with silver nitrate solution. Best results were obtained when the polyethylene bottle rested in an ice-bath and the hydrogen fluoride was con-

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(1) This work was supported by the Office of Naval Research.

(2) The same factor is responsible for the negative ρ-values[3] and small *s*-values[4] generally observed with trityl halides in contrast to the positive ρ-values and large *s*-values with benzoyl halides.

(3) C. G. Swain and W. P. Langsdorf, Jr., THIS JOURNAL, **73**, 2813 (1951).

(4) C. G. Swain and C. B. Scott, *ibid.*, **75**, 141 (1953).

TABLE I

RELATIVE RATES OF SOLVOLYSIS OF ORGANIC CHLORIDES AND FLUORIDES

Compound	Solvent	Temp., °C.	k_1, sec.$^{-1}$	ΔS^*, cal. deg.$^{-1}$	ΔE^*, kcal.	k_{RCl}/k_{RF}
Trityl fluoride[a]	15% H_2O 85% acetone	25	2.7×10^{-6}	−10	22.6	
Trityl chloride[b]	15% H_2O 85% acetone	25	2.7	−17	12.5	1×10^6
t-Butyl fluoride[c]	20% H_2O 80% EtOH	25	1×10^{-10}			
t-Butyl chloride[c]	20% H_2O 80% EtOH	25	9.1×10^{-6}			1×10^5
Acetyl fluoride	25% H_2O 75% acetone	25	1.1×10^{-4}			
Acetyl chloride[d]	25% H_2O 75% acetone	25	8.6×10^{-1}	−14	13.9	7.8×10^3
Benzenesulfonyl fluoride	50% H_2O 50% acetone	25	$<5 \times 10^{-8}$			
Benzenesulfonyl chloride	50% H_2O 50% acetone	25	2.4×10^{-4}	−29	14.3	$>4.8 \times 10^3$
Benzoyl fluoride	50% H_2O 50% acetone	0.5	1.1×10^{-5}			
Benzoyl fluoride[e]	25% H_2O 75% acetone	25	8.2×10^{-6}			
Benzoyl chloride[f]	50% H_2O 50% acetone	0	4.3×10^{-4}	−7.1	18.8	39
Benzoyl chloride[e]	25% H_2O 75% acetone	25	7.2×10^{-4}			88

[a] Calculated from runs in 30% water–70% acetone by Mr. R. B. Mosely. [b] Calculated from runs at −34 and −14°. [c] K. A. Cooper and E. D. Hughes, *J. Chem. Soc.*, 1183 (1937). [d] Calculated from runs at −30 and −11°. [e] Data supplied by Mr. D. E. Bown. [f] G. Berger and S. C. J. Olivier, *Rec. trav. chim.*, **46**, 516 (1927).

TABLE II

RATES WITH HYDROXIDE ION IN 50% WATER–50% ACETONE AT 0.5°

Compound	k_2, M^{-1} sec.$^{-1}$	Relative to solvolysis[a]
Acetyl fluoride	250	3.1×10^7
Benzoyl fluoride	21	5.4×10^7
Benzoyl chloride	15	1.0×10^6
Benzenesulfonyl fluoride	0.11	$>6 \times 10^7$
Benzenesulfonyl chloride	0.68	7.3×10^5

[a] Values of k/k° where $k = k_{OH^-} = k_2$, $k^\circ[H_2O] = k_w = k_1$.

densed by cooling the entrance tube. Frequent swirling of the contents was necessary since the reaction was very vigorous. After the addition, the ice-bath was replaced by a steam-bath until hydrogen fluoride evolution ceased.

TABLE III

SAMPLE KINETIC DATA

Substrate	Concn., $M \times 10^1$	Water, % by volume	Temp., °C.	Added reagent	Concn., $M \times 10^3$	k_1, sec.$^{-1}$	Run no.
Acetyl chloride	6.8	25	−30	......	...	4.1×10^{-3}	254
	7.6	25	−11	......	...	3.3×10^{-2}	255
Acetyl fluoride	3.3	25	25	......	...	1.1×10^{-4}	267
	4.7	50	0.5	......	...	2.2×10^{-4}	248
	8.6	50	.5	$LiClO_4$	100	2.0×10^{-4}	251
	4.9	50	.5	$HClO_4$	100	3.1×10^{-4}	252
	2.8	50	.5	H_3BO_3[a]	20	4.1×10^{-2}	244
				NaH_2BO_3	20		

The dark liquid residue was dissolved in benzene and rapidly extracted with ice-water to remove more hydrogen fluoride. The benzene solution was dried over sodium sulfate and the benzoyl fluoride distilled, yielding 74 g. (60%), b.p. 155–157°, n^{15}D 1.4988.[5]

Acetyl fluoride was prepared from 150 g. (2.1 moles) of acetyl chloride added dropwise from a dropping funnel to 100 g. (0.96 mole) of zinc fluoride (Harshaw technical, dried at 100° for 10 hours under oil-pump vacuum) in a 500-ml. flask at 0° fitted with vertical condenser, sealed stirrer and calcium chloride tubes.[6] After the addition, the water-bath was warmed to 40°. The vapors passed through a 50-cm. vertical condenser held at 20°, then into a condensing head and well-cooled receiver. The distillate was mixed with 3 g. of anhydrous sodium fluoride, redistilled through a small Vigreux column and stored in a polyethylene bottle containing a small test-tube of sodium fluoride to remove hydrogen fluoride; yield 76 g. (1.2 moles, 63%), b.p. 19.5–20.0°.

Benzoyl bromide from Eastman Kodak Co. was redistilled, b.p. 80° (7 mm.), n^{25}D 1.5864.

Benzenesulfonyl fluoride from the Pennsalt Co. was redistilled, b.p. 83° (3 mm.), n^{25}D 1.4897.

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(5) A. I. Mashentsev, *J. Gen. Chem., U.S.S.R.*, **15**, 915 (1945); *C. A.*, **40**, 6443 (1946), reported n^{15}D 1.4988 from a different synthesis.

(6) M. Meslans, *Ann. chim. phys.*, [7] 1, 411 (1894).

TABLE III (*Continued*)

Substrate	Concn., $M \times 10^1$	Water, % by volume	Temp., °C.	Added reagent	Concn., $M \times 10^3$	k_1, sec.$^{-1}$	Run no.
Benzoyl fluoride	4.4	50	.5	......	...	1.1×10^{-5}	156
	1.6	50	.5	$H_3BO_3{}^a$ NaH_2BO_3	20 20	3.6×10^{-3}	149
	2.1	50	.5	$H_3BO_3{}^a$ NaH_2BO_3	10 10	3.2×10^{-3}	151
Benzoyl bromide	3.7	50	.5	......	...	6.3×10^{-2}	265
	4.8	50	.5	$H_3BO_3{}^a$ NaH_2BO_3	20 20	5.3×10^{-2}	263
Benzenesulfonyl fluoride	5.1	50	25.1	......	...	$<5 \times 10^{-8}$	257
	4.9	50	25.1	$HClO_4$	100	$<5 \times 10^{-8}$	260
	6.9	50	0.5	$H_3BO_3{}^a$ NaH_2BO_3	20 20	1.8×10^{-5}	242

[a] Hydroxide ion concentration = 1.6×10^{-4} *N*.

Other reagents were analytical reagent grade or previously described.[4]

Procedure.—Most of the procedure has been described.[4] The rate of hydrolysis of acetyl fluoride in 25% water–75% acetone was determined by allowing a mixture of 150 ml. of acetone and 50 ml. of water to come to 25° in a 250-ml. polyethylene bottle and adding acetyl fluoride directly from a pipet. Aliquots (10 ml.) were shaken with 20 ml. of benzene, the aqueous layer removed, and the benzene extracted twice with 5 ml. of water. The water solutions were combined and titrated for fluoride ion.

The hydrolysis of acetyl fluoride in 50% water–50% acetone was accomplished by cooling a mixture of 45 ml. of acetone and 50 ml. of water at 0.5° in the 100-ml. round-bottomed reaction cell and adding the acetyl fluoride in 5 ml. of cold acetone. The 10-ml. aliquots were shaken with 20 ml. of chloroform and titrated for fluoride ion. When an inert salt or an acid was present, 5 ml. of 2 *N* lithium perchlorate or perchloric acid replaced 5 ml. of water in the solvent.

The hydrolyses of benzoyl fluoride and benzenesulfonyl fluoride were followed in a similar manner. The reaction cell was a 250-ml. polyethylene bottle and the solvent was 100 ml. of acetone and 100 ml. of water. The aliquots for benzoyl fluoride were 20 ml., those for benzenesulfonyl fluoride were 10 ml. Since benzenesulfonyl fluoride hydrolyzed at an extremely slow rate, if at all, the 100% point was found by hydrolyzing a 10-ml. aliquot with sodium hydroxide and titrating for fluoride ion. The reaction proceeded to less than 10% in 2.2×10^6 seconds (26 days). The presence of 0.1 *N* lithium perchlorate or perchloric acid had no apparent effect on the rate.

Table III gives supporting kinetic data in addition to those previously reported.[4]

DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASS.

COMMUNICATIONS TO THE EDITOR

THE FRACTIONATION OF HYDROGEN ISOTOPES IN BIOLOGICAL SYSTEMS[1]

Sir:

Although deuterium has been extensively used as an isotopic tracer in studies of intermediary metabolism,[2] relatively little is known about the H:D fractionation that occurred and its effect on the quantitative interpretation of the metabolic data. Although this factor can be measured in chemical reactions it is inherently difficult to measure in metabolic (*in vivo*) studies utilizing only protium (H) and deuterium. However, the use of precursor compounds labeled with both deuterium and tritium can yield precise values for D:T fractionation effects in such studies and the latter can then be used to estimate these effects for D relative to H.[3] We have administered water containing D and T to rats by intraperitoneal injection in order to bring the deuterium body water level up to about two per cent. and then supplied drinking water having the same T/D ratio for several days to maintain this level. Analysis of the glycogen and fatty acid fractions from the livers of these animals shows a preferential incorporation of the deuterium by approximately 8 and 18 per cent., respectively (Table I). The results for the fatty acids are in qualitative agreement with those recently reported by Glascock and Dunscombe.[4] In the latter experiments, the body fluid isotope

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[1] This work was supported in part by grants-in-aid from the Atomic Energy Commission No. AT(30-1)-910.

[2] R. Schoenheimer, Dynamic State of Body Constituents, Harvard University Press, 1946; M. Kamen, Radioactive Tracers in Biology, Chap. VII, Academic Press, N. Y., 1951.

[3] W. G. Verley, J. R. Rachele, V. du Vigneaud, M. L. Eidinoff and J. E. Knoll, THIS JOURNAL, **74**, 5941 (1952). When methanol containing CD_3OH, CHD_2OH, CH_2DOH and CH_2TOH was administered to rats, the (T/D) ratio in the methyl groups of choline and creatine was greater than the corresponding ratio in the administered methanol.

[4] R. F. Glascock and W. G. Dunscombe, *Biochem. J.*, **51**, August, (1952), xl, Communication to Proceedings of the Biochemical Society.