of the unreacted methyl ω-styryl sulfone; they were combined and dissolved in acetone, and potassium permanganate was added until a purple color persisted. The manganese dioxide was filtered off and the acetone was evaporated. Water was added and the saturated sulfone was extracted with 200 ml. of ether. Evaporation of the ether left a residue, which was recrystallized from methanol and water to give 1.8 g. of methyl β-phenyl-β,*p*-tolylethyl sulfone; m.p. 92–93°, total yield 2.76 g. (37% of theory).

Anal. Calcd. for $C_{16}H_{18}O_2S$: C, 69.82; H, 6.57. Found: C, 69.85; H, 6.75.

Alkylation of Toluene with *p*-Tolyl ω-Styryl Sulfone.—This compound[4] (II) (10 g., 0.039 mole) was added to a stirred mixture of toluene (100 ml.) and 97% sulfuric acid (20 g.) cooled in an ice-bath. The mixture was stirred at ice-bath temperature for 1 hour and then at room temperature for 6 hours. The reaction mixture was poured into ice-water and the aromatic layer was separated. The water layer was extracted with 200 ml. of ether and the combined aromatic layer and ether extract was evaporated to dryness under an air jet. The solid obtained was dissolved in absolute methanol and crystallized in three fractions: (1) 4.3 g., m.p. 130–132°; (2) 1.5 g., m.p. 124–127°; (3) 3.8 g., m.p. 106–111°. A mixture of 0.09 g. of fraction (1) and 0.08 g. of the known sample[3] (m.p. 131–132.5°) had mp. 130.5–132.5°.

Alkylations with Methyl Vinyl Sulfone.—The quantities of reactants used were: 0.1 mole of methyl vinyl sulfone,[9] 0.22 mole of aluminum chloride, and a large excess of the aromatic compound. In all cases a solution of the sulfone in the aromatic compound or in nitrobenzene was added to a stirred mixture of the catalyst and the aromatic compound, in some cases using nitrobenzene as the solvent. The first reactions were run at about 30° for 2 hours generally following the procedure given in ref. (2). In three cases some dry hydrogen chloride was added to the reaction mixtures containing aluminum chloride as a catalyst. When no condensation was obtained the temperature of the subsequent reactions was raised to 90°. The only result of this increase in temperature was an increase in the amount of tar-like product formed. In each case the reaction mixture was d composed by pouring it into an ice-water–hydrochloric acid mixture. The organic layer was then separated, washed with water and steam distilled. The product, if any, should have remained in the pot residue since the sulfone produced by the condensation would be water insoluble and not appreciably steam distillable.

Methyl vinyl sulfone (10.6 g., 0.1 mole), was added to a stirred mixture of benzene (150 ml.) and sulfuric acid (97%, 20 g.) cooled to ice-bath temperature. This mixture was stirred 2 hours at ice-bath temperature, 3 hours at room temperature and 1 hour at 60°. The reaction mixture was hydrolyzed by pouring it into ice-water. The organic layer was separated and evaporated down to a small quantity of oil, which could not be crystallized. The water layer was extracted with two 100-ml. portions of ether and the ether was evaporated; however, no appreciable amount of residue remained.

(9) G. D. Buckley, *et al.*, *J. Chem. Soc.*, 1514 (1947).

LAFAYETTE, INDIANA

NOTES

The Adsorption of Cupric and Mercuric Ions by a Weak-base Anion Exchange Resin

BY JOHN ANDELIN AND NORMAN DAVIDSON

RECEIVED APRIL 25, 1953

Weak-base anion-exchange resins in their "basic" form take up an anion X^- by virtue of the reaction $RNH_2 + H^+ + X^- \rightarrow RNH_3^+ + X^-$, that is, by addition of a proton to the unshared pair of the nitrogen atom. It is reasonable to expect that other cations which form ammine complexes will be adsorbed by the resin. Sussman has briefly reported on the adsorption of cupric ions by an anion-exchange resin.[1] The results of a fragmentary study of the adsorption of Cu^{++} and Hg^{++} ions by the weak-base resin, Amberlite IR-4B, are reported here.

Experimental

A sample of the resin hydrochloride was treated with excess sodium hydroxide, washed with water until on standing for 24 hours the *p*H remained 7–8, and stored over saturated sodium sulfate (vapor pressure 22.3 mm. at 25°, 95% relative humidity).

Cupric solutions were prepared from C.P. copper nitrate and were analyzed iodometrically, using the directions of Swift.[2a] Using 25-ml. portions of solutions at a *p*H of 3–5, Cu^{++} concentrations as low as 2×10^{-4} *M* could be determined to 1% provided the solutions were deoxygenated by bubbling CO_2 gas through them. Mercuric solutions, prepared from mercuric nitrate, were analyzed by thiocyanate titration.[2b] Hydrogen ion in the Hg^{++} solutions was determined by titration with NaOH to a brom thymol blue end-point, in the presence of 0.3 *M* KI to complex the Hg^{++}. The first color change of the indicator gives the end-point, but it subsequently fades.

The adsorption experiments were done at an ionic strength of *ca.* 0.10 volume molar maintained with sodium nitrate. Weighed samples of resin were equilibrated with measured volumes of an aqueous phase in a glass-stoppered erlenmeyer flask by constant end-over-end rotation of the flask. The amount of copper or mercury adsorbed or eluted was computed from the change in concentration of the aqueous phase, and the known initial amount of metal in the resin, or, when necessary, by elution of the copper from the resin with acid. Concentrations of adsorbed constituents in the resin are reported in units of weight molality, *m*, *i.e.*, moles per kilogram of the free base form of the resin. Solution concentrations are reported in units of volume molarity, *M*. Titration curves of the resin, at the ionic strength used in our experiments, are shown in Fig. 1.

Results and Discussion

In order to do experiments at a *p*H at which there was no question about possible precipitation of basic copper compounds, it was necessary to use a resin which was at least 2 *m* in acid. Figure 2 displays the results of a series of experiments on the adsorption and elution of Cu^{++} by the resin. After 5 or 6 days exposure to the aqueous Cu^{++} solution (curves A and B), the rate of adsorption of Cu^{++} fell to a low value. The resin, which was normally amber colored, became blue-green upon adsorbing Cu^{++} and almost black when more

(1) F. C. Nachod (editor), "Ion Exchange," Academic Press, Inc., New York, N. Y., 1949, p. 244.

(2) E. H. Swift, "Introductory Quantitative Analysis," Prentice-Hall, Inc., N. Y., 1950, (a) p. 210, (b), p. 101.

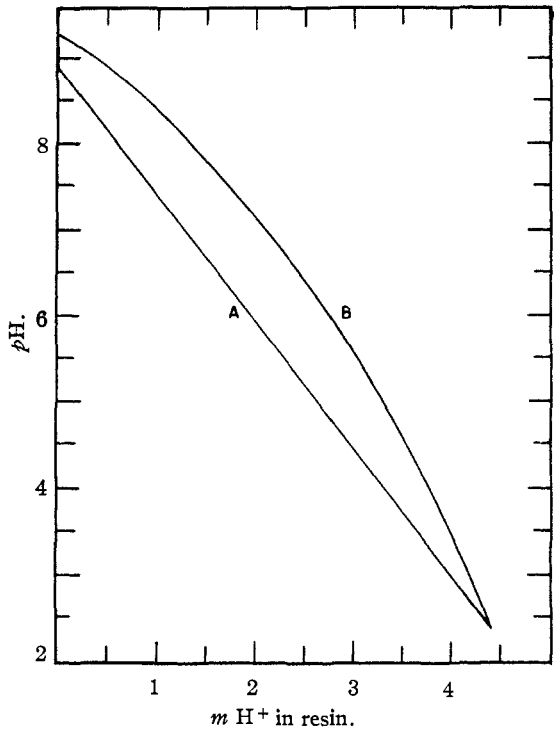

Fig. 1.—Titration curve of Amberlite IR-4B: A, nitric acid added to free base resin; B, NaOH added to resin resulting from the above titration. In each experiment, acid (or base) was added in small batches to 1.00 g. resin in 100 ml. of 0.100 *M* $NaNO_3$ and the *p*H measured after 2 hr. stirring. The final volumes of solutions were 105 ml.; in the course of the back titration, μ increased from 0.10 to 0.14 due to NO_3^- liberated from the resin.

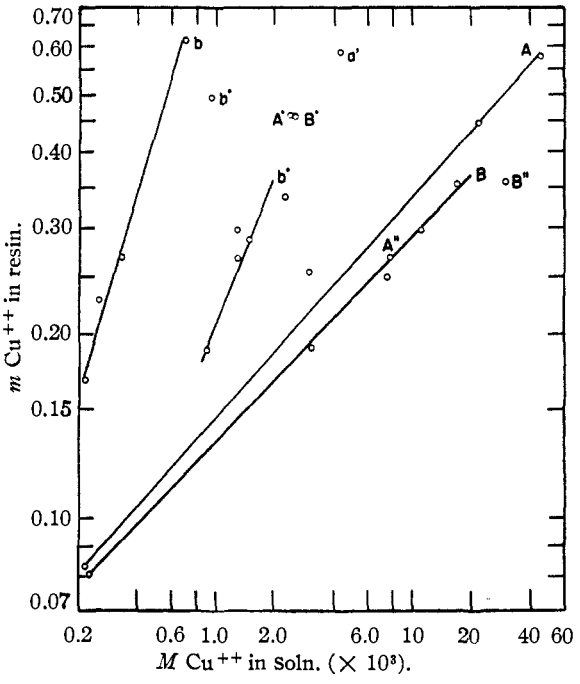

Fig. 2.—Adsorption of Cu^{++} by Amberlite IR-4B: 1 g. resin/100 ml. aqueous phase; constant stirring, 28.3°; final ionic strength, 0.09–0.10 ($NaNO_3$). A", 2 *m* H^+ in resin (one point), adsorption, 5 days; A, (four points), 6 days; A', 90 days; a', elution, 90 days; B", 3 *m* H^+ in resin, adsorption, 5 days; B, (five points), 6 days; B', 90 days; b, elution, 6 days; b', 90 days; b+, elution of 2 *m* H^+ resin with 0.01 *M* HNO_3 so that resin became 3 *m* in H^+. Some typical values of the ratio, *r*, of moles H^+ displaced/moles Cu^{++} adsorbed are: Curve B, (Cu^{++}) = 0.0031 *M*, *r* = 0.045, (Cu^{++}) = 0.017 *M*, *r* = 0.083; B", (Cu^{++}) = 0.030 *M*, *r* = 0.13; B', (Cu^{++}) = 0.0026 *M*, *r* = 0.020. Curve A, (Cu^{++}) = 0.0030 *M*, *r* = 0.0007; (Cu^{++}) = 0.022, *r* = 0.0027; (Cu^{++}) = 0.046, *r* = 0.0043; A", (Cu^{++}) = 0.0024, *r* = 0.0010.

than 0.2 *m* in Cu^{++}. More Cu^{++} was adsorbed from concentrated solutions than from dilute solutions. More Cu^{++} was adsorbed on to a resin that was 2 *m* in H^+ than on to a resin that was 3 *m* in H^+. However, on standing 90 days, much more Cu^{++} was adsorbed and there was no significant difference between the 2 *m* H^+ and 3 *m* H^+ resin (A' and B'). Elution for 90 days of resin samples that were rich in Cu^{++} (points a' and b') with 0.1 *M* $NaNO_3$ gave points which agree, within a factor of 2, with the 90 day adsorption experiments. Therefore, it seems likely that the 90 day adsorption and elution experiments define the equilibrium conditions within a factor of 2.

There was a small but significant release of H^+ by the resin to the aqueous phase as Cu^{++} was adsorbed In effect, therefore, most of the Cu^{++} was adsorbed by the reaction, $RNH_2 + Cu^{++}(aq.) \rightarrow RNH_2Cu^{++}$, rather than by $RNH_3^+ + Cu^{++}(aq.) \rightarrow RNH_2Cu^{++} + H^+(aq.)$. Typical values of the amount of H^+ liberated are listed in the legend to Fig. 2.

The long time required to achieve equilibrium in adsorption indicates that the rate of diffusion of Cu^{++} into the resin particles is quite slow. It is probable therefore that much of the Cu^{++} adsorbed in the 5–6 day experiments was on the outside of the spherical particles. There was a greater release of H^+ per Cu^{++} adsorbed from the 3 *m* resin in the 5–6 day experiments than in the 90 day experiment. This probably indicates that H^+ was displaced from the outside of the resin particles by the concentrated Cu^{++} but was readsorbed as Cu^{++} diffused into the center of the resin particles and the external Cu^{++} decreased.

According to Fig. 1, a resin that is 2 *m* in H^+ is in equilibrium with an aqueous phase at a *p*H of 6–7. Bjerrum[3a] quotes a value of 10^{-7} *M* for the acid constant of $Cu(OH_2)_4^{++}$. At a *p*H of 6–7 there would be a possibility of adsorption of $Cu(OH)^+$ or of precipitation of basic copper salts. However, the acid released in the adsorption experiment decreased the *p*H to 5.5 or less for most of the samples and thus decreased the importance of hydrolysis of Cu^{++}. In effect, adsorption of Cu^{++} increases the acid constant of the resin. The slope of curve A of Fig. 1, $\Delta pH/\Delta (H^+) = -1.45$ m^{-1}. In the 90 day adsorption experiment (point A' of Fig. 2), the *p*H decreased from 6.49 to 5.30 and the resin was 0.46 *m* in Cu^{++}, $\Delta pH/2\Delta (Cu^{++}) = -1.30$ m^{-1}. That is, charge for charge, Cu^{++} and H^+ increase the acid constant of the resin about the same amount.

Figure 2 also displays several elution experiments

(3) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941; (a) p. 69; (b) pp. 128, 173.

on resin samples which were exposed to Cu^{++} for 6 days and then eluted for 6 days. They have only qualitative, descriptive significance, since equilibrium was not reached and since the adsorbed Cu^{++} was probably not uniformly distributed through the resin. When the 3 *m* resin was eluted with 0.10 *M* $NaNO_3$ for 6 days, relatively small amounts of Cu^{++} were extracted into the aqueous phase (curve b). When the 2 *m* resin was treated for 6 days with a solution containing enough HNO_3 to make the resin 3 *m* in H^+, more Cu^{++} was eluted, although curve B was not reached.

The results of the experiments on the adsorption of Hg^{++} are shown in Table I. To avoid possible precipitation of basic mercuric salts, the solutions initially were at a *p*H of *ca.* 2 and the resin was 4 to 5 *m* in H^+. After 8 days, a large fraction of the mercuric ion was taken up by the resin. The ratio of H^+ liberated/Hg^{++} adsorbed was above unity. Table I and the data of Fig. 1 show that on a charge basis Hg^{++} and H^+ are about equally effective in increasing the acid constant of the resin.

TABLE I

ADSORPTION OF Hg^{++} BY AMBERLITE IR-4B

1 g. resin/100 ml. aqueous phase; constant stirring; temp., 28.3°; ionic strength, 0.09 to 0.10; 8 days equilibration.

m Hg^{++} in resin	*M* Hg^{++} in soln.	*m* H^+ in resin	*M* H^+ in soln.	H^+ liberated / Hg^{++} adsorbed
0	0	4.52	0.0040	..
.22	.000025	4.12	.0077	1.7
.50	.00049	3.84	.0100	1.2
.67	.0043	3.35	.0142	1.5
.81	.0029	3.35	.0142	1.2[a]
1.01	.0120	2.55	.0207	1.7
1.13	.0218	2.24	.0221	1.6

[a] 16 days equilibration.

The resin did not appreciably change color on adsorbing Hg^{++}. The greater affinity of the resin for Hg^{++} as compared to Cu^{++} is in accordance with the relative affinity of NH_3 for these two ions; $Hg^{++} + NH_3 = HgNH_3^{++}$, $\log K = 8.8$; $Cu^{++} + NH_3 = CuNH_3^{++}$, $\log K = 4.15$.[3b]

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CONTRIBUTION NO. 1798 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY
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p-Dimethylaminophenylquinaldylcarbinol

BY F. W. BERGSTROM[1] AND ARTHUR FURST

RECEIVED JUNE 18, 1953

Bahner and Pace[2] were unable to isolate *p*-dimethylaminophenylquinaldylcarbinol from the reaction mixture of quinaldine and *p*-dimethylaminobenzaldehyde. Other substituted benzaldehydes did combine with quinaldine under the same conditions. From the Elderfield[3] summary of the various procedures used for the synthesis of these carbinols it is evident that *p*-dimethylaminobenzaldehyde will not condense.

Three of these carbinols were made[4] by treating the lithium derivative of 6-ethoxyquinaldine, 6-methoxyquinaldine and quinaldine itself with *p*-dimethylaminobenzaldehyde. Although the yields were only fair, the by-products formed presented no difficulty in the purification. These carbinols were easily dehydrated to the corresponding styryl derivatives by heating with acetic anhydride or hydrochloric acid.

Experimental

Quinaldines.—Quinaldine was Eastman Kodak Co., white label grade and was used with no further purification. 6-Methoxyquinaldine was prepared by modifying the procedure of Cocker and Turner.[5]

A solution of 123 g. (1.0 mole) of *p*-anisidine in 200 ml. of concentrated hydrochloric acid was cooled to 0°. With constant stirring, 137 g. (1.0 mole) of paraldehyde was added over a period of one-half hour. Stirring was discontinued, and the reaction mixture was allowed to stand overnight. The brown material was refluxed on a water-bath for four hours (additional paraldehyde was added from time to time to compensate for the unavoidable loss through the condenser). The solution was cooled and poured with vigorous stirring into a large excess of 6 *M* ammonium hydroxide (*ca.* 1000 ml.). The dark viscous oil formed was separated, washed three times with 200-ml. portions of water dissolved in ether and dried over KOH. The ether was removed and the remaining oil was distilled under reduced pressure, b.p. 145–150° (3 mm.).[6] The pale yellow oil solidified in the receiver and formed lemon color cubic crystals. Crystallization from ligroin gave white crystals. Yield was 54 g. (31%); m.p. 64°.

6-Ethoxyquinaldine was made by the above method. From 137 g. (1.0 mole) of *p*-phenetidine, 23 g. of 6-ethoxyquinaldine (12.7%) were obtained by fractionating at 173° at 14 mm., the bath temperature being 248°. A white product was obtained from the ether–petroleum ether crystallization; m.p. 71°.[7]

***p*-Dimethylaminophenylquinaldylcarbinol.**—To a phenyl lithium solution[8] cooled in an ice–salt-bath was added an equivalent amount of the quinaldine dissolved in 20 ml. of dry ether; the color turned brick red. Stirring was continued for one-half hour after the addition of the quinaldine. An equal molar amount of *p*-dimethylaminobenzaldehyde suspended in 35 ml. of ether was added portionwise. The color gradually changed from brick-red to orange and then to yellow. The solution was stirred for two hours and then allowed to warm to room temperature by standing overnight. The lithium salt was decomposed with 95% alcohol. The carbinol was isolated by dilution and crystallized from a dilute alcohol solution. Pale straw-yellow plates were obtained which did not turn red if kept dry. Yield was 14.3%; m.p. 130°.

Anal.[9] Calcd. for $C_{19}H_{20}N_2O$: C, 78.02; H, 6.91. Found: C, 78.10; H, 6.90.

***p*-Dimethylaminophenyl-6-methoxyquinaldylcarbinol** was made as described above, but the mixture was refluxed prior to decomposition of the lithium salt until the color changed to yellow. The salt was hydrolyzed with 50% alcohol, the top layer was separated, and the water layer was extracted

(1) Deceased 1946.

(2) C. T. Bahner and E. S. Pace, THIS JOURNAL, **74**, 3932 (1952).

(3) R. C. Elderfield, "Heterocyclic Compounds," Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 295.

(4) A. Furst, Dissertation, Stanford University 1948.

(5) W. Cocker and D. G. Turner, *J. Chem. Soc.*, 143 (1941).

(6) If a sharp fraction is not taken the oil fails to solidify. At times recrystallization from ligroin will give the desired solid, but most of the time two layers separate making redistillation necessary. The dry solid turns pink almost at once and will keep indefinitely. Moist products turn brown and decompose in a matter of days.

(7) W. T. K. Braunholtz, *J. Chem. Soc.*, **121**, 169 (1922).

(8) H. Gilman, E. A. Zoellner and W. M. Selby, THIS JOURNAL, **55**, 1252 (1933).

(9) Microanalysis by C. W. Koch, Albany, California.

with two 50-ml. portions of benzene. The solvents were combined, and evaporated to one-half volume on the steam-bath. On cooling a precipitate formed which was recrystallized from alcohol. Yield was 12.5%; m.p. 151.5°.

Anal.[9] Calcd. for $C_{20}H_{22}N_2O_2$: C, 74.48; H, 6.90. Found: C, 74.30; H, 6.82.

p-Dimethylaminophenyl-6-ethoxyquinaldylcarbinol.—The above procedure was followed. The mixture was refluxed for two hours prior to the hydrolysis until the precipitate turned yellow. Yield was 24.9%; m.p. 153.6°.

Anal.[9] Calcd. for $C_{21}H_{24}N_2O_2$: C, 74.94; H, 7.21. Found: C, 74.69; H, 7.33.

Styryls.—The carbinols were dehydrated to the respective styryl derivatives by boiling in 2 *M* hydrochloric acid for one hour; the carbinol dehydrated *ca.* 99%, the 6-methoxy *ca.* 75% and the 6-ethoxy *ca.* 50%. Mixed melting points showed no depression with the corresponding styryl compounds synthesized by procedures in the literature.[10,11]

Acknowledgment.—We should like to thank Dr. H. S. Mosher for helpful comments in the preparation of this manuscript.

(10) R. S. Tipson, THIS JOURNAL, **67**, 507 (1945).

(11) U. N. Brahmachari and T. Bhattacharjee, *J. Indian Chem. Soc.*, **7**, 527 (1930); *C. A.*, **24**, 5752 (1930).

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A Stable Chloroform Adduct of 11-Epi-17α-hydroxycorticosterone

BY HELMUTH CORDS

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In view of the recent interest in Δ^4-pregnene-11α,17α,21-triol-3,20-dione,[1-7] the 11-epimer of the most important adrenal secretory product 17α-hydroxycorticosterone, we wish to describe a stable adduct of this substance with chloroform. The adduct is formed readily when the steroid is crystallized from chloroform, in which it is very difficultly soluble. It forms colorless platelets, m.p. 206–209°, $[\alpha]^{23}D$ +88 ± 2° (0.5% in ethanol) (calculated for an adduct containing one mole of chloroform: +87.8°).[8] The substance was analyzed after drying *in vacuo* (1 mm.) at 100° for two hours. *Anal.* Calcd. for $C_{21}H_{30}O_5 \cdot CHCl_3$: C, 54.83; H, 6.49; Cl, 22.08. Found: C, 54.92; H, 6.59; Cl, 21.99.

The infrared spectrum of the chloroform adduct, sampled as nujol mull, differs from that of the free 11-epi-17α-hydroxycorticosterone in that it contains a deep band at 13.28 μ, characteristic for chloroform. Moreover, the C_{21}-carbonyl band shifted from 5.83 μ for the free steroid to 5.88 μ for the adduct.

(1) J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, THIS JOURNAL, **74**, 3962 (1952).

(2) J. Romo, A. Zaffaroni, J. Hendrichs, G. Rosenkranz, C. Djerassi and F. Sondheimer, *Chem. and Ind.*, 783, 834 (1952).

(3) D. H. Peterson, S. H. Eppstein, P. D. Meister, B. J. Magerlein, H. C. Murray, H. Marian Leigh, A. Weintraub and L. M. Reineke, THIS JOURNAL, **75**, 412 (1953).

(4) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953).

(5) J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, THIS JOURNAL, **75**, 1277 (1953).

(6) F. Sondheimer, O. Mancera, G. Rosenkranz and C. Djerassi, *ibid.*, **75**, 1282 (1953).

(7) S. Bernstein, R. Littell and J. H. Williams, *ibid.*, **75**, 1481 (1953).

(8) The specific rotation of the free steroid is +117° (0.5% in ethanol).

Its low solubility in chloroform, its well formed crystal shape and its stability to heat and vacuum render the chloroform adduct very suitable for purification of 11-epi-17α-hydroxycorticosterone. Microbiological synthesis of Δ^4-pregnene-11α,17α,21-triol-3,20-dione[1] generally yields slightly colored material. Recrystallization of this material from chloroform produces an almost colorless chloroform adduct (well formed platelets). The steroid can be readily freed of chloroform by crystallization from the lower alcohols, acetone or ethyl acetate.

Δ^4-Pregnene-11α,17α,21-triol-3,20-dione 11,21-diacetate[2-6,9,10] forms a similar complex with chloroform, whereas the corresponding 21-monoacetate, which was obtained in crystalline form from acetic acid–water, crystallizes from chloroform without solvate formation.

Another adduct has been observed with 5,16-pregnadiene-3β-ol-20-one and chloroform. One mole of chloroform is attached here to two moles of the steroid. This adduct, colorless platelets, is obtained by crystallization of the steroid from chloroform, and is stable to a vacuum of 1 mm., yet labile to heat. The melting point is unchanged from that of the free compound. The optical rotation, $[\alpha]^{23}D$ 20 ± 2° (0.5% in ethanol) differs, as expected, 19% from that of the free steroid. *Anal.* Calcd. for $C_{21}H_{30}O_2 \cdot {}^1/_2CHCl_3$: C, 69.02; H, 8.22; Cl, 14.22. Found: C, 69.27; H, 8.34; Cl, 14.21.

The adduct shows the strong band at 13.32 μ, characteristic for chloroform, and three additional bands at 10.54, 11.78 and 11.99 μ. The C_{20}-carbonyl shifted from 6.05 to 6.02 μ in the adduct, and four minor bands of the free steroid (8.62, 10.45, 12.41 and 12.52 μ) appeared at slightly lower wave lengths. The band at 9.86 μ is missing in the complex.

$\Delta^{5,16}$-Pregnadiene-3β-ol-20-one acetate does not form a similar adduct.

I am indebted to Dr. N. Coy for the spectrometric measurements and to Mr. J. Alicino for the microanalytical determinations.

(9) A. Lardon and T. Reichstein, *Pharm. Acta Helv.*, **27**, 287 (1952).

(10) H. L. Herzog, E. P. Oliveto, M. A. Jevnik and E. B. Hershberg, THIS JOURNAL, **74**, 4470 (1952).

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Phenyl Esters

BY ALFRED R. BADER AND ANTHONY D. KONTOWICZ

RECEIVED JUNE 22, 1953

The preparations of phenyl esters have hitherto been rather tedious as they have involved the use of acid chlorides, acid anhydrides or $POCl_3$, or in the case of phenyl esters of reactive acids such as acrylic[1] or methacrylic acid,[2] somewhat circuitous synthetic routes. Phenyl esters even of reactive acids have recently been prepared with trifluoro-

(1) E. M. Filachione, J. H. Lengel and C. H. Fisher, THIS JOURNAL, **66**, 494 (1944).

(2) E. M. Filachione, J. H. Lengel and W. P. Ratchford, *ibid.*, **72**, 839 (1950).

TABLE I

PHENYL ESTERS

Acid	Formula	M.p., °C.	Solvent of cryst.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
Benzoic	$C_{13}H_{10}O_2$	70–71					
Diglycolic	$C_{16}H_{14}O_5$	73–75	Heptane–toluene	67.12	67.45	4.93	4.91
Levulinic	$C_{11}H_{12}O_3$	32	Methanol–water	68.73	68.92	6.30	6.46
Maleic[a]	$C_{16}H_{12}O_4$	71–72	Heptane–toluene	71.63	72.01	4.51	4.72
Methacrylic[b]	$C_9H_{10}O_2$	17					
Phthalic[a,c]	$C_{20}H_{14}O_4$	73–74	Toluene–acetone				
Salicylic	$C_{13}H_{10}O_3$	42–43	Methanol				
Stearic[d]	$C_{24}H_{40}O_2$	51–52	Toluene				

[a] The anhydride was used as the starting material. [b] This agreed in b.p. and n_D with the lit. values,[2] and crystallized easily in the ice-box. [c] E. Huntress and S. P. Mulliken, "Identification of Pure Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 291. [d] *Ibid.*, p. 287.

acetic anhydride[3] which is not, however, readily accessible.

Phenyl esters of most carboxylic acids can be prepared easily and in good yields simply by heating the free acids with phenol in the presence of polyphosphoric acid on the steam-bath. In all cases tried the phenyl esters were easily separated from unreacted starting materials through their insolubility in dilute aqueous alkali. All solid phenyl esters crystallized beautifully, and we are tempted to suggest them as derivatives for the characterization of acids.

Experimental

Table I lists the esters prepared.

In a representative experiment, 50 g. of salicylic acid, 150 g. of phenol and 100 g. of polyphosphoric acid (Victor Chemical Co.) were stirred and heated on the steam-bath for 24 hours. The cooled mixture was diluted with water, extracted with toluene; and the toluene solution was extracted with aqueous sodium bicarbonate solution from which 14 g. of unreacted salicylic acid was recovered on acidification. The toluene solution was washed, stripped *in vacuo* and the residue distilled to yield unreacted phenol and 53 g. (95% based on unrecovered salicylic acid) of phenyl salicylate, b.p. 108–110° at 0.4 mm., which crystallized in the receiver. There was no flask residue. One crystallization from methanol yielded 49 g. of pure product melting at 42–43°.

Substantial quantities of polyphosphoric acid are desirable. In the preparation of diphenyl phthalate, phenolphthalein also was formed, and during the separations of phenyl levulinate and phenyl methacrylate, the aqueous alkali extracted products other than only starting materials. The yields (based on unrecovered organic acid) of phenyl levulinate and phenyl methacrylate were 35 and 55%, respectively, and in the other preparations yields ranged from 85 to 98%.

(3) (a) E. J. Bourne, M. Stacey, J. C. Tatlow and J. M. Tedder, *J. Chem. Soc.*, 2976 (1949); (b) A. H. Ahlbrecht and D. W. Codding, THIS JOURNAL, **75**, 984 (1953).

THE RESEARCH LABORATORIES
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Preparation of 1-Methyl-4-phenyl-4-(aminomethyl)- and 1-Methyl-4-phenyl-4-(methylaminomethyl)-piperidine

BY F. F. BLICKE AND EU-PHANG TSAO

RECEIVED JUNE 15, 1953

When 1-methyl-4-phenyl-4-cyanopiperidine was heated with sodium and ethanol, Bergel, *et al.*,[1] obtained 1-methyl-4-phenylpiperidine. Provinciala[2] stated that sodium and ethanol, at 0°, reduced the 4-cyano to the corresponding 4-aminomethyl derivative which was claimed to be an active analgesic; a hydrochloride was obtained which melted at 190–192°. Kwartler and Lucas[3] prepared the 4-aminomethyl compound in 66.7% yield by hydrogenation of the 4-cyano derivative, in the presence of Raney nickel and ammonia, under 500 pounds pressure. According to them,[3a] the compound has negative analgesic activity; their dihydrochloride melted at 287–288°.

We found also that when 1-methyl-4-phenyl-4-cyanopiperidine was heated with sodium and ethanol, the cyano radical was replaced by hydrogen. The hydrochloride of the 1-methyl-4-phenylpiperidine obtained melted at 191–193°.[4] However, reduction with lithium aluminum hydride yielded the 4-aminomethyl compound in 83% yield; the dihydrochloride melted at 291–292°.

Formylation of the 4-aminomethyl derivative with chloral[5] produced the N-formyl derivative which was reduced with lithium aluminum hydride to the 4-methylaminomethyl compound.

Tested for analgesic activity in the Parke, Davis and Company laboratories under the direction of Dr. C. V. Winder, both the 4-aminomethyl and the 4-methylaminomethyl compound were found to be inactive as analgesics at the aspirin dose level.

Experimental

1-Methyl-4-phenyl-4-(aminomethyl)-piperidine.—1-Methyl-4-phenyl-4-cyanopiperidine (20.0 g.), dissolved in 50 cc. of ether, was reduced by adding the solution to 3.0 g. of lithium aluminum hydride, dissolved in 150 cc. of ether, in the usual manner. After the addition of 6 cc. of water, the mixture was filtered. From the filtrate there was obtained 16.6 g. (83%) of the desired product; b.p. 109–112° (1 mm.).[6]

The dihydrochloride precipitated when an ethereal solution of the base was treated with hydrogen chloride; m.p. 291–292° (dec.) after recrystallization from methanol.[7]

Anal. Calcd. for $C_{13}H_{22}N_2Cl_2$: N, 10.11; Cl, 25.63. Found: N, 10.37; Cl, 25.70.

(1) F. Bergel, J. W. Haworth, A. L. Morrison and H. Rinkerknecht, *J. Chem. Soc.*, 261 (1944).

(2) C. Provinciala, *Boll. chim. farm.*, **85**, 228 (1946); *C. A.*, **41**, 1328 (1947).

(3) (a) C. E. Kwartler and P. Lucas, THIS JOURNAL, **69**, 2582 (1947); (b) U. S. Patent 2,538,107; *C. A.*, **45**, 6664 (1951).

(4) O. Eisleb (*Ber.*, **74**, 1433 (1941)), who decarboxylated 1-methyl-4-phenylpiperidine-4-carboxylic acid, stated that the hydrochloride melted at 196–197°.

(5) F. F. Blicke and C. J. Lu, THIS JOURNAL, **74**, 3933 (1952).

(6) Ref. 3, b.p. 170–172° (12.5 mm.).

(7) Ref. 3, m.p. 287–288°.

1-Methyl-4-phenyl-4-(formamidomethyl)-piperidine.—Chloral (4.5 g.) was added slowly to 6.1 g. of the 4-aminomethyl compound with occasional cooling. After 12 hours at room temperature, the solid product was recrystallized from benzene; m.p. 108.5–110°; yield 4.4 g. (63.8%).

The dihydrochloride was recrystallized from ethanol; m.p. 276–277° (dec.).

Anal. Calcd. for $C_{14}H_{22}ON_2Cl_2$: N, 9.18; Cl, 23.23. Found: N, 9.22; Cl, 23.50.

1-Methyl-4-phenyl-4-(methylaminomethyl)-piperidine.—The formyl derivative (5.0 g.) was added in small portions to a stirred solution of 1.0 g. of lithium aluminum hydride in 50 cc. of ether. After the mixture had been stirred and refluxed for 4 hours, it was cooled, 2 cc. of water was added, dropwise, and the mixture filtered. The product obtained from the filtrate boiled at 117–119° (1.5 mm.); yield 3.8 g. (81%).

The dihydrochloride was recrystallized from ethanol; m.p. 256–257° (dec.).

Anal. Calcd. for $C_{14}H_{24}N_2Cl_2$: N, 9.62; Cl, 24.35. Found: N, 9.37; Cl, 24.68.

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Preparation of Cycloöctanone

BY F. F. BLICKE, J. AZUARA, N. J. DOORENBOS AND E. B. HOTELLING

RECEIVED JUNE 22, 1953

It was found that cycloöctanone can be obtained quite readily, in relatively large amounts, from the dimethyl ester of azelaic acid. This method is especially advantageous since azelaic acid has become a cheap commercial chemical.

The cyclization of the ester was attempted by Dieckmann[1] but he obtained only a resinous mass. By the use of sodium hydride[2] and high dilution technique, we converted dimethyl azelate into 2-carbomethoxycycloöctanone in 47.5% yield. Simultaneous hydrolysis and decarboxylation of the β-keto ester yielded cycloöctanone.[3]

Cycloöctanone was prepared also by a second procedure in which cycloheptanone cyanohydrin[4] was reduced with lithium aluminum hydride to 1-(aminomethyl)-cycloheptanol and the latter compound was then treated with nitrous acid.

Experimental

Dimethyl Azelate.—A practical grade of azelaic acid[5] was recrystallized from benzene with the use of Norite. After a second recrystallization from benzene, the acid melted at 94–97° and was sufficiently pure for the preparation of the ester.

A mixture of 752.8 g. of azelaic acid, 3.2 liters of methanol and 128 g. of concd. sulfuric acid was refluxed for 48 hours and then most of the solvent was removed under reduced pressure, the residue was poured into ice-water, the product extracted with ether, the ether solution shaken first with a saturated bicarbonate solution and then with water. The solution was dried with magnesium sulfate, the solvent removed and the ester distilled, yield 695.0 g. (80%), b.p. 145–153° (12 mm.).

2-Carbomethoxycycloöctanone.—To the middle neck of a 3-necked, 5-liter flask there was attached a jacketed 4" condenser. The upper end of the condenser was fitted with a ball joint lubricated with glycerol to which a short tube was attached. A Hershberg stirrer passed through the joint and the condenser into the flask. A piece of rubber tubing connected to the top of the short tube made a seal between the tube and the shaft of the stirrer. To the other necks of the flasks a nitrogen inlet tube and a dilution apparatus[6] were attached.

After the whole apparatus had been filled with dry nitrogen, 60.0 g. (2.5 moles) of sodium hydride, 60 g. of 5-mm. glass beads and 2.5 liters of xylene[7] were placed in the flask. A very slow stream of nitrogen was passed through the apparatus throughout the experiment. The suspension was stirred rapidly and 2 cc. of absolute methanol was added. The flask was heated and as soon as the mixture refluxed vigorously, the addition of 216.0 g. (1 mole) of dimethyl azelate, dissolved in 1.8 liters of xylene, was begun. The addition was made at the rate of about nine drops per minute and required about nine days. After the addition had been completed, the mixture was refluxed for one hour, and then allowed to cool to room temperature. The nitrogen inlet tube was replaced by a dropping funnel, and 150.0 g. (2.5 moles) of acetic acid was added to the stirred solution at such a rate that the reaction mixture did not become warm. After the mixture had been stirred for one hour, 142 cc. of water was added slowly. A few crystals of sodium acetate were added to induce the precipitation of the sodium acetate in the mixture. The precipitate was filtered and washed with xylene. The xylene solution was washed with concd. sodium bicarbonate solution, dried over anhydrous magnesium sulfate and then fractionated through a 15-cm. Vigreux column. The product which boiled at 129–135° (17 mm.) weighed 87.0 g. (47.5%); about 90% of this material boiled at 130–133° (17 mm.).[8]

Cycloheptanone Cyanohydrin.—Cycloheptanone[9] (224.0 g., 2 moles) and 98.0 g. (2 moles) of sodium cyanide were placed in a 2-liter, 3-necked flask equipped with a thermometer, stirrer and a dropping funnel. The mixture was stirred, cooled to 0° and a mixture of 115 cc. of concd. sulfuric acid and 460 cc. of water was added dropwise, at such a rate that the temperature of the mixture could be maintained below 5° by the use of an ice–salt-bath. After the addition was completed, water was added to dissolve the inorganic salts, the product was extracted with ether, the extract was washed free from acid and then dried with anhydrous magnesium sulfate. The dried extract was used for the next experiment.[10]

1-(Aminomethyl)-cycloheptanol.—The ether solution of the cyanohydrin was added, dropwise, to a stirred mixture of 100 g. of lithium aluminum hydride and 1 liter of ether. After the mixture had been stirred and refluxed for 36 hours, 120 cc. of water was added dropwise. The mixture was stirred for one-half hour, filtered and the inorganic salts washed with ether. From the ether solution there was obtained 124 g. (44% based on cycloheptanone) of product; b.p. 125–129° (17 mm.).[11] The hydrochloride melted at 217–218° after recrystallization from isopropyl alcohol–ether.

Cycloöctanone. (A).—A mixture of 174.0 g. (0.95 mole) of 2-carbomethoxycycloöctanone and 120.0 g. (3 moles) of sodium hydroxide, dissolved in 2.28 liters of water, was

(1) W. Dieckmann, *Ann.*, **317**, 49 (1901).

(2) Used previously in the Dieckmann cyclization by N. Green and L. B. LaForge, THIS JOURNAL, **70**, 2287 (1948).

(3) The procedure used successfully for the preparation of suberone (cycloheptanone) (F. F. Blicke, N. J. Doorenbos and R. H. Cox, *ibid.*, **74**, 2924 (1952)) could not be utilized for cycloöctanone since suberone condensed with nitromethane to form 1-(nitromethyl)-cycloheptanol in only 3% yield.

(4) Cyclohexanone cyanohydrin has been reduced with lithium aluminum hydride by H. R. Nace and B. B. Smith, *ibid.*, **74**, 1861 (1952).

(5) Purchased from The Matheson Company.

(6) For a description see N. J. Leonard and R. C. Sentz, *ibid.*, **74**, 1708 (1952). To neck D of this apparatus we connected a drip-tip condenser to which a calcium chloride tube was attached and a 1-liter Hershberg dropping funnel was inserted into neck E.

(7) All of the xylene used had been distilled from sodium.

(8) V. Prelog, L. Ruzicka, P. Barman and L. Frenkiel, *Helv. Chim. Acta*, **31**, 92 (1948), found 120° (12 mm.).

(9) F. F. Blicke, N. J. Doorenbos and R. H. Cox, THIS JOURNAL, **74**, 2924 (1952).

(10) B. Tchoubar (*Compt. rend.*, **215**, 224 (1942)), reported that the cyanohydrin boils at 138–139° (15 mm.). We found that upon distillation, under these conditions, about one-half of the cyanohydrin decomposed into cycloheptanone.

(11) B. Tchoubar (*Bull. soc. chim. France*, 160 (1949)), obtained this product in 50% yield by reduction of the cyanohydrin with hydrogen in the presence of platinum oxide; b.p. 124° (15 mm.); hydrochloride, m.p. 223°. Previously, B. Tchoubar (ref. 10) reported the melting point of the hydrochloride to be 185°.

stirred at room temperature until a clear solution had been obtained. The solution was stirred for 2 hours and then 400 g. (4 moles) of concd. hydrochloric acid was added slowly to the stirred mixture. After the latter had been stirred for 30 minutes, it was heated on a steam-bath for 3 hours. The mixture was extracted thoroughly with ether, the extract washed with concd. sodium bicarbonate solution, the ether removed and the residue treated with concd. sodium bisulfite solution. The latter converted the lower ketones into their bisulfite addition products but did not affect the cycloöctanone. The mixture was triturated thoroughly with ether to extract the cycloöctanone, filtered, the ether layer separated and the aqueous layer extracted with ether. The combined ethereal solutions were washed with concd. sodium bicarbonate solution and dried over anhydrous magnesium sulfate. Upon distillation, 92.0 g. of product, b.p. 115–120° (65 mm.), m.p. 26–29°, was obtained. This material was shaken for several hours with a saturated sodium bisulfite solution, the mixture filtered and the solid material washed with sufficient ether to dissolve all of the ketone in the filtrate. From the ether solution there was obtained 13.0 g. of ketone, b.p. 115–118° (64 mm.), m.p. 23° and 72.5 g. (61%) of ketone, b.p. 118–120° (64 mm.),[12] m.p. 38–39°.[10]

(B).—1-(Aminomethyl)-cycloheptanol (124.0 g.) dissolved in 400 cc. of 10% hydrochloric acid was stirred, cooled to 0–5° and maintained at this temperature while 69.0 g. of sodium nitrite, dissolved in 300 cc. of water, was added dropwise. During a period of 2 hours, the mixture was stirred and allowed to warm to room temperature. The mixture was heated on a steam-bath for 1 hour, cooled, the oily layer was separated and the aqueous layer extracted with ether. The combined oil and extract were dried and distilled, b.p. 85–87° (17 mm.), m.p. 32–34°, yield 67.1 g. (26.4% based on cycloheptanone).

Five grams of 1-(hydroxymethyl)-cycloheptanol was isolated from the high boiling fraction; b.p. 142–147° (22 mm.), m.p. 50–51° after recrystallization from heptane.[13]

(12) E. P. Kohler, M. Tishler, H. Potter and H. T. Thompson (THIS JOURNAL, **61**, 1057 (1939)), obtained the pure ketone by hydrolysis of the semicarbazone, b.p. 115–115.5° (60 mm.), m.p. 43.8°.

(13) O. Wallach, *Ann.*, **345**, 148 (1906), found b.p. 135–140° (16 mm.), m.p. 50–51°.

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3-Trichloromethanesulfenyloxazolidine- and Thiazolidine-2,4-diones

BY W. J. CROXALL, CHIEN-PEN LO AND ELWOOD Y. SHROPSHIRE

RECEIVED MARCH 14, 1953

Because of the appearance of 3-trichloromethane sulfenyloxazolidine- and thiazolidine-2,4-diones in the recent literature,[1] we wish to report our independent work on compounds of this and related types.

Our method of preparation was essentially the same as that of Kittleson,[1] namely, the reaction of trichloromethanesulfenyl chloride (perchloromethylmercaptan) with the sodium or potassium salts of the appropriate oxazolidine- or thiazolidine-2,4-diones. By this method, we have prepared four 3-trichloromethanesulfenyl-5-alkyl- and/or 5,5-dialkyloxazolidine-2,4-diones (Table I); three 3-trichloromethanesulfenyl-5-alkyl- and/or 5,5-dialkylthiazolidine-2,4-diones (Table I) and twelve 3-trichloromethanesulfenyl-5-alkylidene- and/or 5-aralkylidenethiazolidine-2,4-diones (Table II).

Incidental to this work, the preparations of 5-alkylidenethiazolidine-2,4-diones were investigated. Condensation of aromatic and related aldehydes such as furfural and cinnamaldehyde, with thiazolidine-2,4-dione in acetic acid containing sodium acetate, in general, gave 5-aralkylidene-2,4-diones in good yields.[2] However, when this method was applied to certain aliphatic aldehydes, the desired 5-alkylidene derivatives were obtained in much lower yields (see Experimental section). Attempts to condense aliphatic ketones with thiazolidine-2,4-dione have been unsuccessful. Since Brown, Bradsher, McCallum and Potter[3] have reported the successful condensation of ketones with rhodanine to give 5-alkylidenerhodanines and we have found that rhodanine can be transformed into thiazolidine-2,4-dione by the treatment of chloroacetic acid,[4] three of the 5-alkylidene- (namely, isopropylidene-, *s*-butylidene- and cyclohexylidene-) rhodanines reported by Brown and co-workers were thus converted to the corresponding 5-alkylidenethiazolidine-2,4-diones. The product thus obtained apparently contained some unchanged rhodanines as evidenced by their melting points and sulfur analyses. The separation of the two materials by recrystallization was found to be difficult. However, the crude products were satisfactory for subsequent reaction with trichloromethanesulfenyl chloride.

Experimental[9]

Materials.—The sodium salts of the oxazolidine-2,4-diones were prepared according to the method of Stoughton.[10] They were not isolated but used directly in the reaction with trichloromethanesulfenyl chloride (see below).

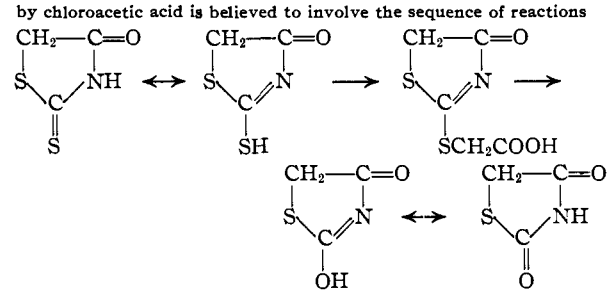
Thiazolidine-2,4-dione,[11] 5-methyl-[12] and 5,5-dimethyl-[13] thiazolidine-2,4-diones were prepared by known methods.

(1) (a) R. S. Healey, A. R. Kittleson and P. V. Smith, U. S. Patent, 2,553,775 (1951). (b) A. R. Kittleson, *Science*, **115**, 84 (1952).

(2) (a) F. Kucera, *Monatsh.*, **35**, 137 (1914); (b) D. Libermann, J. Hienberl and L. Hengl, *Bull. soc. chim., France*, 1120 (1948); (c) C. P. Lo, E. Y. Shropshire and W. J. Croxall, THIS JOURNAL, **75**, 4845 (1953).

(3) F. C. Brown, C. K. Bradsher, S. G. McCallum and M. Potter, *J. Org. Chem.*, **15**, 174 (1950).

(4) Many examples of "desulfurization" of heterocyclic compounds containing mercapto group by means of chloroacetic acid are known in the literature. For example, this method has been successfully applied to thiohydantoins,[5] thiouracils,[6] thiopyrimidines[7] and mercaptoquinolines.[8] The transformation of rhodanine to thiazolidine-2,4-dione by chloroacetic acid is believed to involve the sequence of reactions



(5) (a) T. B. Johnson, G. M. Pfau and W. W. Hodge, THIS JOURNAL, **34**, 1041 (1912); (b) T. B. Johnson and S. E. Hadley, *ibid.*, **37**, 171 (1915); (c) T. B. Johnson and R. Wrenshall, *ibid.*, **37**, 2133 (1915); (d) T. B. Johnson, A. J. Hill and E. B. Kelsey, *ibid.*, **42**, 1711 (1920).

(6) (a) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908); (b) T. B. Johnson and E. H. Hemingway, THIS JOURNAL, **37**, 398 (1915).

(7) (a) T. B. Johnson and A. W. Joyce, *ibid.*, **38**, 1385 (1916); (b) A. R. Todd, *J. Chem. Soc.*, 357 (1946); (c) D. J. Brown, *J. Soc. Chem. Ind.* (London), **69**, 353 (1950).

(8) R. V. Jones and H. R. Henze, THIS JOURNAL, **46**, 1669 (1942).

(9) All melting points are uncorrected.

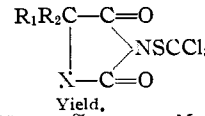
(10) R. W. Stoughton, THIS JOURNAL, **63**, 2376 (1941).

(11) J. Volhard, *J. prakt. Chem.*, [2] **9**, 9 (1874).

(12) H. L. Wheeler and B. Barnes, *Am. Chem. J.*, **24**, 78 (1900).

(13) H. Erlenmeyer and H. von Meyenburg, *Helv. Chim. Acta*, **20**, 1390 (1937).

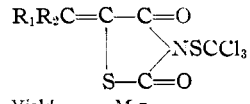
TABLE I



R_1	R_2	X	Method	Solvent[a]	Yield, %	M.p., °C.	Formula	Kjeldahl nitrogen, % Calcd.	Found
H	H	O	I	A	40	119–120	$C_4H_2Cl_3NO_3S$	5.6	5.7
CH_3	H	O	I	..	70	Oil	$C_5H_4Cl_3NO_3S$	5.3	5.3
CH_3	CH_3	O	I	B	57.5	91–93[b]	$C_6H_6Cl_3NO_3S$	5.0	5.0[c]
C_2H_5	CH_3	O	I	B	55	86–88[d]	$C_7H_8Cl_3NO_3S$	4.8	4.7[e]
H	H	S	II	C	72.5	117–118[f]	$C_4H_2Cl_3NO_2S_2$	5.3	5.1[g]
CH_3	H	S	II	..	76	Oil	$C_5H_4Cl_3NO_2S_2$	5.0	5.2
CH_3	CH_3	S	II	D	86	70–71	$C_6H_6Cl_3NO_2S_2$	4.8	4.7[h]

[a] Solvent for recrystallization: A, ethanol; B, methanol; C, acetone + petroleum ether; D, petroleum ether. [b] Kittleson (ref. 1b) reported a m.p. of 88–89°. [c] *Anal.* Calcd.: S, 11.5. Found: S, 11.6. [d] Kittleson (ref. 1b) reported a m.p. of 83–84°. [e] *Anal.* Calcd.: S, 10.9. Found: S, 10.9. [f] Hanley, Kittleson and Smith (ref. 1a) reported a m.p. of 111–116°. [g] *Anal.* Calcd.: S, 24.0. Found: S, 23.9. [h] *Anal.* Calcd.: S, 21.7. Found: S, 22.1.

TABLE II



R_1	R_2	Method	Solvent[a]	Yield, %	M.p., °C.	Formula	Nitrogen,[b] % Calcd.	Found	Sulfur, % Calcd.	Found
$(CH_3)_2CH-$	H	II	A	56	54–56	$C_5H_8Cl_3NO_2S_2$	4.4	4.4	19.9	20.2
$C_8H_{17}-$[c]	H	II	B	42	52–53.5	$C_{13}H_{18}Cl_3NO_2S_2$	3.6	3.5	16.4	16.8
OCH=CHCH=C—	H	II	C	62	149–150	$C_9H_4Cl_3NO_3S_2$	4.1	4.1	18.5	18.4
C_6H_5-	H	III	C	72	159–161	$C_{11}H_6Cl_3NO_2S_2$	4.0	3.9	18.0	18.0
$2\text{-}ClC_6H_4-$	H	III	D	55	141–143	$C_{11}H_5Cl_4NO_2S_2$	3.6	3.4	16.4	16.8
$4\text{-}ClC_6H_4-$	H	II	E	41.5	170–172	$C_{11}H_5Cl_4NO_2S_2$	3.6	3.7	16.4	16.8
$3\text{-}NO_2C_6H_4-$	H	II	E	50	148–150	$C_{11}H_5Cl_3N_2O_4S_2$	7.0	7.2[d]	16.0	15.9
$4\text{-}CH_3OC_6H_4-$	H	II	C	57	189–190	$C_{12}H_8Cl_3NO_3S_2$	3.7	3.7	16.6	16.9
$3,4\text{-}(OCH_2O)C_6H_3-$	H	II	E	65	178–179	$C_{12}H_6Cl_3NO_4S_2$	3.5	3.6	16.0	16.3
$C_6H_5CH=CH-$	H	III	C	60	173–174	$C_{13}H_8Cl_3NO_2S_2$	3.7	3.8	16.8	17.0
CH_3-	CH_3-	II	B	50	114–115	$C_7H_6Cl_3NO_2S_2$	4.6	4.9	34.8[e]	34.4[e]
$-CH_2CH_2CH_2CH_2CH_2-$		II	F	67	169–171	$C_{10}H_{10}Cl_3NO_2S_2$	4.1	4.1	18.4	18.8

[a] Solvent for recrystallization: A, ethanol; B, methanol; C, acetone + methanol; D, chloroform + acetone; E, chloroform; F, carbon tetrachloride + petroleum ether. [b] Kjeldahl method. [c] $(CH_3)_3CCH_2CH(CH_3)CH_2-$. [d] By A.O.A.C. salicylic acid + sodium thiosulfate method. [e] Chlorine, %.

The 5-aralkylidenethiazolidine-2,4-diones were prepared in good yields by the condensation of thiazolidine-2,4-dione with aromatic aldehydes in acetic acid containing sodium acetate.[2] By applying this method to isobutyraldehyde and 3,5,5-trimethylhexaldehyde, two new compounds were prepared.

5-Isobutylidenethiazolidine-2,4-dione, m.p. 69–71°, yield 19%. *Anal.* Calcd. for $C_7H_9NO_2S$: N, 8.2. Found: N, 7.8.

5-(3,5,5-Trimethylhexylidene)-thiazolidine-2,4-dione, m.p. 69–72°, yield 16.2%. *Anal.* Calcd. for $C_{12}H_{19}NO_2S$: N, 5.8. Found: N, 5.7.

Conversion of Rhodanine to Thiazolidine-2,4-dione.—A mixture of rhodanine (27 g.), chloroacetic acid (30 g.) and water (100 ml.) was heated under reflux for 18 hr. The solid which separated upon cooling was collected and washed with water. The air-dried product weighed 11.5 g. (49%) and melted at 121–123°. A mixture of this and an authentic sample of thiazolidine-2,4-dione showed no depression of melting point.

The following three 5-alkylidenethiazolidine-2,4-diones were similarly prepared from the corresponding 5-alkylidenerhodanines.[14]

5-Isopropylidenethiazolidine-2,4-dione, m.p. 160–162°,[15] yield 77%.

5-*s*-Butylidenethiazolidine-2,4-dione, m.p. 141–145°, yield 38%. *Anal.* Calcd. for $C_7H_9NO_2S$: N, 8.2. Found: N, 8.2.

5-Cyclohexylidenethiazolidine-2,4-dione, m.p. 139–142°,[16] yield 69%.

3-Trichloromethanesulfenyloxazolidine-2-4-dione. Method I.—A mixture of *n*-butyl glycolate (72 g.), urea (32 g.), sodium methoxide (29 g.) and anhydrous ethanol (250 ml.) was stirred and heated under reflux on a steam-bath for two hours while a slow current of air was drawn through the mixture. The ethanol was removed by distillation under diminished pressure. Water (200 ml.) was added to the residue and the mixture again concentrated under reduced pressure to ensure the complete removal of the ethanol. To the cooled solution of the sodium salt of oxazolidine-2,4-dione was slowly added a solution of trichloromethanesulfonyl chloride (100 g.) in petroleum ether (100 ml.) and the mixture stirred at room temperature for three hours. The white solid was collected, washed with petroleum ether and air-dried. The product weighed 53 g. and melted at 117–118°. Recrystallization from ethanol raised the m.p. to 119–120°.

3-Trichloromethanesulfenyl-5,5-dimethylthiazolidine-2,4-dione. Method II.—To a cooled and stirred solution containing 5,5-dimethylthiazolidine-2,4-dione (24.6 g.), sodium hydroxide (6.8 g.) and water (100 ml.) was slowly added a solution of trichloromethanesulfenyl chloride (31.4 g.) in carbon tetrachloride (70 ml.). After the addition was completed, the mixture was stirred at room temperature for several hours. The organic layer was separated and the

(14) By using ammonium hydroxide alone as the condensing agent in the reaction of ketones and rhodanine,[3] we were able to obtain 5-isopropylidenerhodanine in 80% and 5-*s*-butylidenerhodanine in 74% yield.

(15) C. C. J. Culvenor, W. Davies, J. A. Maclaren, P. F. Nelson and W. E. Savige, *J. Chem. Soc.*, 2373 (1949), prepared this compound by desulfurization of 5-isopropylidenerhodanine with lead acetate and reported a m.p. of 166°.

(16) D. Libermann, *et al.*,[2b] obtained this compound (m.p. 147°) by the direct condensation of cyclohexanone with thiazolidine-2,4-dione. This method yielded a purer product and is therefore preferred.

aqueous layer extracted with carbon tetrachloride. The combined carbon tetrachloride solution was evaporated under reduced pressure. The residue was an amber oil (43 g.) which solidified upon standing in the cold. The solid after recrystallization from petroleum ether weighed 37 g.

3-Trichloromethanesulfenyl-5-benzylidenethiazolidine-2,4-dione. Method III.—A mixture of the potassium salt of 5-benzylidenethiazolidine-2,4-dione[2c] (20 g.), trichloromethanesulfenyl chloride (15.3 g.) and carbon tetrachloride (150 ml.) was stirred for three hours. The solid was collected and recrystallized from a mixture of acetone and methanol. The 3-trichloromethanesulfenyl-5-benzylidenethiazolidine-2,4-dione thus obtained weighed 21 g.

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Cholesterol and Companions. VII. Steroid Dibromides

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Among many reported instances of the bromination of Δ^5-stenoids, occasional reference has been made to the use of pyridine[1] or sodium acetate[2] as a buffer to neutralize traces of hydrogen bromide. Being unaware of any prior comparison, I wish to report that whereas bromination of cholesterol (150-g. lots) in ether by addition of a solution of bromine in acetic acid (Windaus[3] procedure) gave the dibromide (as the acetic acid complex) in 72–74% yield, the yield rose to 84% on addition of 0.14 equivalent of sodium acetate.

Windaus' method[3] of debromination with zinc dust in boiling acetic acid is applicable, with some limitations,[4,5] to stenyl acetate dibromides[6] and to the conversion of 5,6-dibromo-3-ketones into Δ^4-stene-3-ones,[3] but not to free sterol dibromides because of the ready acetylation of sterols in hot acetic acid.[7] Since newer methods of debromination utilizing sodium iodide,[8] ferrous chloride[9] or chromous chloride[5] did not seem well adapted to rapid, large-scale operation, the Windaus method was reinvestigated and a simple modification found that eliminates the difficulties: a suspension of the dibromide in ether containing a small amount of acetic acid is stirred at room temperature and zinc dust is added. A vigorous, exothermic reaction reminiscent of the formation of a Grignard reagent sets in and is soon complete; the yield of cholesterol from the dibromide is 93%. This material is free from cholestanol, 7-dehydrocholesterol, and lathosterol[7]; the first-crop material from methanol is free also from cerebrosterol[10] and from 25-hydroxycholesterol, a product of autoxidation that has been found present in old samples.

(1) A. Serini and W. Logemann, *Ber.*, **71**, 1362 (1938).
(2) J. Hier and K. Miescher, *Helv. Chim. Acta*, **34**, 359 (1951).
(3) A. Windaus, *Ber.*, **39**, 518 (1906).
(4) A. Butenandt and U. Westphal, *ibid.*, **67**, 2085 (1934).
(5) P. L. Julian, W. Cole, H. Magnani and E. W. Meyer, THIS JOURNAL, **67**, 1728 (1945).
(6) A. Windaus and A. Hauth, *Ber.*, **39**, 4378 (1906).
(7) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953).
(8) R. Schoenheimer, *Z. physiol. Chem.*, **192**, 86 (1930); *J. Biol. Chem.*, **110**, 461 (1935).
(9) H. Bretschneider and M. Ajtai, *Monatsh.*, **74**, 57 (1943).
(10) A. Ercoli and P. de Ruggieri, THIS JOURNAL, **75**, 3284 (1953).

The new procedure is also applicable to the debromination of 5,6-dibromocholestanone, obtainable on a large scale in 96.5% yield by oxidation of cholesterol dibromide with sodium dichromate in place of chromic acid.[11,12] The reaction with zinc dust in ether–acetic acid proceeds rapidly at 15–20° and Δ^5-cholestene-3-one of high purity is obtained in 88% yield. Debromination to the Δ^5-stenone with zinc dust and boiling ethanol[11] proceeds satisfactorily on a small scale but on a large scale gives material of inferior quality.

The three steps leading to Δ^5-cholestene-3-one seemed so satisfactory for preparative purposes that the further conversion to Δ^4-cholestene-3-one was explored. Isomerization catalyzed by a mineral acid or a base, while applicable on a micro scale,[11] gave inferior material as applied to 100-g. lots. However, oxalic acid in ethanol effected isomerization smoothly and afforded in 98% yield cholestenone corresponding in melting point (81–82°) and extinction coefficient to material purified by chromatography[13]; the over-all yield from cholesterol is 69%. Because of the high purity of the product and since all the steps from cholesterol can be completed in a few hours, this route rivals direct Oppenauer oxidation, which affords cholestenone, m.p. 77–79°, in 70–81% yield.[14]

Experimental

Cholesterol Dibromide.—One hundred and fifty grams of commercial cholesterol was dissolved in 1 l. of absolute ether by brief boiling in a 4-l. beaker, the solution was cooled to 25°, and a solution of 5 g. of anhydrous sodium acetate and 68 g. of bromine in 600 cc. of acetic acid was added. The solution turned yellow and a stiff paste of dibromide promptly resulted. The mixture was cooled to 20° and the product collected and washed with acetic acid (500 cc.) until the filtrate was colorless. A second crop of satisfactory material was obtained by adding 800 cc. of water to the combined yellow filtrate and washings, filtering the precipitate and washing it free of yellow mother liquor with acetic acid. When spread out on a paper and let dry in a hood at room temperature overnight, the material reaches a weight unchanged by drying for another day or two and appears from the infrared spectrum to be a 1:1 acetic acid complex, and percentage yields are calculated on this basis. Yields obtained in the first and second crops are: 182.4, 14.7 g.; 171.5, 25.2 g.; total yield 197.1, 196.7 g. (84%).

The infrared spectrum of the air-dried dibromide in chloroform resembles that of the 2:1 cholesterol–oxalic acid complex. In each case the band in the hydroxyl region is minor and shifted to about 3.0 μ, bands ordinarily associated with free carboxyl group and ester groups are absent, and a prominent band at 5.79–5.81 μ and a less intense band at 5.6–5.7 μ probably are characteristic of a carbonyl group in this particular type of acid–alcohol complex.

Cholesterol already purified through the dibromide afforded dibromide in only slightly higher yield (85%). In numerous earlier brominations made in exactly the same way but without addition of the small amount of sodium acetate the yield in the first crop was only 72–74% and the second crop contained much unbrominated material. Doubling of the amount of sodium acetate specified produced no change in the result.

Cholesterol from the Dibromide.—The acetic acid–moist dibromide from 150 g. of cholesterol was suspended in 1.2 l. of ether in a flask equipped with a stirrer and with provision for ice cooling when required. Fresh zinc dust (40 g.) was added in the course of 5 min. The first 5–10 g. was added without cooling; when the reaction had started, as evi-

(11) A. Butenandt and J. Schmidt-Thomé, *Ber.*, **69**, 882 (1936).
(12) H. H. Inhoffen, *ibid.*, **69**, 1134 (1936).
(13) E. R. H. Jones, P. A. Wilkinson and R. H. Kerlogue, *J. Chem. Soc.*, 392 (1942).
(14) R. V. Oppenauer, *Org. Syntheses*, **21**, 18 (1941).

denced by solution of part of the dibromide, the cooling bath was raised during the remainder of the addition. At the end, the ice-bath was removed and the mixture, which soon set to a heavy paste of white solid,[15] was stirred for 15 min. longer. Then 50 cc. of water was added to dissolve the zinc salt and the ethereal solution was decanted into a separatory funnel and washed with 400 cc. of water containing 25 cc. of 36% hydrochloric acid. After three more washings with 400 cc. of water, the solution was shaken with 300 cc. of water and 150 cc. of 25% sodium hydroxide solution and the ether layer tested to make sure it contained no trace of acetic acid (which readily acetylates the sterol during evaporation). The solution was then dried, evaporated to about 600 cc., 600 cc. of methanol was added, and the solution boiled down to the point of incipient crystallization (about 1 l.). After cooling (4°), the main crop of purified cholesterol was collected and dried: 108.4 g., m.p. 149.5–150°; a second crop of 8.4 g., m.p. 148–149°, was obtained after evaporation to a volume of 250 cc.; total yield 116.8 g. (93%). The residual mother liquor afforded about 4 g. of material containing bromine not removed by repetition of the treatment with zinc dust. If air-dried dibromide is used, 25 cc. of acetic acid should be added to the ethereal suspension before addition of zinc.

5α,6β-Dibromocholestane-3-one.—The moist dibromide from 150 g. of cholesterol was suspended in 2 l. of acetic acid in a 5-l. flask equipped with a stirrer and mounted over a bucket of ice and water that could later be raised, and a solution, preheated to 90°, of 80 g. of sodium dichromate dihydrate in 2 l. of acetic acid was poured into the stirred suspension (at 25°). The temperature of the mixture reached 55–58° during the oxidation and the solid all dissolved in 3–4 min. After another 2 min. the ice bucket was raised so that the flask was completely immersed and the stirrer was stopped for 10 min. to allow the dibromoketone to separate in easily filterable crystals. With stirring resumed, the temperature was brought to 25° and then, after addition of 400 cc. of water, to 15°. The product was collected, washed with methanol until the filtrate was colorless (500–600 cc.) and the white crystals, m.p. 73–75° dec., $[\alpha]^{25}D$ −46.8° Chf (*c* 2.11) were either used while still moist or dried in a dark cupboard at room temperature; yield 170.9 g. (96.5% in the oxidation, 81% from cholesterol). Butenandt[11] and Inhoffen[12] report m.p. (dec.) 80° and 68–69°.

Δ⁵-Cholestene-3-one.—The methanol-moist dibromocholestanone from 150 g. of cholesterol was covered with 2 l. of ether, 25 cc. of acetic acid was added, and the mixture stirred mechanically in an ice-bath and the temperature lowered to 15°. Then 40 g. of fresh zinc dust was added in portions in the course of 5 min. with maintenance of a temperature of 15–20° by cooling. When the exothermic reaction was over, the ice-bath was removed and stirring continued for 10 min. Then 70 cc. of pyridine was added and the resulting suspension of white complex stirred briefly; the solution was then filtered by suction and the filter cake washed well with ether. The colorless filtrate was washed three times with water and once with 600 cc. of 5% bicarbonate solution (to remove a trace of acetic acid), dried, and evaporated to a volume of 1 l. After addition of 500 cc. of methanol, evaporation was continued to a volume of 1.2 l. and the product let crystallize. It separated in large, pure white prisms, m.p. 126–129° (camphor-like), $[\alpha]D$ −2.5° Chf (*c* 2.03), no selective absorption at 242 mμ. The yield in the first crop was 87–94 g., and concentration of the mother liquor afforded 12–19 g. more of colorless material melting in the range 118–124° and suitable for conversion to the conjugated ketone; total yield 106 g. (88%, 71% from cholesterol).

Debromination with zinc and ethanol according to Butenandt and Schmidt-Thomé[11] when conducted on the same scale as above afforded crude Δ⁵-cholestene-3-one in 81–85% yield, but the material melted at 116–120°. Chromatography of the ethanolic mother liquor afforded Δ⁴-cholestene-3-one, Δ⁴-cholestene-3,6-dione and Δ⁴-cholestene-6β-ol-3-one; the last two products must be derived from cholesterol formed from the dibromide during the oxidation.

Δ⁴-Cholestene-3-one.—One hundred grams of Δ⁵-cholestene-3-one and 10 g. of anhydrous oxalic acid were dissolved in 800 cc. of 95% ethanol and the colorless solution was warmed for 10 min. on the steam-bath and then let cool to room temperature and seeded. The main crop of conjugated ketone (91.1 g.) separated as large, colorless prismatic needles, m.p. 81–82°, $[\alpha]D$ +92.0° Chf (*c* 2.01), λ^{EtOH} 242 mμ (17,000); constants reported[13] for material purified by chromatography are: m.p. 81–82°, λ^{EtOH} 240.5 mμ (18,000). Further crops were obtained first by concentration of the mother liquor and then by dilution with water, and these on recrystallization gave 6.8 g. of colorless product, m.p. 81–82°; total yield 97.9% (69% from cholesterol).

Isomerization of Δ⁵-cholestene-3-one in ethanol with either hydrochloric acid or sodium hydroxide (followed by neutralization of the yellow enolate solution with acetic acid) proved unsatisfactory on a large scale since a permanent yellow color developed and the first-crop material was yellowish and melted at 78–80°.

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(15) This solid, m.p. about 170° dec., contains zinc and affords cholesterol on crystallization from methanol or acetone; it was not obtained in a form suitable for analysis.

Oxidation of 10-Acyl- and 10-Alkylphenothiazines

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A number of acid chlorides react readily with phenothiazine in pyridine to give 10-substituted phenothiazines.[1] Later reports[2] have shown that phenothiazine, and some of its nuclearly substituted derivatives, react with various haloacyl halides when heated in refluxing benzene or toluene to give the corresponding 10-haloacylphenothiazines in good yield. In this work, the 10-acylphenothiazines (Table I) were prepared by allowing the acid chloride to react with phenothiazine in the presence of dioxane and sodium carbonate.

A variety of oxidizing agents has been used to oxidize the sulfur of a number of phenothiazine derivatives to the sulfoxide or the sulfone. Those which oxidized the sulfur to the sulfoxide were potassium permanganate,[3,4] 30% hydrogen peroxide in ethanol,[5] sodium nitrite[6] and nitric acid.[6] In the latter case nitration also resulted. The sulfur has been oxidized to the sulfone by potassium permanganate,[4] 30% hydrogen peroxide in glacial acetic acid[7,8] and hypochlorous acid.[8] This note includes additional studies made on the oxidation of the sulfur of the phenothiazine nucleus.

The reaction of concentrated nitric acid with 10-chloroacetylphenothiazine in glacial acetic acid gave 3-nitrophenothiazine-5-oxide (I) and 10-chloroacetylphenothiazine-5-oxide but apparently no 3-nitro derivative of the latter compound. It appears that the acyl derivative was first oxidized by nitric acid to give the monoxide and that this reaction was then followed by hydrolysis and nitration resulting in the formation of I. Previous reactions using nitric acid and phenothiazine, or some of its derivatives, gave the corresponding

(1) S. E. Hazlet and C. E. Roderuck, THIS JOURNAL, **67**, 495 (1945).

(2) R. Dahlbom and T. Ekstrand, *Acta Chem. Scand.*, **5**, 102 (1951); T. Ekstrand, Swedish Patent 127,566 [*C. A.*, **45**, 1886 (1951)].

(3) E. DeB. Barnett and S. Smiles, *J. Chem. Soc.*, **97**, 188 (1910).

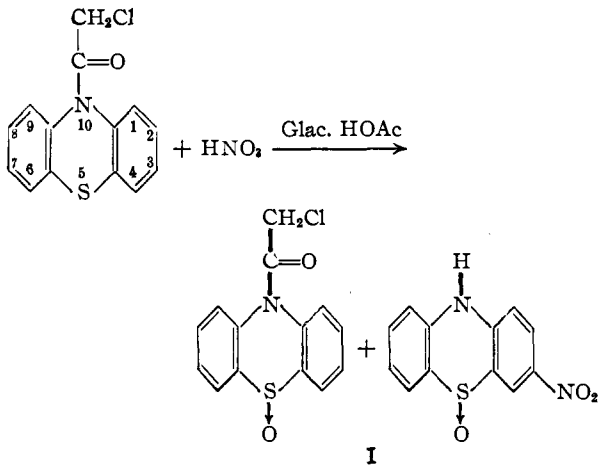
(4) H. I. Bernstein and L. R. Rothstein, THIS JOURNAL, **66**, 188 (1944).

(5) H. Gilman and D. A. Shirley, *ibid.*, **66**, 888 (1944); D. F. Houston, E. B. Kester and F. DeEds, *ibid.*, **71**, 3819 (1949).

(6) F. Kehrmann and P. Zybs, *Ber.*, **52B**, 130 (1919).

(7) N. L. Smith, *J. Org. Chem.*, **16**, 415 (1951).

(8) J. G. Michels and E. D. Amstutz, THIS JOURNAL, **72**, 888 (1950).



I

nitromonoxides[5,6,9]; consequently, both oxidation and nitration had occurred. One exception involved a tetrachlorophenothiazine.[10] In that case only oxidation of the sulfur was observed, doubtless because the chlorine atoms occupied the positions normally affected by nitration reactions. The nitric acid oxidation was extended to other 10-acylphenothiazines.

In a second experiment, 10-chloroacetylphenothiazine gave an 86% yield of the monoxide and very little, if any, I (Table II). Under similar conditions, 10-phenacetylphenothiazine gave 50% of the oxide; 10-acetylphenothiazine gave a 19% yield of 10-acetylphenothiazine-5-oxide, as well as some I; and the 10-dichloroacetyl derivative was unaffected as evidenced by the almost quantitative recovery of starting material. Thus, it is evident that the group attached to the nitrogen of phenothiazine affects the success of the oxidation of the sulfur. This observation was made also by Bernstein and Rothstein.[4] They found that 10-ethylphenothiazine was converted to the sulfone and that 10-(*p*-toluenesulfonyl)-phenothiazine was oxidized to the monoxide by potassium permanganate in boiling water, whereas the *p*-acetamidobenzenesulfonyl derivative did not undergo reaction. Neither of the latter two derivatives was oxidized by hydrogen peroxide in acetone.

Pummerer and Gassner[11] reported the formation of phenothiazine-5-oxide in 75% yield by the oxidizing action of 30% hydrogen peroxide on phenothiazine dissolved in hot ethanol containing some potassium hydroxide. An attempt to repeat the reaction was unsuccessful. However, by carrying out the reaction in the absence of potassium hydroxide, phenothiazine-5-oxide was obtained in an almost quantitative yield.

A number of 10-acylphenothiazine-5-oxides was prepared by the action of excess 30% hydrogen peroxide on the acyl derivative in refluxing ethanol. There was no indication that any dioxide had been formed. That alkali could not have been used to catalyze the foregoing reactions was shown by the rapid hydrolysis of the acyl group upon addition of 10% sodium hydroxide to a hot ethanolic solution of the 10-acylphenothiazine-5-oxide.

(9) A. Bernthsen, *Ann.*, **230**, 73 (1885).
(10) O. Unger and K. A. Hofmann, *Ber.*, **29**, 1362 (1896).
(11) R. Pummerer and S. Gassner, *ibid.*, **46**, 2322 (1913).

An exception to the above reactions was the oxidation of 10-ethylphenothiazine by hydrogen peroxide in refluxing ethanol. Two products were isolated from the reaction mixture: the monoxide in 62% yield and the dioxide in 15.5% yield. The rather large excess of hydrogen peroxide used might account for the fact that the dioxide was formed. Early reports[12] stated that the oxidation of sulfides by excess 30% hydrogen peroxide in acetone or aqueous solutions at ordinary temperatures gave only the sulfoxides. These conditions are not strictly comparable to those used for the production of sulfoxides in this investigation. Thus, the observations of Smiles and Hinsberg do not preclude the formation of small amounts of the dioxide by carrying out the oxidation reaction in refluxing ethanol solution.

10-Methyl- and 10-ethylphenothiazine, as well as various 10-acyl derivatives, were oxidized to the corresponding dioxides (Table III), in fair to excellent yields, by means of excess 30% hydrogen peroxide in glacial acetic acid. In addition, a number of the monoxide derivatives was oxidized to the respective dioxides by the same procedure.

One method of forming peracetic acid[13] is by heating a mixture of 30% hydrogen peroxide and glacial acetic acid. Since hydrogen peroxide in ethanol oxidized the sulfur of the phenothiazine derivatives to the sulfoxide, and the use of hydrogen peroxide in acetic acid resulted in the oxidation of the sulfur to the sulfone, it seems possible that in the latter reaction peracetic acid would be formed *in situ* and thus behave as the oxidizing agent. This very likely might account for the difference in degree of oxidation upon using ethanol or glacial acetic acid as the solvent.

Potassium amide reacts readily with aryl halides in liquid ammonia at $-33°$.[14] In view of this fact and also of the fact that 10-sodiophenothiazine reacts readily with ethyl bromide in liquid ammonia,[15] the reaction of 10-sodiophenothiazine with iodobenzene was tried. Under corresponding conditions the reaction was found to be unsuccessful. Thus, it seems that the amide ion is a stronger attacking agent than the 10-phenothiazyl ion in the nucleophilic displacement reaction on carbon.

Methylation of 3-methylphenothiazine with dimethyl sulfate in acetone containing sodium hydroxide was successful.[16] It was found that phenothiazine could also be methylated by dimethyl sulfate.

The authors are grateful to Parke, Davis and Company for arranging for the testing of some of the compounds. The results of these tests will be reported elsewhere.

Experimental

10-Acetylphenothiazine.[17]—This example is typical of procedures used to prepare three other acyl derivatives which are described in Table I.

(12) M. Gazdar and S. Smiles, *J. Chem. Soc.*, **93**, 1833 (1908); O. Hinsberg, *Ber.*, **41**, 2836 (1908).
(13) D. Swern, *Chem. Revs.*, **45**, 1 (1949).
(14) F. W. Bergstrom and W. C. Fernelius, *ibid.*, **12**, 98 (1933).
(15) H. Gilman, R. D. Nelson and J. F. Champaigne, Jr., THIS JOURNAL, **74**, 4205 (1952).
(16) Ng. Ph. Buu-Hoï and Ng. Hoán, *J. Chem. Soc.*, 1834 (1951).
(17) The compound was also prepared in 99% yield by refluxing a solution of phenothiazine in acetic anhydride. See reference 9.

TABLE I

10-ACYLPHENOTHIAZINES

Acyl group	Recrystallizing solvent	M.p., °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
$COCH_2Cl$[a]	Benzene–pet. ether (b. 60–70°)	113.5–114.5	45	$C_{14}H_{10}ClNOS$	...	.....
$COCHCl_2$[b]	Ethanol	149–151	23	$C_{14}H_9Cl_2NOS$	4.52[c]	4.87, 4.66
$COCH_2C_6H_5$	Benzene	152–153	66	$C_{20}H_{15}NOS$	4.46	4.43

[a] Reference 2. [b] On using dichloroacetic acid anhydride a 32% yield of the product, m.p. 154–155°, was obtained. It was purified by chromatographic adsorption on a column of alumina. The reaction of the acid chloride in pyridine or the reaction of ethyl dichloroacetate with phenothiazine so far has not given the desired derivative. [c] Chlorine analysis. Calcd.: Cl, 22.86. Found: Cl, 22.32, 22.42, 22.42.

Two and four-tenths grams (0.03 mole) of acetyl chloride in 20 ml. of dioxane was slowly added with stirring to a solution of 2 g. (0.01 mole) of phenothiazine in 40 ml. of dioxane in which there was suspended 4 g. of anhydrous sodium carbonate. The mixture was stirred at room temperature for a few minutes and then slowly heated to a gentle reflux. The heating was continued for 40 minutes. The mixture was poured into very dilute hydrochloric acid to precipitate 2.2 g. (91%) of yellow solid, m.p. 184–188°. The crude product was recrystallized from ethanol giving 1.9 g. (79%) of light yellow crystals, m.p. 197–198°.

10-Acetylphenothiazine-5-oxide.—The following procedures are typical of the methods by which the oxides listed in Table II were prepared.

TABLE II

10-ACYLPHENOTHIAZINE-5-OXIDES

Acyl group	Method	M.p., °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
$COCH_2Cl$[a]	A	186–187	86	$C_{14}H_{10}ClNO_2S$	4.80[b]	4.72
$COCH_2C_6H_5$	A	141–141.5[c]	50	$C_{20}H_{15}NO_2S$	4.20	4.39
$COCH_2C_6H_5$	B	138–140[d]	70			

[a] In the first experiment, 3-nitrophenothiazine-5-oxide and a small amount of 10-chloroacetylphenothiazine-5-oxide were isolated. [b] Chlorine analysis. Calcd.: Cl, 12.15. Found: Cl, 12.36, 12.08. [c] The compound was purified by chromatographic adsorption on a column of alumina. [d] The product was recrystallized from ethanol only.

Method A.—Five milliliters of concentrated nitric acid (sp. gr. 1.42) was added slowly to a solution of 5 g. (0.021 mole) of 10-acetylphenothiazine in 105 ml. of glacial acetic acid cooled in an ice-bath. After 20 minutes an additional milliliter of nitric acid was added. The reaction mixture was stirred occasionally while standing in the ice-bath for 30 minutes and then poured into water. After a few minutes a dark red solid began to separate. This was filtered off and shown to be 3-nitrophenothiazine-5-oxide[9] (mixed m.p.). The filtrate was poured over ice and 2.5 g. (46%) of light brown powder, m.p. 156–159°, separated. This product was purified by five recrystallizations from ethanol to give 1 g. (19%) of flat, white needles, m.p. 169.5–170°.

Anal. Calcd. for $C_{14}H_{11}NO_2S$: N, 5.45. Found: N, 5.47.

Method B.—A solution of 5 g. (0.021 mole) of 10-acetylphenothiazine, 500 ml. of ethanol and 60 ml. of 30% hydrogen peroxide was refluxed for 5 hours. Another 20 ml. of hydrogen peroxide was added and the refluxing continued for 30 minutes. After the solution had stood at room temperature for a few hours, approximately 450 ml. of solvent was removed by distillation. Water was added to the residual solution and 4.7 g. (88%) of white solid, m.p. 169–170°, separated. The mixed melting point with the 10-acetylphenothiazine-5-oxide prepared by the former method was undepressed. There was no evidence that any of the dioxide had been formed in the reaction.

Hydrolysis of 10-Acetylphenothiazine-5-oxide.—A solution of 1.0 g. (0.0039 mole) of the oxide in 15 ml. of ethanol and 2 ml. of 10% sodium hydroxide was refluxed for a few minutes. The color of the solution immediately became brown. After a short time colorless platelets crystallized. The solution was cooled and 0.6 g. (72%) of solid, m.p. 250–251° dec., filtered off. The mixed melting point with an authentic specimen of phenothiazine-5-oxide was undepressed.

The other 10-acylphenothiazine-5-oxides were hydrolyzed, by a similar procedure, to give phenothiazine-5-oxide.

Phenothiazine-5-oxide.[11]—A solution of 24.5 g. (0.123 mole) of phenothiazine, 800 ml. of ethanol, 8 ml. of 10% ethanolic potassium hydroxide and 24 ml. of 30% hydrogen peroxide was heated with stirring on a steam-bath for 3 hours.[11] A small amount of solid was filtered off. Since very little more solid separated after 10 days at room temperature, the solution was poured into 3 l. of water to give 24.1 g. of solid, m.p. 160–163° dec. Apparently the reaction did not go to completion since phenothiazine-5-oxide melts at 250° dec.[18] Thus, the solid, m.p. 160–163° dec., was redissolved in 750 ml. of ethanol, and 35 ml. of 30% hydrogen peroxide was added to the refluxing solution. The refluxing was continued for 4 hours. Most of the solvent was removed by distillation and the remaining solution was poured into water; 25.4 g. (96%) of yellow solid, m.p. 242–242.5° dec., separated. Recrystallization of the solid did not change its melting point. The mixed melting point with an authentic sample of the oxide was undepressed.

Hydrogen Peroxide Oxidation of 10-Ethylphenothiazine.—Three hundred milliliters of 30% hydrogen peroxide was added to a hot solution of 29.5 g. (0.13 mole) of 10-ethylphenothiazine[15] in 1200 ml. of absolute ethanol. The mixture was refluxed for a total of 8 hours. After 5 hours, more hydrogen peroxide (100 ml.) was added. The solution stood for 15 hours and then was concentrated by distilling off 700 ml. of solvent. The remaining solution was poured into 2 l. of ice-water to precipitate 25.5 g. of white solid, m.p. 146–149°. Recrystallization of the product from 125 ml. of absolute ethanol did not change the melting point. Since this one recrystallization from ethanol did not seem to purify the product, the total amount of crude material was dissolved in 800 ml. of benzene and the solution chromatographed on a 38 × 196 mm. column of alumina (Alcoa Activated Alumina, F-20). The column was eluted with benzene and finally absolute ethanol, the eluate being collected in 125-ml. portions. This procedure resulted in the isolation of 5.2 g. (15.5%) of 10-ethylphenothiazine-5-dioxide,[4] m.p. 161–163°, and 19.7 g. (62%) of 10-ethylphenothiazine-5-oxide, m.p. 162–163°. The dioxide passed through the column in the first portions of the eluate. The mixed melting point of the two products was depressed to 138–141°.

Anal. Calcd. for $C_{14}H_{13}NOS$: N, 5.76. Found: N, 5.85.

10-Acetylphenothiazine-5-dioxide.—The dioxides listed in Table III were prepared by the following procedure.

TABLE III

10-SUBSTITUTED PHENOTHIAZINE-5-DIOXIDES

R	M.p., °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
CH_3[a]	220–221[b]	44	$C_{13}H_{11}NO_2S$	..	..
CH_2CH_3[c]	162–164	95	$C_{14}H_{13}NO_2S$	..	..
$COCH_2Cl$	211[b]	51	$C_{14}H_{10}ClNO_3S$	4.56	4.60
$COCHCl_2$	211–212[b]	80	$C_{14}H_9Cl_2NO_3S$	4.09	4.12
$COCH_2C_6H_5$[d]	215–216[b]	70	$C_{20}H_{15}NO_3S$	4.01	4.22

[a] Reference 9. [b] Decomp. [c] Reference 4. [d] The product was purified by recrystallization from xylene. It was also prepared in a 77% yield by the hydrogen peroxide oxidation of the monoxide in glacial acetic acid.

Five milliliters of 30% hydrogen peroxide was added with stirring to a solution of 5 g. (0.021 mole) of 10-acetylphenothiazine in 150 ml. of glacial acetic acid at room temperature. After heating the solution at 60–70° for 15 minutes another 3 ml. of hydrogen peroxide was added. The heat-

(18) E. DeB. Barnett and S. Smiles, *J. Chem. Soc.*, **95**, 1253 (1909).

ing with stirring was continued for 1.5 hours. A major portion of the solvent was then removed by distillation under reduced pressure. Four and three-tenths grams (80%) of white solid, m.p. 200–216° dec., crystallized from the cooled solution. Recrystallization of the product from ethanol gave 3.9 g. (68%) of small, flat, white crystals, m.p. 216–217°.

Anal. Calcd. for $C_{14}H_{11}NO_3S$: N, 5.13. Found: N, 5.18.

The dioxide was also prepared in 30% yield by hydrogen peroxide oxidation of 10-acetylphenothiazine-5-oxide in glacial acetic acid.

Hydrolysis of 10-Acetylphenothiazine-5-dioxide.—One and one-half milliliters of 10% sodium hydroxide was added to a hot solution of 0.50 g. (0.0018 mole) of the dioxide in 30 ml. of absolute ethanol. The color of the solution immediately became yellow. After a few minutes, part of the solvent was removed by distillation. The addition of water to the residual solution precipitated 0.42 g. (100%) of yellow solid, m.p. 255–257° dec. The mixed melting point with an authentic sample of phenothiazine-5-dioxide[19] was undepressed.

The other 10-acylphenothiazine-5-dioxides were hydrolyzed, in a similar fashion, to give phenothiazine-5-dioxide.

10-Methylphenothiazine.[4]—Fifteen milliliters of dimethyl sulfate was added with stirring to a mixture of 10 g. (0.05 mole) of phenothiazine, dissolved in 100 ml. of dioxane, and 50 g. of anhydrous potassium carbonate. The color of the mixture immediately turned brown and soon after heating to reflux, the color became yellow. After 3.5 hours of refluxing with stirring, another 10 ml. of dimethyl sulfate was added. The mixture was refluxed for a total of 24 hours. It was carefully poured into about 400 ml. of warm water, and after standing overnight, 10.5 g. of tan solid, m.p. 75–80°, was filtered off. After extracting this solid with hot ethanol, a tar remained. From the ethanol extract there crystallized 4.3 g. (40%) of light yellow needles, m.p. 91–94°. This solid was recrystallized from 95% ethanol giving 2.8 g. (26%) of yellow needles, m.p. 99–100°.

(19) A. Bernthsen, *Ber.*, **39**, 1807 (1906).

DEPARTMENT OF CHEMISTRY
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Ion-exchange Chromatography of Pteroylglutamic Acid and Aminopterin[1]

BY M. R. HEINRICH, VIRGINIA C. DEWEY AND G. W. KIDDER

RECEIVED MAY 26, 1953

A separation of pteroylglutamic acid (PGA, folic acid) and 4-amino-PGA (Aminopterin) has been effected on columns of the anion exchanger Dowex-1. The procedure is of use in the analysis and purification of these compounds.

A "standard" PGA sample showed a single peak upon chromatography, and thus appeared to be pure (Fig. 1). The three samples of Aminopterin tested, however, contained approximately 20% of an impurity with the elution properties of PGA (Fig. 2). Total recoveries in the Aminopterin runs, based on optical density, were 80–90%, probably indicating further impurity which is not eluted.

In order to further characterize the impurity in the Aminopterin, some of the fractions from the second peak (Fig. 2) were combined, neutralized with sodium hydroxide, and evaporated to dryness in a vacuum desiccator. Paper chromatography of this material showed it to have the properties of PGA. Samples from both chromatographic peaks were also tested with *Tetrahymena pyriformis* (*gelei*). Material from the major (Aminopterin) peak gave neither inhibition nor stimulation of growth, contrary to the earlier report of 17% activity of (impure) Aminopterin for growth.[2] This confirms the complete separation of the two components, as shown in the ion-exchange chromatogram. Material from the minor (PGA) peak gave the growth stimulation with *Tetrahymena* expected from the PGA calculated to be present.

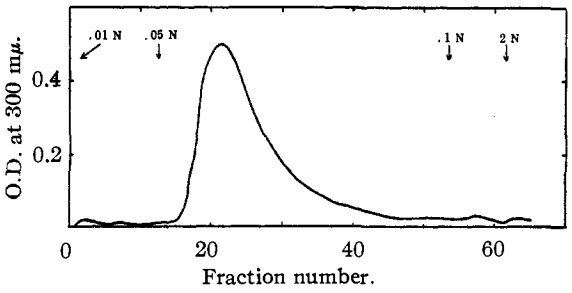

Fig. 1.—Pteroylglutamic acid (2.5 mg.) on column of Dowex-1-chloride 56 × 9 mm.: eluted at 0.6 ml./min. with hydrochloric acid of concentrations shown; 30-min. fractions, 93% recovery.

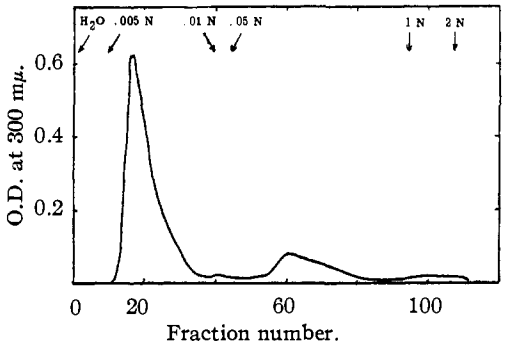

Fig. 2.—Aminopterin (2.5 mg.) on column of Dowex-1-chloride 50 × 9 mm.: eluted at 0.33 ml./min. with hydrochloric acid of concentrations shown; 45-min. fractions, the second peak is PGA.

PGA is not produced by deamination of Aminopterin during the separation, as shown by rechromatographing the pure Aminopterin fractions.

These studies confirm previous reports of the presence of PGA in Aminopterin,[3–5] and present a procedure for the purification of these compounds. Work is continuing on these and related materials.

The authors are grateful to the Lederle Laboratories for supplies of the compounds used.

Experimental

Columns of approximately 55 × 9 mm. were prepared from Dowex-1-chloride (200–400 mesh[6]) in the usual manner.[7] Solutions of PGA or analog were prepared in water, at a concentration of 0.5 mg./ml., neutralized to *p*H 7 with sodium hydroxide. Immediately before adsorption on the column, this solution was brought to *p*H 8–9 with ammonium hydroxide. Dilute hydrochloric acid was used for elution, 0.005 *N* for Aminopterin and 0.05 *N* for PGA in

(1) Supported in part by Contract No. AT(30-1)-1351 with the U. S. Atomic Energy Commission.

(2) G. W. Kidder, V. C. Dewey and R. E. Parks, Jr., *Proc. Soc. Exp. Biol. Med.*, **78**, 88 (1951).

(3) F. Weygand, A. Wacker, H.-J. Mann and E. Rowold, *Z. Naturforsch.*, **6b**, 174 (1951).

(4) D. J. Hutchison and J. H. Burchenal, *Proc. Am. Assoc. Cancer Res.*, **1**, 26 (1953).

(5) D. J. Hutchison, J. H. Burchenal, H. P. Broquist and A. R. Kohler, in manuscript.

(6) Kindly supplied by the Dow Chemical Company.

(7) W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950).

these studies. The column and fraction collector were covered to exclude light; other operations were shielded insofar as possible. The optical density of samples at 300 mμ was determined in a Beckman DU spectrophotometer. Samples to be used later were neutralized to prevent decomposition. After each run the column was washed with about 200 ml. of 2 *N* hydrochloric acid, followed by water. The capacity of these columns for pterins has not been accurately determined, but it is much lower than their capacity for purines and pyrimidines.

Paper chromatography was carried out on strips of Whatman no. 1 paper in 1% aqueous dipotassium phosphate.[8]

To determine whether or not PGA was produced by deamination of Aminopterin during the ion-exchange procedure, combined fractions from the Aminopterin peak (285 ml.) were made alkaline with ammonium hydroxide and readsorbed by running through the column by gravity, in the dark. This required 28 hr. at room temperature. Elution in the same manner used previously gave only an Aminopterin peak, with a recovery of about 95% and no evidence of PGA.

(8) O. P. Wieland, B. L. Hutchings and J. H. Williams, *Arch. Biochem. Biophys.*, **40**, 205 (1952).

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The Oxidation of Dialuric Acid by *o*-Iodosobenzoic Acid[1]

BY LESLIE HELLERMAN AND WENDELL T. CARAWAY[2]

RECEIVED MARCH 25, 1953

The kinetics of oxidation of dialuric acid by oxygen has been studied by Hill[3] and by Richardson.[4] The dissociation constants and oxidation–reduction potentials of dialuric acid and ascorbic acid are of similar magnitude. Tautomeric formation of an enediol configuration is conceivable. In addition, the half-life of oxidized dialuric acid (alloxan)[5] is practically the same as the half-life of dehydroascorbic acid.[6] These similarities in properties suggested a study of the rate of oxidation of dialuric acid by *o*-iodosobenzoic acid to supplement work previously reported for ascorbic acid.[7]

Experimental

Dialuric acid was prepared by the method of Biltz and Damm.[8] General procedures for the preparation of reaction mixtures were the same as those previously described by us for the studies of oxidation of ascorbic acid. The rate of the reaction was followed by the spectrophotometric method using spirocyclohexylporphyrexide.[7,9] Solutions of dialuric acid were found to be too susceptible to oxidation by oxygen to permit use of the titrimetric procedure that utilizes porphyrindine.

Results and Discussion

Dialuric acid (DA) was found to be oxidized by *o*-iodosobenzoic acid (RIO) in a second-order process expressed by the differential equation

$$-\mathrm{d(DA)/dt} = k''\mathrm{(DA)(RIO)} \qquad (1)$$

The integrated form of equation 1 was used to evaluate k'' from the experimental data. Typical results are shown in Table I.

TABLE I

OXIDATION OF DIALURIC ACID BY *o*-IODOSOBENZOIC ACID

Temperature 15.0°

No.	Buffer	*p*H	(DA) × 10⁴, moles per l.	(RIO) × 10⁴, moles per l.	k'', l. mole^{-1} min.$^{-1}$
1	Phosphate, 0.10 *M*	7.05	6.91	9.43	47
2	Phosphate, .10 *M*	7.05	6.99	5.66	49
3	Phosphate, .10 *M*	7.05	13.69	9.43	46
4	Phosphate, .10 *M*	7.05	7.03	9.43	48
5[a]	Phosphate, .60 *M*	6.97	6.97	9.43	104
6[b]	Phosphate, .10 *M*	6.69	6.84	9.43	53
7	Phosphate, .10 *M*	6.04	6.91	9.43	47
8	Phosphate, .10 *M*	7.68	6.83	9.43	24
9[c]	Phosphate, .10 *M*	7.05	6.91	9.43	96
10[d]	Phosphate, .10 *M*	7.05	6.96	9.43	61
11	Veronal, 0.05 *M*	6.96	7.02	9.43	19
12[e]	Phosphate, 0.10 *M*	7.05	6.99	13.58	116

[a] Ionic strength, 1.44. [b] Ionic strength, 1.42 by addition of KCl. [c] $FeSO_4$, 1×10^{-5} *M*. [d] $CuSO_4$, 1×10^{-5} *M*. [e] Temperature, 25.0°.

In expt. 1–3, the concentrations of dialuric acid and *o*-iodosobenzoic acid were varied independently over a limited range owing to the low solubility of *o*-iodosobenzoic acid. The agreement of the values for k'' indicates that the reaction is first order with respect to each of the reactants. The value of k'' increased from 48 to 104 as the concentration of phosphate buffer was increased from 0.10 to 0.60 *M* (expt. 4 and 5). This increase in rate is not associated primarily with an increase in ionic strength since addition of potassium chloride to produce an equivalent ionic strength had no significant effect on the rate (expt. 6). In phosphate buffer, the rates were similar at *p*H 6 and 7 but decreased at *p*H 7.7 (expt. 7 and 8). In veronal buffer at *p*H 7 (expt. 11) the rate was much less than in phosphate buffer. No experiments were conducted in nonbuffered solutions. Iron was a more effective catalyst than copper (expt. 9 and 10). In 0.10 *M* phosphate buffer at *p*H 7.05, k' increased from 48 to 116 liters mole^{-1} min.$^{-1}$ as the temperature was increased from 15 to 25° (expt. 12).

Analyses of solutions after reactions were completed indicated that, under all conditions studied, one mole of *o*-iodosobenzoic acid had been reduced for each mole of dialuric acid oxidized. There was no evidence of formation of any products from alloxan capable of reducing either iodine or *o*-iodosobenzoic acid.

These preliminary results suggest that the oxidation of dialuric acid by *o*-iodosobenzoic acid is similar in some respects to the oxidation of ascorbic acid. The principal reactions are second order; the rate is proportional to the concentration of buffer but is independent of the ionic strength; both reactions exhibit catalysis by copper and iron.

Points of difference also may be noted. Under the same conditions, dialuric acid is oxidized at a rate fifteen times that of ascorbic acid. No reducing substance is formed from oxidized dialuric acid. The catalytic effect of copper is much greater than

(1) One of several investigations supported in part by a research grant from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

(2) Predoctorate Research Fellow of the National Institutes of Health, 1949–1950.

(3) E. S. Hill, *J. Biol. Chem.*, **85**, 713 (1930); **92**, 471 (1931).

(4) G. M. Richardson, *Biochem. J.*, **26**, 1959 (1932).

(5) J. W. Patterson, A. Lazarow and S. Levy, *J. Biol. Chem.*, **177**, 187 (1949).

(6) E. G. Ball, *ibid.*, **118**, 219 (1937).

(7) W. T. Caraway and L. Hellerman, THIS JOURNAL, **75**, 5334 (1953).

(8) H. Biltz and P. Damm, *Ber.*, **46**, 3662 (1913).

(9) C. C. Porter and L. Hellerman, THIS JOURNAL, **66**, 1652 (1944).

iron on the rate of oxidation of ascorbic acid but with dialuric acid the effect is reversed. These observations are consistent with the suggestion that copper acts more effectively than iron as a catalyst for the oxidation of the enediol configuration (ascorbic acid); conversely, iron may act more effectively as a catalyst for the oxidation of the α-keto-hydroxy configuration (dialuric acid).

Levy[10] has concluded that ascorbic acid exists in its ketonic form in acid solution and Huelin and Stephens[11] have observed that the relative catalytic effects of copper and iron on the oxidation of ascorbic acid are reversed as the *p*H of the solution is decreased from 3.0 to 0.4. The specificity of copper as a catalyst for the enediol group has been discussed by Dodds.[12]

(10) L. F. Levy, *Nature*, **152**, 693 (1943).
(11) F. E. Huelin and I. M. Stephens, *ibid.*, **158**, 703 (1946).
(12) M. L. Dodds, *Arch. Biochem.*, **18**, 51 (1948).

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
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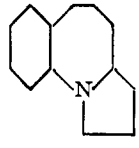
A New Synthesis of 4a(H)-Dodecahydrobenzo(c)-quinolizine

BY L. M. JAMPOLSKY AND W. E. SOLODAR[1]

RECEIVED JUNE 26, 1953

Of the four racemic modifications possible in 4a-(H)-dodecahydrobenzo(c)quinolizine, Clemo and co-workers[2] have reported the preparation of two, "A" and "B." Subsequent work by Leonard and Wildman[3] has shown that the precursor to Clemo's compound "A," 1-keto-6,7-hexahydrobenzoquinolizidine, had undergone disproportionation of two rings during a Clemmensen reduction to give a structure shown by I.



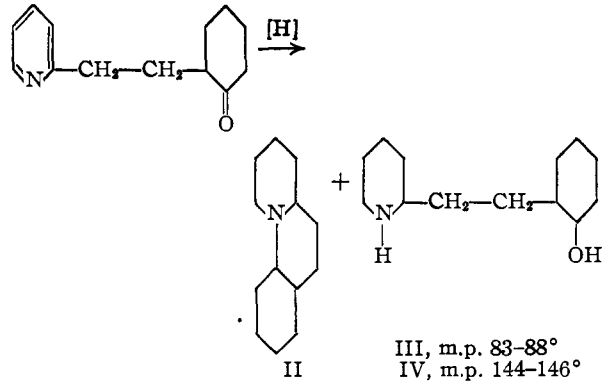
I

Our compound has physical constants differing from those of Clemo's compound "B" and is, therefore, presumed to be a heretofore unknown racemate.

Results and Discussion

Reduction of 2-(2-ethylpyridyl)-cyclohexanone in aqueous HCl over PtO_2 gave three products, of which one IV, was formed to the extent of about one per cent. under all temperatures employed. The ratio of II and III formed was found to be a function of the temperature, with II predominating at higher temperatures at the expense of III.

The uncyclized alcohol III could not be dehydrated to give the cyclic 4a(H)-dodecahydrobenzo-(c)quinolizine (II) when subjected to the same conditions prevalent at the formation of II. It is



II III, m.p. 83–88°
IV, m.p. 144–146°

possible that this racemic form of the alcohol III is not an intermediate in the formation of II.

Reaction of II with methyl iodide gave two methiodides which could not be completely separated by fractional crystallization. The partially separated fractions had melting points of 185–186° and 242–249° and both analyzed for the quaternary ammonium compound expected from 4a(H)-dodecahydrobenzo(c)quinolizine and one equivalent of methyl iodide. The fact that II forms a single, constant-melting picrate is taken as proof that it is a single racemic modification; the two methiodides represent a pair of diastereoisomers which owe their existence to the asymmetric nitrogen atom.

Experimental Part

2-(2-Ethylpyridyl)-cyclohexanone was prepared by the procedure of Levine and Wilt[4] in 38% yield, b.p. 137–143° (1 mm.), $n^{20}D$ 1.5311.

2-(2-Ethylpiperidyl)-cyclohexanol (IV).—Fifty-one grams (0.25 mole) of 2-(2-ethylpyridyl)-cyclohexanone was reduced in aqueous HCl at 55–60° over one gram of PtO_2 at 1500 p.s.i. of hydrogen. The theoretical uptake of hydrogen (4 equivalents) required 5 hours. The solution was decanted from the catalyst, made basic with aqueous alkali, and extracted with benzene. The benzene was evaporated, ether was added, and the mixture kept at 0° for 2 days. A precipitate of white crystals was filtered off (1.5 g., m.p. 107–112°) and recrystallized twice from ether, giving 0.52 g. of fine white needles, m.p. 144–146°.

Anal. Calcd. for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63. Found: C, 73.70; H, 11.54; N, 6.82.

The *p*-nitrobenzoate (N-*p*-nitrobenzoyl), prepared in the usual manner, crystallized from ethanol in yellow prisms, m.p. 144–146°. An intimate mixture of it and the starting alcohol IV melted at 132–140°.

Anal. Calcd. for $C_{27}H_{31}N_3O_7$: C, 63.64; H, 6.13; N, 8.25. Found: C, 63.44; H, 6.03; N, 8.31.

4a(H)-Dodecahydrobenzo(c)quinolizine (II).—The ether filtrate from the filtration of IV above was evaporated and distilled, giving 38 g. (79%) of colorless liquid, b.p. 113–115° (1.5 mm.) and a small amount of higher boiling material, subsequently identified as compound III. Redistillation gave 30 g. of colorless liquid, b.p. 71–74° (0.5 mm.), $n^{24}D$ 1.5080.

Anal. Calcd. for $C_{13}H_{23}N$: C, 80.76; H, 11.99; N, 7.25. Found: C, 80.52; H, 12.25; N, 7.38.

The picrate, prepared in ether, crystallized from an ethanol–water mixture in fine yellow crystals, m.p. 178–180°.

Anal. Calcd. for $C_{19}H_{26}N_4O_7$: C, 54.02; H, 6.20; N, 13.26. Found: C, 54.45; H, 6.03; N, 13.67.

The methiodides were prepared by refluxing the base with excess methyl iodide in ethyl acetate for 0.5 hour. Repeated fractional crystallization from methanol–acetone mixtures gave two fractions which could not be purified to constant melting points.

Anal. Calcd. for $C_{14}H_{26}NI$: C, 50.15; H, 7.81; N, 4.18. Found: fraction a, m.p. 242–249°: C, 49.73; H, 7.58; N,

(1) Abstracted from a thesis submitted by Warren E. Solodar in partial fulfillment of the Master of Science degree, Stevens Institute of Technology.
(2) G. R. Clemo, J. G. Cook and R. Raper, *J. Chem. Soc.*, 1318 (1938).
(3) N. J. Leonard and W. C. Wildman, THIS JOURNAL, **71**, 3089 (1949).
(4) R. Levine and M. H. Wilt, *ibid.*, **74**, 342 (1952).

4.74. Fraction b, m.p. 185–186°: C, 50.48; H, 7.94; N, 3.93.

2-(2-Ethylpiperidyl)-cyclohexanol (III).—The reduction of 2-(2-ethylpyridyl)-cyclohexanone was carried out as in the preparation of IV above, but at room temperature (50 hours). The reduction mixture was decanted from the catalyst, made basic with aqueous alkali, and extracted with chloroform. The chloroform was dried, evaporated, and the residue distilled, giving 57% of a thick, colorless liquid, b.p. 145–148° (2 mm.). This was crystallized from Skellysolve B, the crystals taken up in boiling ether, and set at 0° for 2 days. A small amount of IV was filtered off, and the ether filtrate evaporated. The residue was recrystallized from Skellysolve B, giving a 40% yield of white solid, m.p. 83–88°.

Anal. Calcd. for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63. Found: C, 73.83; H, 12.14; N, 6.91.

The *p*-nitrobenzoate (N-*p*-nitrobenzoyl), prepared in the usual manner, crystallized from ethanol in pale yellow prisms, m.p. 158–160°.

Anal. Calcd. for $C_{27}H_{31}N_3O_7$: C, 63.64; H, 6.13; N, 8.25. Found: C, 63.96; H, 6.32; N, 8.30.

Acknowledgments.—The authors wish to thank the Scientific Management of Hoffmann–La Roche, Inc., Nutley, N. J., for the use of their facilities during this study, Dr. Al Steyermark of the Microchemical Department at Roche, for the microanalyses reported in this paper, and Professor N. J. Leonard, for his comments.

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Carotenoids in *Phycomyces*

BY G. MACKINNEY, C. O. CHICHESTER AND PATRICIA S. WONG

RECEIVED JULY 29, 1953

A marked effect on β-carotene production in *Phycomyces* may be shown by addition of β-ionone to cultures.[1] Recent experiments with purified α-ionone indicate that this isomer also enhances β-carotene production. By contrast, we reported that citral and pseudoionone had a slight effect on lycopene and that methylheptenone had no effect. This requires revision. Control and ionone-treated cultures, harvested 60 to 100 hours after inoculation on a glucose–yeast autolysate medium give extracts whose carotenoid spectrum is essentially that of β-carotene. Methylheptenone-treated cultures show weak pigmentation, when compared with controls, but the carotenoid spectrum is radically different. After chromatography of the crude petroleum ether extracts on MgO–SiO_2 columns and spectrophotometric estimation of the components, the following effects of methylheptenone may be shown: production of β-carotene is halved; the phytofluene content is increased 6- to 15-fold; ζ-carotene which is not demonstrable in control cultures grown under our conditions is found in significant amount, neurosporene is detected, and the lycopene content is also increased.

Culture conditions and procedures have already been described.[1] Methylheptenone, 20 μl., (Fritzsche Bros.) was applied to each culture at times varying from 6 to 27 hours after inoculation. To minimize adverse growth effects, the methylheptenone should be applied 12 to 24 hours after inoculation. If applied immediately germination of the spores is unduly delayed.

The following results are typical of several independent runs. Figures for the heptenone-treated cultures precede values for the controls, in μg. carotenoid per g. of dry mycelium, each value representing five plates: (1) cultures treated 24 hr. after inoculation, harvested in 60 hr., *vs.* controls; dry weights, 0.400, 0.573 g.; phytofluene 46, 3.7; β-carotene 118, 253; ζ-carotene 60, not detected. (2) Cultures treated 17.5 hr. after inoculation, harvested in 112 hr., *vs.* controls; dry weights 0.555, 0.582 g.; phytofluene 88.5, 5.8; β-carotene 195, 392; ζ-carotene 43.3, trace.

In no case was neurosporene detected in the controls, though present in the treated cultures. The lycopene zone was definitely more prominent in the treated cultures, but at best was still a minor component, not exceeding 5 to 10 μg./g.

The striking effects of methylheptenone are therefore three: reduction of the β-carotene, a marked increase in phytofluene and the appearance of ζ-carotene as a major constituent. Re-examination of the absorption curves from extracts of citral-treated cultures makes it apparent that they are intermediate between controls and methylheptenone-treated cultures. Loss in β-carotene is not so marked, nor is production of phytofluene so enhanced, under comparable conditions.[2] These qualitative interpretations are supported also by the observation that the fluorescence of the citral-culture extracts is intermediate in intensity. We cannot as yet comment on possible additional effects ascribable to pseudoionone.

We have hitherto been puzzled by failure to detect numerous minor components observed by Goodwin.[3] He obtained phytofluene yields of 70 to 109 μg. per 250 ml. culture solution (Table 9, ref. 2) after 9 days growth on a 3% glucose–0.2% asparagine medium, in the presence of diphenylamine. Our 5 plates, collectively containing 100 ml. medium, produced 49 μg. of phytofluene (run 2), from 0.555 g. of dry matter, in a total of 112 hr., when treated, compared with 3.4 μg. for the controls. It is clear that great variation may be anticipated in the proportions of the different carotenoids comprising the mixture.

(1) G. Mackinney, T. Nakayama, C. O. Chichester and C. D. Buss, THIS JOURNAL, **74**, 3456 (1952); **75**, 236 (1953).

(2) The culture response to citral is affected more by the mode of application than is the response to ionone.

(3) T. W. Goodwin, *Biochem. J.*, **50**, 550 (1952).

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The Reaction of Diphosphopyridine Nucleotide with Sodium Borohydride—A Correction and Extension

BY MARTIN B. MATHEWS AND ERIC E. CONN

RECEIVED JUNE 25, 1953

It was previously observed[1] that diphosphopyridine nucleotide (DPN) was quantitatively reduced by sodium borohydride, in agreement with values obtained by reduction with sodium

(1) M. B. Mathews, *J. Biol. Chem.*, **176**, 229 (1948).

hydrosulfite and with formic dehydrogenase[2] and that the reduced products were only 50% enzymatically oxidizable. Subsequent results obtained with other preparations of the same reactants disagreed in part with the original findings. The reaction was thoroughly reinvestigated and the new data are reported in the present communication.

Experimental

Materials and Methods.—Sodium borohydride, potassium borohydride and sodium trimethoxyborohydride ($NaBH(OCH_3)_3$) were purchased from Metal Hydrides, Inc. DPN was obtained from various commercial sources. The enzymes and other chemicals were standard preparations suitably identified or purchased as C.P. reagents.

Most of the methods used have been described previously.[1] Chromatographic purification of the nucleotides was carried out using Dowex-1 resin.[3]

Assay of DPN with Sodium Borohydride.—The observation that a sample of DPN assayed with sodium borohydride gave considerably less than the value expected from enzymatic assay prompted the investigation of this effect with other preparations of DPN, as well as with some preparations of triphosphopyridine nucleotide (TPN). The results obtained are shown in Table I. The average obtained by assay of the first nine samples of DPN with sodium borohydride is 66% of the value obtained by enzyme assay. A probable error of 3% indicates the degree of consistency obtained with samples ranging in purity from 30 to 90%.

TABLE I

Sample	Purity by assay by enzyme,[a] %	Assay[b] by sodium boro-hydride, % of enzyme value	Assay[b] by sodium hydro-sulfite, % of enzyme value	Enzymatic reoxidation[c] of samples reduced with sodium borohydride % of optical density at 340 mμ
DPN, NB-1[d]	61	68		
DPN, SG 11-38[e]	62	60		
DPN, S 4903[f]	42	72		
DPN, SA[f]	63	60		
DPN, SG 51-30[e]	65	71		
DPN, SB[f]	30	68		
DPN, SC[f]	40	66		
DPN, CPA[g]	90	63	100	49
DPN, CPB[g]	80	66	100	49
DPN, SGX[e]	40	100	138	36
DPN, SGX 30[h]	90	69		50
DPN, SGX 20[h]	0	+	+	
TPN, PA[i]	90	71	100	
TPN, AM[j]	40	65		

[a] Crystalline alcohol dehydrogenase prepared according to Racker[4] used for DPN samples; Zwischenferment was used for TPN samples.[5] [b] Previously described.[1] [c] With lactic dehydrogenase. [d] Nutritional biochemicals product. [e] Sigma chemical product. [f] Schwartz Laboratories product. [g] Chromatographically purified preparation. [h] Chromatographic fractions of DPN, SGX. [i] Chromatographically purified. [j] Armour and Co. product from bovine liver.

The highly purified samples of DPN, CPA and CPB (see Table I), obtained by chromatography of commercial DPN were assayed with sodium hydrosulfite, and yielded values equal to those obtained with enzyme. The reduction products obtained by the action of sodium borohydride on these samples were reoxidized enzymatically on the average only 49% (a result in agreement with previous findings[1]).

Of all samples examined only sample SGX assayed 100% of the enzyme value with sodium borohydride. However, when sample SGX was assayed with sodium hydrosulfite, a value of 138% of the enzyme value was found. This result suggested contamination with compounds capable of reduction with absorption in the 340 mμ region (*e.g.*, nicotinamide nucleoside and nicotinamide nucleotide). The contaminants were isolated, in part, by chromatography of sample SGX, and designated SGX20. SGX20 was reduced by both sodium borohydride and sodium hydrosulfite with resulting absorption at 340 mμ, but was not reduced by enzyme. Sample SGX30, also obtained by chromatography from SGX, had a DPN purity of 90% and reacted with sodium borohydride as did the other preparations of DPN listed. It thus appeared that the unusual results obtained with sample SGX were due to reducible impurities. Consistent with this view were the facts that the assay by sodium borohydride was 72% of the assay by sodium hydrosulfite and that after correction for reducible impurities, the extent of enzymatic reoxidation was 50% of the optical density at 340 mμ due to reduction of DPN by sodium borohydride. The presence of *d*-ribose, adenosine-5-monophosphate, or nicotinamide at concentrations of 2×10^{-4} *M* had no measurable influence upon the formation of the band at 340 mμ. In the reaction with DPN, potassium borohydride or sodium trimethoxyborohydride were quantitatively equivalent to sodium borohydride.

Reoxidation of Reduction Products.—It was noted above that the products of reduction of DPN by sodium borohydride were reoxidized enzymatically only to the extent of 50%. With the reduction products obtained by the action of sodium borohydride on highly purified DPN, it was noted that addition of iodine caused essentially complete removal of absorption at 340 mμ. Also, as was previously reported,[1] the amount of iodine absorbed in titration of the products of reaction between DPN and sodium borohydride correspond, within experimental error, to that required for quantitative oxidation of an amount of reduced DPN which could be calculated from the optical density at 340 mμ.

Although the oxidation by iodine is apparently a stoichiometric reaction, oxidized DPN is not a product since subsequent addition of sodium borohydride fails to increase the optical density at 340 mμ. If, however, potassium ferricyanide in slight excess is used in place of iodine and sodium borohydride subsequently added, the increase in optical density at 340 mμ is found to be, on the average, 66% of that present before oxidation by ferricyanide.[6] It appears probable therefore that the original reduction products formed by action of sodium borohydride on DPN are restored by ferricyanide to their original state, at least so far as concerns that portion of the DPN molecule responsible for the band at 340 mμ.

Discussion

For a variety of DPN samples it has been found that reaction with sodium borohydride produces only about $^2/_3$ of the absorption at 340 mμ corresponding to complete reduction of the DPN by enzyme. This result disagrees with a previous finding of complete reduction by sodium borohydride.[1] No ready explanation of the discrepancy appears, although it is conceivable that some yet unknown contaminant in the first crude DPN preparations was responsible for the original results.

It has been confirmed that the spectrophotometrically measurable products of the reaction of DPN and sodium borohydride can be reoxidized enzymatically to the extent of close to 50%. This fact, taken in conjunction with the observation of close to $^2/_3$ apparent reduction of DPN by sodium borohydride, suggests the possibility that this reaction may involve reduction of the nicotinamide ring of DPN at the 2-, 4- and 6-positions.

(2) M. B. Mathews and B. Vennesland, *J. Biol. Chem.*, **186**, 667 (1950).

(3) Chromatography was accomplished according to an unpublished procedure of A. Kornberg and B. L. Horecker.

(4) E. Racker, *J. Biol. Chem.*, **184**, 313 (1950).

(5) E. Kornberg, *ibid.*, **182**, 805 (1950).

(6) Ferricyanide oxidizes reduced DPN and is itself reduced to ferrocyanide. Subsequent addition of sodium borohydride reduces any remaining excess of ferricyanide to ferrocyanide. Thus, the absorption of this solution at a given wave length less the absorption of a solution containing the same concentration of ferrocyanide yields the absorption due to the products of reaction of sodium borohydride with oxidized DPN.

The products of reduction at the 2- and the 6-positions may absorb less strongly in the 340 mμ region than does the product of reduction at the 4-position. Only the latter product would be expected to show enzymatic activity.[7]

(7) M. E. Pullman, *Federation Proc.*, **12**, 255 (1953).

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Preparation of Highly Compressed Samples for Adsorption Studies[1]

BY S. V. R. MASTRANGELO,[2] R. J. TYKODI AND J. G. ASTON

RECEIVED JULY 13, 1953

While determining the B.E.T.[3] surface area of a highly compressed sample of TiO_2, it was discovered that equilibrium time for coverages between $0.5V_m$ and saturation was very much greater than usual, 8–10 hours as compared to 1–2 hours in less compact samples. When the B.E.T. surface area plot was made a non-linear curve was obtained as shown by the dotted curve through the circles with a left bar in Fig. 1. The surface area determined

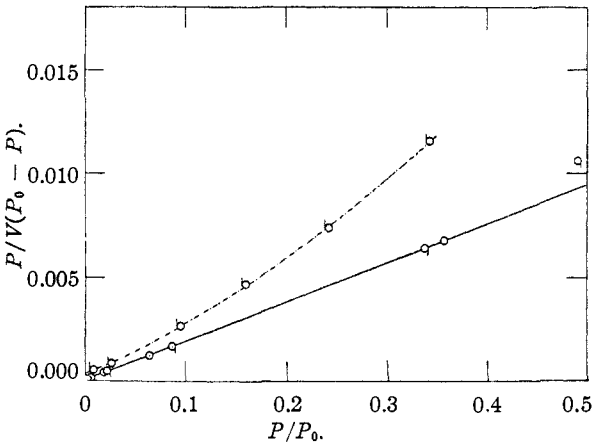

Fig. 1.—B.E.T. plot for nitrogen on TiO_2: o, points for loosely packed test sample; b, points for highly compressed samples; ϙ, points for highly compressed samples after vibration.

by means of a straight line through the low pressure points is 134.5 m.²/g. However, a portion of the same sample had already been run in a small testing chamber, and this sample gave an excellent linear B.E.T. plot as shown by the plain circles in Fig. 1. This test sample was loosely packed relative to the first mentioned sample, and equilibrium was reached in 1–2 hours. The surface area obtained for the test sample is 230.0 m.²/g.

From these results it was concluded that some TiO_2 was not available to the gas in the first sample and that the slow equilibration was caused by gas leaking through solid cakes resulting from high compression. The packed calorimeter was vibrated by means of an ordinary electric vibrating machine. The calorimeter was clamped above the vibrator with a rubber stopper placed between to absorb most of the shock. It was not necessary to use the vibrating mechanism; the vibration of the body of the machine was apparently sufficient to loosen the packing. In order to prevent the machine from getting too hot, the sample was vibrated periodically for about 8 hours.

After the treatment described above the B.E.T. surface area determination was made again with nitrogen. This time, equilibrium was attained in less than 1 hour and in 0.5 hour for some points, all above coverages of $0.5V_m$. The points below $0.5V_m$ were, as usual, slow. The B.E.T. plot obtained for this run is not only linear, but falls on the same plot as for the test sample. These data are shown by the circles with a right bar in Fig. 1.

The authors wish to recommend this technique for preparing highly compressed samples used in adsorption studies at very low temperatures where the dead space corrections are large.

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(1) This Research was carried out under Contract N6ONR, Task Order X, of the Office of Naval Research.

(2) Postdoctoral Fellow, 1950–1951. Barrett Division of Allied Chemical and Dye Corporation, Genolden, Pa.

(3) S. Brunauer, P. H. Emmett and E. Teller, THIS JOURNAL, **60**, 309 (1938).

The Reactions of *p*-Arsanilic Acid and 4-Hydroxyphenylarsonic Acid with Brominated Fatty Acids[1,2]

BY ROBERT L. MCGEACHIN AND MARVIN GREENWALD

RECEIVED JULY 13, 1953

In the course of a study of the preparation of homologs and analogs of Tryparsamide, the condensations of a number of brominated fatty acids with *p*-arsanilic acid and 4-hydroxyphenylarsonic acid were attempted. Standard conditions known to give satisfactory results in the preparation of N-4-arsonophenylglycine[3] and several variations from these conditions (including the use of sodium iodide as a catalyst) all failed to bring about the condensation of α-bromobutyric, α-bromoisobutyric, α-bromovaleric and α-bromoisovaleric acids with *p*-arsanilic acid. However, condensations of *p*-arsanilic acid with α-bromopropionic and β-bromopropionic acids were successful under the standard conditions[3] giving N-(4-arsonophenyl)-α-aminopropionic acid (I) and N-(4-arsonophenyl)-β-aminopropionic acid (II). These products did not recrystallize from hot water as readily as the lower homolog, N-4-arsonophenylglycine, making their isolations somewhat more difficult and resulting in lower yields.

Preparation of α-(4-arsonophenoxy)-propionic acid (III) and β-(4-arsonophenoxy)-propionic acid (IV) were carried out following conditions outlined[4] for 4-arsonophenoxyacetic acid but the yield for the β-isomer was very low. Here again recrystallization and purification were more difficult than with the lower homolog.

The ethyl esters of I, II and III were prepared using the method given by Jacobs and Heidelberger[5] for the ethyl and methyl esters of N-4-arsonophenylglycine. The amide of I (a higher

(1) This work was aided by a grant to the University of Louisville from the Kentucky State Medical Research Commission.

(2) From the M.S. Thesis of Marvin Greenwald, 1952.

(3) German Patent 204,644.

(4) H. Gilman and A. H. Blatt, "Organic Syntheses," Coll. Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1947, p. 75.

(5) W. A. Jacobs and M. Heidelberger, THIS JOURNAL, **41**, 1950 (1919).

homolog of Tryparsamide) was prepared from its ethyl ester.[5]

Experimental

Preparation of I and II.—These compounds were prepared by the method given for N-4-arsonophenylglycine.[3] One variation in purification that was found necessary to remove final traces of unreacted bromopropionic acids was washing the products several times with ether; yields I (65%), II (41%).

Anal. Calcd. for $C_9H_{12}AsNO_5$: As, 25.99. Found: (I) As, 25.50; (II) As, 25.91.

Preparation of III and IV.—These compounds were prepared by the method given for 4-arsonophenoxyacetic acid[4]; yields III (37%), IV (5%).

Anal. Calcd. for $C_9H_{11}AsO_6$: As, 25.86. Found: (III) As, 25.90; (IV) As, 25.80.

Preparation of Ethyl Esters of I, II and III.—Prepared by the method used for the esterification of N-4-arsonophenylglycine[5]; yields I (45%), II (60%), III (50%).

Anal. Calcd. for $C_{11}H_{16}AsNO_5$: As, 23.69. Found: (I) As, 23.30; (II) As, 23.60. Calcd. for $C_{11}H_{15}AsO_6$: As, 23.63. Found: (III) As, 23.50.

Preparation of the Amide of I.—Prepared by ammonolysis of the ethyl ester using the method given for the amide of N-4-arsonophenylglycine[5]; yield 52%.

Anal. Calcd. for $C_9H_{13}AsN_2O_4$: As, 26.04; N, 9.72. Found: As, 26.0; N, 9.15.

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Preparation of 3-(6-Methoxynaphthyl-2)-2-cyclohexen-1-one and Related Compounds

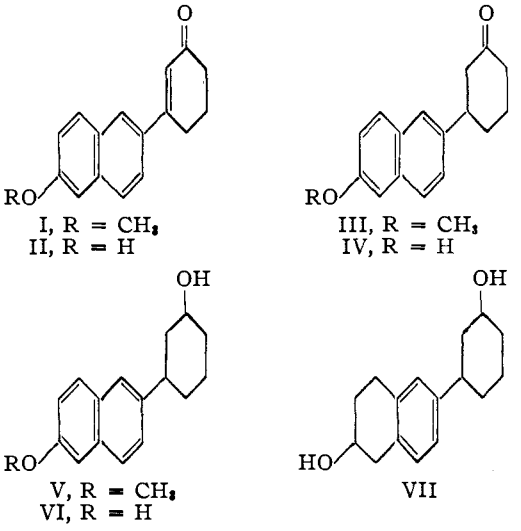
BY FREDERICK C. NOVELLO AND MARCIA E. CHRISTY

RECEIVED JULY 14, 1953

The condensation reaction between aryl β-dialkylamino ketones and acetoacetic ester and related compounds containing an active methylene group offers a convenient method for preparing 3-aryl-2-cyclohexen-1-one derivatives.[1] This procedure was employed to prepare 3-(6-methoxynaphthyl-2)-2-cyclohexen-1-one (I) and the related compounds, II–VII, which were of interest in view of the estrogenic activity of 1-ethyl-2-(4-hydroxyphenyl)-6-hydroxy-1,2,3,4-tetrahydronaphthalene and 1-methyl-2-(4-hydroxyphenyl)-6-methoxy-3,4-dihydronaphthalene.[2]

Synthesis of I was accomplished by condensation between 2-(β-dimethylaminopropionyl)-6-methoxynaphthalene hydrochloride and acetoacetic ester in the presence of alcoholic potassium hydroxide. Demethylation to II was effected by brief treatment with aluminum chloride in boiling xylene.[3] Conversion of I and II to the cyclohexanone and cyclohexanol derivatives, III–VII, was carried out by catalytic hydrogenations. Separation of stereoisomers was not attempted although V and VI were isolated as apparent homogeneous crystalline entities; VII was obtained as a glass. In the course of these hydrogenation studies it was found that vigorous stirring was superior to conventional methods of agitation and in the reduction of 3-(6-methoxynaphthyl-2)-2-cyclohexen-1-one to the cyclohexanone III, a sixfold increase in the reaction rate was realized when vigorous stirring was employed.

Of this series, 3-(6-hydroxynaphthyl-2)-2-cyclohexen-1-one (II) was the most active and gave a positive estrogenic response at a dosage level of 500 μg. in the vaginal cornification assay procedure.[4]



Experimental[5]

2-(β-Dimethylaminopropionyl)-6-methoxynaphthalene Hydrochloride.—A mixture of 37.5 g. (0.19 mole) of 2-acetyl-6-methoxynaphthalene,[6] 16.3 g. of dimethylamine hydrochloride, 8.8 g. of paraformaldehyde in 100 ml. of ethanol and 5 drops of concentrated hydrochloric acid was refluxed for 48 hours and concentrated to dryness *in vacuo*. The residual solid was suspended in ether, collected on a funnel and crystallized from ethanol; yield 39.1 g. (70%) of yellow needles, m.p. 180–184°. After further recrystallizations, pale yellow needles were obtained, m.p. 184–185.5°.

Anal. Calcd. for $C_{16}H_{20}O_2NCl$: C, 65.41; H, 6.86; N, 4.77. Found: C, 65.21; H, 7.04; N, 4.73.

The free base was obtained as colorless plates, m.p. 77–79°, from ether–petroleum ether.

Anal. Calcd. for $C_{16}H_{19}O_2N$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.47; H, 7.41; N, 5.41.

3-(6-Methoxynaphthyl-2)-2-cyclohexen-1-one (I).—A solution of 11.5 g. of potassium hydroxide in 150 ml. of isopropyl alcohol was added to a well-stirred mixture of 30 g. (0.1 mole) of 2-(β-dimethylaminopropionyl)-6-methoxynaphthalene hydrochloride and 13.1 g. of methyl acetoacetate in 150 ml. of isopropyl alcohol. The mixture was heated to reflux and stirred until the mixture became too viscous to allow further stirring (4–5 hours). After heating for a total of 72 hours, the mixture was poured into 3 l. of water and allowed to cool. The product was collected on a funnel, sucked dry, and then digested on the steam-bath with 200 ml. of benzene. The filtered benzene solution was washed with 10% hydrochloric acid, water and dried over sodium sulfate. After removal of solvent, the residue was distilled at 0.5 mm. and the product crystallized from acetone; yield 18.0 g. (70%) of yellow needles, m.p. 138–141°.

An analytical sample was obtained by repeated recrystallizations from acetone as pale yellow needles, m.p. 142.3–143.3°.

Anal. Calcd. for $C_{17}H_{16}O_2$: C, 80.92; H, 6.39; OCH_3, 12.30. Found: C, 81.00; H, 6.45; OCH_3, 12.24.

The oxime derivative crystallized from alcohol as pale yellow plates, m.p. 171.2–172.5°.

Anal. Calcd. for $C_{17}H_{17}O_2N$: C, 76.38; H, 6.41. Found: C, 76.40; H, 6.45.

(1) F. C. Novello, M. E. Christy and J. M. Sprague, THIS JOURNAL, **75**, 1330 (1953).

(2) W. Salzer, *Z. physiol. Chem.*, **274**, 39 (1942).

(3) K. Fries and K. Schimmelschmidt, *Ber.*, **58**, 2835 (1925).

(4) The authors are indebted to Dr. Roland K. Meyer and Dr. Elva S. Meyer, University of Wisconsin, for the estrogenic assays.

(5) The authors are indebted to Mr. Kermit B. Streeter and his associates, Miss J. L. Pyett and Mr. J. P. Laux for the analytical data.

(6) R. Robinson and H. N. Rydon, *J. Chem. Soc.*, 1399 (1939).

3-(6-Hydroxynaphthyl-2)-2-cyclohexen-1-one (II).—A solution of 2 g. of I in 20 ml. of xylene was refluxed with 4.0 g. of aluminum chloride for 5 minutes and poured onto ice and dilute hydrochloric acid. The solid was collected on the filter and taken up in acetone–benzene. The organic extract was washed with water and then extracted with 5% sodium hydroxide. The alkaline solution was acidified and the product collected on the filter and purified through its sparingly water-soluble sodium salt; yield 1.2 g. (63.5%) tan plates, m.p. 210–212°. Repeated crystallizations from alcohol gave pale yellow plates, m.p. 215.1–217.4°.

Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92. Found: C, 80.70; H, 5.95.

An oxime was obtained as pale yellow needles from alcohol–hexane, m.p. 234.7–238.4°.

Anal. Calcd. for $C_{16}H_{15}O_2N$: C, 75.87; H, 5.97. Found: C, 75.75; H, 6.12.

3-(6-Methoxynaphthyl-2)-cyclohexanone (III).—A solution of 30 g. (0.12 mole) of 3-(6-methoxynaphthyl-2)-2-cyclohexen-1-one in 160 ml. of purified dioxane[7] was hydrogenated at atmospheric pressure in the presence of 7.0 g. of 5% palladium-on-charcoal catalyst[8] with vigorous stirring[9] at 55°. Reduction ceased upon saturation of the ethylenic double bond. After removal of catalyst and solvent, the product was crystallized from ethanol; yield 18.4 g. (60%) of colorless needles, m.p. 116–119°. Further recrystallizations from ethanol afforded a sample melting at 124–125°.

Anal. Calcd. for $C_{17}H_{18}O_2$: C, 80.28; H, 7.13; OCH_3, 12.20. Found: C, 80.29; H, 7.05; OCH_3, 12.28.

An oxime was obtained as colorless needles from alcohol, m.p. 152.5–154°.

Anal. Calcd. for $C_{17}H_{19}O_2N$: C, 75.80; H, 7.11. Found: C, 75.53; H, 7.12.

3-(6-Methoxynaphthyl-2)-cyclohexanol (V).—A solution of 1 g. of 3-(6-methoxynaphthyl-2)-2-cyclohexen-1-one in 50 ml. of ethanol was hydrogenated in the presence of 150 mg. of platinum catalyst until hydrogen absorption ceased (24 minutes). The product crystallized from alcohol–hexane as colorless needles; yield 0.55 g., m.p. 122–124°. Further recrystallizations from alcohol–water gave colorless plates, m.p. 127.6–129.2°.

Anal. Calcd. for $C_{17}H_{20}O_2$: C, 79.65; H, 7.87. Found: C, 79.72; H, 8.02.

3-(6-Hydroxynaphthyl-2)-cyclohexanone (IV).—A solution of 0.5 g. of 3-(6-hydroxynaphthyl-2)-2-cyclohexen-1-one in 35 ml. of glacial acetic acid was hydrogenated in the presence of 100 mg. of palladium black catalyst until reduction ceased (50 minutes). After removal of catalyst and solvent, the residue was distilled at 0.3 mm. and the distillate crystallized from benzene–hexane; yield 200 mg. of pale yellow needles, m.p. 142.6–145.3°.

Anal. Calcd. for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.89; H, 6.81.

3-(6-Hydroxynaphthyl-2)-cyclohexanol (VI).—A solution of 1 g. of 3-(6-hydroxynaphthyl-2)-2-cyclohexen-1-one in 100 ml. of ethanol was hydrogenated in the presence of 1 g. of WF-7 nickel catalyst[10] at atmospheric pressure until reduction ceased. After removal of catalyst and solvent, the residue was taken up in ether, washed with water and dried. The product was obtained from acetone–petroleum ether as pale yellow plates; yield 250 mg., m.p. 183–194°. Further recrystallizations from alcohol–water afforded colorless needles, m.p. 217.1–218.4°.

Anal. Calcd. for $C_{16}H_{18}O_2$: C, 79.31; H, 7.49. Found: C, 79.45; H, 7.56.

2-Hydroxy-6-(3-hydroxycyclohexyl)-tetralin (VII).—A solution of 2 g. of 3-(6-hydroxynaphthyl-2)-2-cyclohexen-1-one in 50 ml. of alcohol containing 20 mg. of sodium hydroxide was hydrogenated in a bomb in the presence of 600 mg. of Raney nickel catalyst at 130° for 12 hours. The colorless alcoholic solution was filtered and concentrated on the steam-bath. The concentrate was taken up in ether and washed with dilute sodium hydroxide, water and dried over sodium sulfate. The product was obtained by distillation at 0.5 mm. as a colorless glass; yield 1.6 g.

Anal. Calcd. for $C_{16}H_{22}O_2$: C, 78.01; H, 9.00. Found: C, 77.95; H, 9.14.

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MERCK AND CO., INC.
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(7) L. F. Fieser, "Experiments in Organic Chemistry," 2nd Edition, D. C. Heath and Company, Boston, Mass., 1941, p. 368.

(8) Baker and Co., Inc., Catalysts, Newark, N. J.

(9) A 3-necked round bottom flask served as the reaction vessel and stirring was accomplished with a stainless steel stirrer made gas tight by means of a stainless steel stuffing box and driven by a ½ h.p. drill press at 1750 r.p.m.

(10) H. R. Billica and H. Adkins, *Org. Syntheses*, **29**, 24 (1949).

Studies in Low Concentration Chemistry. V. The Spontaneous Deposition of Silver-111 on Various Metals

BY GEORGE K. SCHWEITZER AND DALE L. WILHELM

RECEIVED JULY 2, 1953

In the past fifty years, numerous publications have indicated an interest in the spontaneous deposition of carrier-free radionuclides from solution onto metal foils. A detailed bibliography of these researches has been compiled by Bonner.[1] Most of the investigators assumed that the process involved was a simple electrochemical displacement. If one makes this assumption, a knowledge of the critical deposition potential of the carrier-free nuclide should allow a prediction to be made as to which solution–metal foil conditions would bring about deposition of the tracer. Such a deposition potential for silver-111 has been measured and a value of −0.77 v. is reported.[2] In most previous experiments, no metal salts of the metal foils were added to the solutions, making it difficult to assign a metal foil potential, and to predict deposition behavior. Erbacher,[3] Joliot[4] and Camarcat, Bouissieres and Haissinsky[5] have helped to clarify the situation by measuring the potentials of various metal foils in solutions to which no corresponding metal salts had been added. However, several depositions have been reported which should not occur according to the relationship of these so-called "anomalous metal potentials" to the deposition potentials of the ions.[1]

This research was undertaken in order to investigate the spontaneous deposition of carrier-free silver-111 onto various metal foils in the absence of ions of the metal.

Experimental

Materials.—The silver-111 isotope was produced by neutron irradiation of palladium foil at the Oak Ridge National Laboratory. Perchloric acid solutions of this radionuclide were prepared by the procedure of Schweitzer and Nehls.[6] Spectrographic analysis of the palladium foil indicated that the silver solutions had a concentration of about 10^{-8} *M*. All inactive chemicals used in these experiments were reagent grade and all solutions were prepared using distilled water. All metal foils were cut square 2.0 cm. on an edge with a short strip 0.3 cm. wide appended by which they could be attached to a support. The foil thicknesses ranged

(1) A. C. Wahl and N. A. Bonner, "Radioactivity Applied to Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1951, pp. 169–171, 450–459.

(2) L. Rogers, D. Krause, J. Greiss and D. Ehrlinger, *J. Electrochem. Soc.*, **95**, No. 2, 33 (1949).

(3) O. Erbacher, *Z. physik. Chem.*, **156A**, 135 (1931).

(4) F. Joliot, *J. chim. phys.*, **27**, 119 (1930).

(5) M. Camarcat, G. Bouissieres and M. Haissinsky, *ibid.*, **46**, 153 (1949).

(6) G. K. Schweitzer and J. W. Nehls, THIS JOURNAL, **74**, 6186 (1952).

from 0.002 to 0.005 cm. Previous to the potential measurements, the surfaces of all foils, except the platinum ones, were polished with 4/0 flint paper until a completely new surface was exposed. The sheets from which the foils were cut had been secured from the J. T. Baker Company.

Apparatus.—Potential measurements were made with a Fisher Type S Potentiometer with a Rubicon Company galvanometer attached. An Eppley Laboratory standard cell was employed as a reference electromotive force. Leads from the potentiometer circuit were attached to a Beckman fiber type calomel electrode and to the metal foil under investigation, both of these being dipped in the solution. Depositions were carried out in a jacketed 30-ml. beaker. A Model B-1 Eastern centrifugal pump was used to circulate water of the desired temperature through the outer jacket of the beaker. For the 25° measurements, temperature control was provided by a thermostated Sargent heater and circulator in a large bath. For the 3° measurements and the 89° measurements, the reservoirs were a large beaker of ice-water and a large beaker of water just below the boiling point, respectively. A Precision-Scientific Company Mag-mix magnetic stirring apparatus was used to stir the solutions in the 30-ml. beaker.

Radioactivity measurements were made with standard counting apparatus consisting of a Nuclear D-33 end-window counting tube, mounted in a Tracerlab SC-10 sample holder, attached to a Tracerlab P-4 preamplifier and a Tracerlab SC-2A scaler. All measurements of *p*H were made with a Beckman Model G *p*H Meter.

Sampling.—Silver ion concentration changes in the solutions were determined by the removal of 0.100-ml. samples at regular time intervals. The number of samples taken in each run was adjusted so that the error incurred by the removal of the silver from the bulk of the solution was less than the error inherent in the radioactivity measurements. The samples were evaporated to dryness on metal planchets in preparation for counting.

Results

Potential Measurements.—The potential existing between metal and solution was measured for various metal foils in distilled water made up to a *p*H of 3.0. These measurements were made at 25.0°. The trends in potential with respect to time and the magnitudes of these values referred to the standard hydrogen electrode are indicated in Fig. 1. At least two duplicate runs were made on all metals. The greatest disagreement in values of successive runs occurred with aluminum, for which the standard deviation of the plotted values is 0.04 v. This standard deviation is based on three runs.

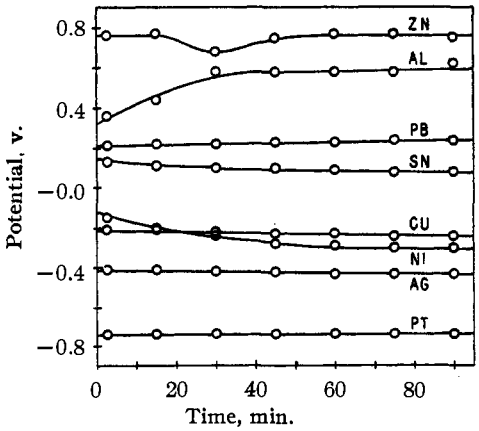

Fig. 1.—Metal foil potentials measured in 0.001 *N* perchloric acid solution plotted as a function of time; temp., 25°.

Deposition.—Deposition of tracer radiosilver was attempted on all metals for which metal foil potentials had been measured, except silver. The depositions were carried out under the same solution conditions which existed in the potential measurements. All metals which were investigated removed tracer radiosilver from solution. In each case, after approximately 120 min. immersion in the solution, the percentage removed had reached a maximum value. All plots of the percentage deposited as a function of time resembled the ones shown in Fig. 2. Table I represents values of percentage removal at the maximum for various metal foils and also a measure of the rate of deposition, expressed as the time required for one-half the maximum percentage deposited to be removed from solution.

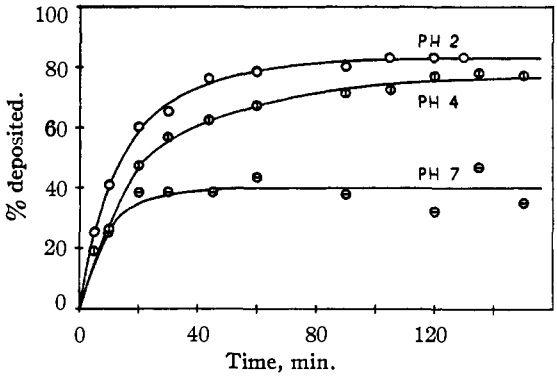

Fig. 2.—Per cent. radiosilver deposited on platinum as a function of time for several *p*H values; temp., 25°.

Surface Effects.—Additional depositions were made on copper foils which had been polished with the flint paper. No general reproducibility could be attained. Foils were then prepared by electroplating copper foils in an acidic copper sulfate bath using copper foil anodes. Then copper foils were prepared by anodic etching in a similar bath using a current of 1.5 amp. for five min. Neither of these methods produced reproducibility in the data. It was found that runs reproducible within 5% could be obtained if a single platinum foil were used. The deposited radiosilver could not be removed from the foil by allowing the foil to stand in the solution, but it was found that hot concentrated nitric acid effected a satisfactory removal.

***p*H and Temperature Effects.**—The rates of deposition of radiosilver onto platinum from perchloric acid solutions with *p*H values of 2.0, 4.0 and 7.0 at 25° were observed and the results are shown in Fig. 2. The data for plotting these curves were the averages of two or more runs at each *p*H value, the data being duplicated within 5% at *p*H values of 2.0 and 4.0. The values determined in successive runs at a *p*H value of 7.0 varied widely, disagreeing as much as 15%. Then the rates of deposition onto platinum from perchloric acid solutions at a *p*H value of 2.0 were observed at 3° and at 89°. The results of the temperature experiments are shown in Fig. 3. These curves were plotted from averaged values of the data from two or more runs, all runs agreeing within 5%.

Effect of Added Ions.—Depositions of tracer radiosilver onto copper foils from solutions 0.1 and 0.5 *M* in copper(II) perchlorate made up

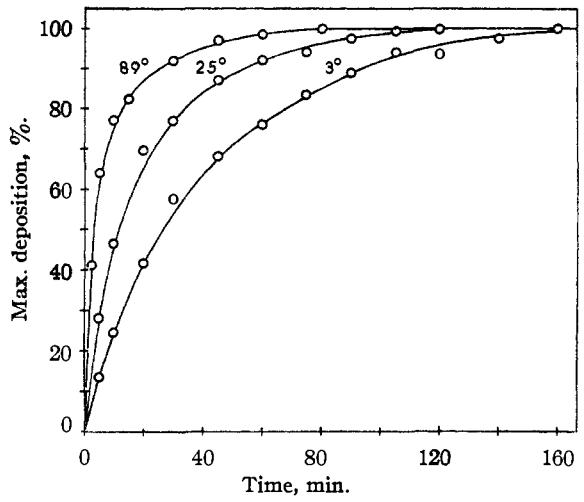

Fig. 3.—Per cent. of maximum deposition of radiosilver on platinum as a function of time at several temperatures.

to a *p*H value of 2.0 with perchloric acid could not be reproduced. However, all curves were of the type as shown in Figs. 2 and 3.

TABLE I

DEPOSITION ON VARIOUS METALS AT 27°

Metal	1st run Max. removed,[a] %	1st run Half-time, min.[b]	2nd run Max. removed,[a] %	2nd run Half-time, min.[b]
Zn	75	25	..	..
Al	65	20	..	..
Pb	45	15	60	10
Sn	15	15	25	30
Cu	85	15	75	30
Ni	85	15	35	25
Pt	75	10	70	15

[a] Given to the nearest 5%. [b] Given to the nearest 5 min.

Discussion

The anomalous metal foil potentials measured in perchloric acid at a *p*H value of 3.0 are in reasonable agreement with those measured in 0.1 *M* hydrochloric acid,[3] in 0.125 *M* nitric acid,[4] and in dilute hydrofluoric acid,[5] except for silver. A value of −0.42 v. has been obtained in this investigation as compared to previously measured values of −0.29 v. in 0.1 *M* hydrochloric acid and −0.26 v. in 0.125 *M* nitric acid. It is interesting to note that none of the anomalous silver foil potentials are even close to the deposition potential of tracer radio-silver, which is reported as −0.77 v.[2]

Table I indicates that it is difficult to draw any conclusion relating either high rates of deposition or large percentages deposited to electropositive character in the metals. It seems that the lack of reproducibility in the various runs might be assigned to differences in the surfaces of the individual metal foils. Since determinations of anomalous metal potentials can be duplicated using different foils that have undergone pretreatment with flint paper, while deposition determinations cannot be reproduced using similarly prepared foils, one questions whether there is any direct relation between the anomalous potentials and the deposition behavior. Camarcat, Bouissieres and Haissinsky,[5] from their data on the spontaneous deposition of protactinium, conclude that there is more of a relation between deposition behavior and the standard electrode potentials of the metals than between the deposition behavior and the anomalous potentials. This investigation shows no apparent relation to either potential.

The results of the surface effect studies seem to indicate that the surface plays an important role in the deposition behavior. Just exactly what this role is cannot be concluded from the present work. Perhaps differences in adsorption of silver ions or atoms onto the surfaces may be involved. Numerous authors[7] have treated this possibility from a theoretical viewpoint, and conclude that this is a likely explanation.

The data from the deposition on the platinum foil indicate that the maximum amount deposited decreased as the *p*H value went from 2.0 to 7.0. This effect may be due to the tendency of tracer silver to form radiocolloids as the *p*H of the solution is increased.[6] With increases in temperature, the rate of deposition onto platinum increased. The fact that hot concentrated nitric acid was necessary to remove the radiosilver from the platinum indicates that the silver has been deposited as an atom and probably does not exist in the form of an adsorbed ion.

Some investigators have hypothesized that the lack of reproducibility in spontaneous deposition behavior was due to the absence of any intentionally added ions corresponding to the metal foil under consideration. To test this hypothesis in the case of silver, depositions onto copper from solutions containing macro amounts of copper(II) ion were run. The results could not be duplicated, thus lending no support to the hypothesis.

(7) M. Haissinsky, "Electrochimie des substances radioactives et des solutions extremement diluées," Herman and Cie, Paris, 1946, pp. 27-34; J. Byrne, L. Rogers and J. Greiss, *J. Electrochem. Soc.*, **98**, 447, 452, 457 (1952); M. Haissinsky, *Experientia*, [4] **8**, 125 (1952).

DEPARTMENT OF CHEMISTRY
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Acylhydrazones of *o*-Oxy- and *o*-Aminoaldehydes and Ketones as Tridentate Complexing Agents

BY LUIGI SACCONI

RECEIVED JUNE 26, 1953

In the course of previous works on chemical reactions of complexes[1] a number of acylhydrazones of salicylaldehyde, 5-bromosalicylaldehyde, *o*-oxynaphthaldehyde, *o*-oxyacetophenone and *o*-aminobenzaldehyde were prepared. These products are able to react as tridentate groups and to yield, with nickel, bicyclic and polynucleate complexes.

The data obtained for a series of acylhydrazones are given in Table I.

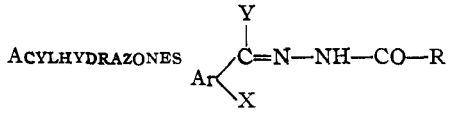
Experimental

Preparation of the Acylhydrazones.—The solution of aldehyde or ketone and acylhydrazines (1 mole:1 mole) in alcohol was heated under reflux on steam-bath for about one hour and cooled. Water was often added in order to obtain a larger yield of crystalline precipitate. The products were purified by recrystallization from 95% or dilute alcohol.

(1) L. Sacconi, THIS JOURNAL, **74**, 4503 (1952); *Z. anorg. allgem. Chem.*, **277**, 176 (1953); *Gazz. chim. ital.*, in course of being printed.

TABLE I

ACYLHYDRAZONES



Y = H, CH_3; X = OH, NH_2; R = $(CH_2)_{12}$–CH_3, C_6H_5, CH_2–C_6H_5, $C_6H_4NO_2$; Ar = C_6H_4, $C_{10}H_6$.

Hydrazone	Formula	M.p., °C.	Crystals	Nitrogen, % Calcd.	Found
Salicylaldehyde myristyl	$C_{21}H_{34}O_2N_2$	104–105	White	8.10	8.23
Salicylaldehyde *o*-nitrobenzoyl	$C_{14}H_{11}O_4N_3$	175–177	Yellow	14.73	15.17
o-Oxynaphthaldehyde myristyl	$C_{25}H_{36}O_2N_2$	129–130	Yellow	7.07	7.00
o-Oxynaphthaldehyde benzoyl	$C_{18}H_{14}O_2N_2$	211–212	White	9.74	9.75
o-Oxynaphthaldehyde fenylacetyl	$C_{19}H_{16}O_2N_2$	204–206	Yellow	9.20	9.12
o-Oxyacetophenone benzoyl	$C_{15}H_{14}O_2N_2$	180–181	White	11.06	10.92
o-Aminobenzaldehyde myristyl	$C_{21}H_{35}ON_3$	103–104	White	12.16	12.00
o-Aminobenzaldehyde benzoyl	$C_{19}H_{13}ON_3$	180–181	Yellow	17.57	17.67
o-Aminobenzaldehyde fenylacetyl	$C_{15}H_{15}ON_3$	164–165	Yellow	16.59	16.50
Salicylaldehyde picolinyl	$C_{13}H_{11}O_2N_3$	171–173	White	17.42	17.37
o-Oxynaphthaldehyde picolinyl	$C_{17}H_{13}O_2N_3$	189–190	Yellow	14.43	14.35
o-Oxyacetophenone picolinyl	$C_{14}H_{13}O_2N_3$	184–186	White	16.47	16.35
o-Aminobenzaldehyde picolinyl	$C_{13}H_{12}ON_4$	216–218	Yellow	23.33	23.42
Salicylaldehyde nicotinyl	$C_{13}H_{11}O_2N_3$	175–177	White	17.42	17.57
o-Oxynaphthaldehyde nicotinyl	$C_{17}H_{13}O_2N_3$	252–253	Yellow	14.43	14.53
o-Oxyacetophenone nicotinyl	$C_{14}H_{13}O_2N_3$	183–185	White	16.47	16.43
o-Aminobenzaldehyde nicotinyl	$C_{13}H_{12}ON_4$	208	Yellow	23.33	23.28
5-Bromosalicylaldehyde nicotinyl	$C_{13}H_{10}O_2N_3Br$	216–217	Yellow	13.12	13.09
Salicylaldehyde isonicotinyl	$C_{13}H_{11}O_2N_3$	244–245	White	17.42	17.48
o-Oxynaphthaldehyde isonicotinyl	$C_{17}H_{13}O_2N_3$	155–157	Yellow	14.43	14.15
o-Oxyacetophenone isonicotinyl	$C_{14}H_{13}O_2N_3$	235–237	White	16.47	16.43
o-Aminobenzaldehyde isonicotinyl	$C_{13}H_{12}ON_4$	232–233	Yellow	23.33	23.20
5-Bromosalicylaldehyde isonicotinyl	$C_{13}H_{10}O_2N_3Br$	251–252	Yellow	13.12	13.10

All hydrazones, except those of *o*-aminobenzaldehyde, are soluble in dilute sodium hydroxide and in aqueous ammonia with yellow color.

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Chemical Interactions of Amino Compounds and Sugars. VIII.[1] Influence of Water[2]

BY M. L. WOLFROM AND C. S. ROONEY

RECEIVED APRIL 15, 1953

This Laboratory has been concerned with a rather extended study of the color-producing (browning) reaction between a reducing sugar (such as D-xylose) and an amino acid (such as glycine, in excess) in dilute aqueous solution. From an applied food product standpoint, most of these browning reactions occur in relatively low water concentrations and such environments have been recently studied extensively by Lea and associates.[3] While our immediate program is not concerned with these applied aspects, it was nevertheless considered of interest to investigate the parameter of water concentration in our model system. To this end solid mixtures, each containing D-xylose and glycine in 1:5 molar ratio, were heated at 65°, under nitrogen and with mechanical stirring, with various proportions of water. The degree of color formation was determined by measuring the optical density at 490 mμ at a suitable standard dilution after heating periods of four, six and eight hours. The results are plotted in the accompanying figure. Below the abscissa value of about 18.6 (65% water) the reactions were heterogeneous. The degree of coloration increases with time but follows the same

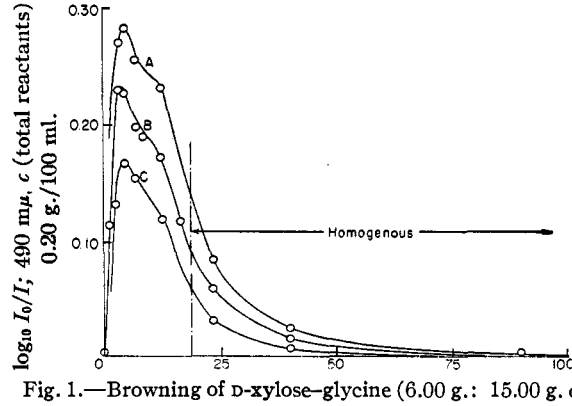

Fig. 1.—Browning of D-xylose–glycine (6.00 g.: 15.00 g. or 1:5 molar ratio) mixtures mechanically stirred (except at 0–1.8 on abscissa) at 65° under nitrogen at various water ratios: curve A, 8 hr. reaction time; curve B, 6 hr.; curve C, 4 hr.; Lumetron (Model 402E) photoelectric colorimeter; 1-cm. cell.

(1) Previous communication in this series: M. L. Wolfrom, Doris K. Kolb and A. W. Langer, Jr., THIS JOURNAL, **75**, 3471 (1953).

(2) This paper represents research undertaken in coöperation with the Quartermaster Institute for the Armed Forces under Contract No. DA11-009-qm-13294 with The Ohio State University Research Foundation (Project 477), and has been assigned number 420 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

(3) C. H. Lea and R. S. Hannan, *Biochem. et Biophys. Acta*, **3**, 313 (1949); **4**, 518 (1950); V. M. Lewis and C. H. Lea, *ibid.*, **4**, 532 (1950); C. H. Lea, R. S. Hannan and D. N. Rhodes, *ibid.*, **7**, 366 (1951); R. S. Hannan and C. H. Lea, *ibid.*, **9**, 293 (1952).

type of curve for the three reaction times measured. The amount of coloration increases rapidly from about zero in the anhydrous state to a maximum at about 4.3 g. of water per 10 g. of solids (30% water). A more gradual decrease occurs to the right of this peak with the degree of coloration approaching zero at a water concentration of about 90%. The homogeneous reactions follow the expected pattern of a roughly first-order increase in the rate of coloration with an increase in reactant concentration. Those portions of the curve in the heterogeneous reaction region are difficult to interpret and are undoubtedly affected by rates of solution and by diffusion. Nevertheless, our model system demonstrates that browning is at a minimum at high and low water concentrations and passes through a maximum value at an intermediate point of rather low (*ca.* 30%) water concentration. The retardation with an increase in the water content has been recorded in related model systems.[4]

(4) G. P. Volgunov and M. T. Pokhno, *Biokhimiya*, **15**, 67 (1950); M. F. Mashkovtsev, *ibid.*, **16**, 615 (1951).

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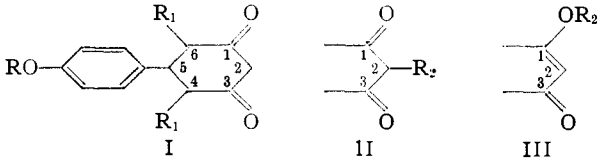
Acylation[1] of 5-(*p*-Acetoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3

BY PHILIPPOS E. PAPADAKIS, JOSEPH SCIGLIANO AND SEBASTIAN PIRRUCCELLO

RECEIVED MAY 11, 1953

A previous publication[2] described the synthesis of 5-(*p*-acetoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 (I) and some of its derivatives. The present paper reports a study of the acylation of this substance, presumably leading to both C-acylation (II) and O-acylation (III).



I II III

R = CH_3CO, R_1 = $COOC_2H_5$
R_2(II) = $COCH_3$, COC_2H_5, $COCH_2CH_2N$, $COCH_2CH_2COOCH_3$, or $SO_2C_6H_4NHCOCH_3$
R_2(III) = $COCH_3$ or COC_2H_5

C-Acylation at the 2-position gives compounds which are structurally similar to usnic acid and chalcones, both of which show antibiotic activity[3,4] against gram-positive organisms and against human and bovine tubercle bacilli.

The procedures outlined by Claisen[5] and Dieckmann and Stein[6] for the acylation of 1,3-dicarbonyl compounds were used. These authors report C-acylation with acid halides and sodium alkoxide, or by acid anhydrides and the sodium salt of the acid; and O-acylation with the anhydride. The conversion of O-acyl into C-acyl derivatives by potassium carbonate, pyridine, or sodium acetate in acetic anhydride was also reported; the two latter methods were used in this work.

The acyl derivatives obtained can be divided into two classes: those which give a yellow color (presumably the C-acyl derivatives) and those which give a reddish purple color when treated with alcoholic ferric chloride. The O-acyl derivatives were found to be more soluble in ether and in benzene than the C-acyl. This difference in solubility proved helpful in the separation of these compounds.

Dieckmann and Stein[6] showed that O-acyl derivatives can be hydrolyzed by alkali, while the C-acyl group under similar conditions is not affected. These investigators claimed also that when C-acetyldimethylhydroresorcinol was boiled with dilute sulfuric acid, it was cleaved to dimethylhydroresorcinol, m.p. 144°, and acetic acid. In the present work, cleavage of the C-acetyl group of 5 - (*p* - acetoxyphenyl) - 2 - (C - acetyl) - cyclohexanedione-1,3 did not occur when the compound was refluxed with sodium carbonate solution, and the mixture then acidified and refluxed again. This is in contrast to Dieckmann and Stein's finding.

p-Acetaminobenzene sulfones have been used in the therapy of tuberculosis and leprosy. The sodio derivatives of I and of the *m*-methoxy derivative of I were heated with *p*-acetaminobenzenesulfonyl chloride to give the corresponding sulfones, presumably at position 2 of the cyclohexanedione.

Experimental

The general experimental procedures parallel those of Dieckmann and Stein[6] in expts. 1 to 10, and those of Claisen[5] in expts. 11 to 18.

In expts. 1 to 10, 3 g. of the cyclohexanedione (I) and 7 ml. of the acid anhydride were used (acetic anhydride in 1 to 5; propionic anhydride in 6 to 10). The other specific reagents were 0.15 g. of sodium acetate in 1; 0.15 g. of sodium propionate in 6; excess pyridine (2 ml.) in 3 and 8; and the calculated quantity of pyridine (0.65 ml.) in 4, 5, 9 and 10. In all of these cases ice-water was added to the mixture after reaction, and the solid product was isolated and recrystallized from absolute ethanol.

In expts. 11 to 15 about 5 to 10 g. of the cyclohexanedione (I) was converted into the sodium salt with an equivalent of sodium methylate. The dry sodium salt was isolated and then heated with potassium iodide and an equivalent amount of the appropriate acid halide in dry ether; no potassium iodide was used in expt. 14. In expt. 16 the sodio derivative of 5-(*p*-methoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 and cinnamoyl chloride were used. After the ether was removed, the residue was washed free of halide ion; then the dried residue was extracted with small portions of ether. As the O-acyl derivatives are more soluble in ether than the C-acyl, the residue consisted mostly of the C-acyl which was finally recrystallized from ethanol. A little petroleum ether was added to the ether solution to precipitate any C-acyl which was filtered off. The filtrate, upon evaporation, gave the O-acyl derivative.

In expt. 17 the sodium salt of I and in expt. 18 the sodium salt of the *m*-methoxy derivative of I were heated with *p*-aminobenzenesulfonyl chloride in dry dioxane. The dioxane was then removed and the residue treated as in expts. 11 to 15.

Conversions.—The compound melting at 145° (obtained in expts. 2 and 11 and assumed to be an O-acetyl derivative) was converted into that melting at 116° (obtained in expts. 1, 3 and 4 and assumed to be a C-acetyl derivative) by the action of acetic anhydride and sodium acetate for eight hours at the temperature of the water-bath.

(1) Part of this paper was presented at the 116th meeting of the American Chemical Society at Atlantic City, N. J., Sept. 1949. The paper is based on a manuscript submitted to THIS JOURNAL, May 9, 1950.

(2) P. E. Papadakis, THIS JOURNAL, **67**, 1799 (1945).

(3) Tynosin Ukita, Tomie Tamura, Reiko Matsuda and Etsuko Kashiwabara, *Japan J. Exptl. Med.*, **20**, 109 (1949).

(4) A. Marshak, G. T. Barry and L. C. Craig, *Science*, **106**, 394 (1947).

(5) L. Claisen and E. Haase *Ber.*, **33**, 1242, 3778 (1900).

(6) W. Dieckmann and R. Stein, *ibid.*, **37**, 3370, 3384 (1904).

TABLE I

ACYL DERIVATIVES OF CYCLOHEXANEDIONE (I)

R_2 = $COCH_3$[a]				Analyses, %											
Expt.	Time[b]	Color[c]	M.p., °C.[d]	Carbon[e] Found	Hydrogen[e] Found	Expt.	R_2[a]	Time[b]	Color[c]	M.p., °C.[d]	Formula	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
	10	Y	116	60.76	5.70				RP	145					
1		Y	148			11	$COCH_3$	10	Y	162	$C_{22}H_{24}O_9$	61.10	61.38	5.59	5.72
	10	RP	145	61.48	5.76				Y	163	$C_{23}H_{26}O_9$	61.87	61.56	5.87	5.99
2	10	RP	115			12	$COCH_2CH_3$	22	RP	115					
3	48	Y	116			13	$COCH_2CH_2I$	26	Y	145	$C_{23}H_{25}O_9I$	48.44	48.95	4.45	4.89
	16	Y	116			14	$COCH_2CH_2Cl$	21	Y	150	$C_{23}H_{25}O_9Cl$	57.44	57.62	5.24	5.47
4	12	RP	115	61.19	5.52	15	$COCH_2CH_2COOCH_3$	5	Y	133	$C_{25}H_{28}O_{11}$	59.52	59.74	5.59	5.96
5	10	Y	148	60.95	5.72	16[f]	$COCH{=}CHC_6H_5$	24	Y	140	$C_{28}H_{28}O_8$	68.26	68.57	5.75	5.90
R_2 = CH_3CH_2CO[a]				$C_{23}H_{26}O_9$[e]											
6	10	Y	129	62.30	6.31	17	$SO_2C_6H_4NHCOCH_3$	6	Y	203	$C_{28}H_{29}O_{11}SN$	57.23	56.74	4.97	4.92
	10	RP	102	62.31	6.06	18[g]	$SO_2C_6H_4NHCOCH_3$	6	Y	175	$C_{27}H_{29}O_{11}SN$	56.33	56.66	5.07	5.46
7		RP	115	62.35	5.93										
8	48	Y	163												
9	16	RP	102												
10	10	Y	129												

[a] Acyl substituent. [b] Reaction time in hours. Expts. 3, 4, 8 and 9 were run at room temperature, the others at approximately 100°. [c] Color in ferric chloride test: Y = yellow, RP = reddish purple. [d] Mixed melting points indicated that, in general, the compounds with the same melting point and empirical formula are identical: 116 in expts. 1, 3 and 4; 148 in 1 and 5; 129 in 6 and 10; 145 in 2 and 11; 115 in 2 and 4; 102 in 7 and 9; 115 in 7 and 12; and 163 in 8 and 12. [e] Calcd. for $C_{22}H_{24}O_9$: C, 61.10; H, 5.59. Calcd. for $C_{23}H_{26}O_9$: C, 61.87; H, 5.87. [f] 5-(*p*-Methoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 was used instead of I. [g] The *m*-methoxy derivative of I was used.

A portion of the substance melting at 145° (obtained in expt. 2) was dissolved in sodium carbonate solution. It was allowed to stand at room temperature 24 hours, refluxed for 12 hours, acidified and further refluxed for seven hours. Upon cooling, a precipitate formed which was filtered and recrystallized from boiling distilled water. The product, dried over phosphorus pentoxide in a vacuum desiccator, was found to be identical with 5-(*p*-hydroxyphenyl)-cyclohexanedione-1,3.[2] The O-acyl at position 1 (or 3) was hydrolyzed.

5-(*p*-Acetoxyphenyl)-2-acetylcyclohexanedione-1,3, m.p. 117°, prepared from 5-(*p*-hydroxyphenyl)-cyclohexanedione-1,3[2] by the method[6] of expt. 5, was refluxed for five hours with sodium carbonate solution. The mixture was acidified and refluxed again for one hour. After cooling, the mixture was extracted with 20 ml. of each of the following solvents: benzene, ethyl acetate and ether. The combined solvents were evaporated; water followed by a little ethanol was added to the viscous residue. The crystals formed were recrystallized from ethanol, m.p. 179°. The analysis indicates that the product retained one acetyl group.

Anal. Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.77. Found: C, 68.08; H, 6.04.

The compound melting at 115° (obtained in expts. 2 and 4 and assumed to be an O-acetyl derivative) was converted into the isomer melting at 148° (obtained in 1 and 5 and assumed to be a C-acetyl derivative) by the action of acetic anhydride and pyridine for ten hours on the water-bath. Similarly, the compound melting at 102° (obtained in expts. 7 and 9 and assumed to be O-propionyl) was converted into the isomer melting at 129° (obtained in expt. 10 and assumed to be a C-propionyl derivative) by the use of propionic anhydride and pyridine.

Acknowledgment.—The authors wish to express their appreciation to Dr. C. L. Kenny for the interest he has shown in this investigation.

DEPARTMENT OF CHEMISTRY
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5-(*p*-Hydroxyphenyl)-4,6-dicarboxy-2-(β-diethylaminopropanol)-cyclohexanedione-1,3 and Derivative[1]

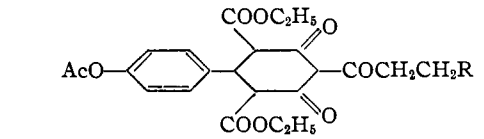
BY PHILIPPOS E. PAPADAKIS AND JOSEPH SCIGLIANO

RECEIVED MAY 18, 1953

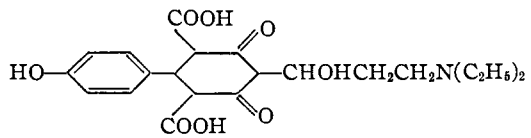
In the search for antimalarials, a great many substituted diethylaminoalkanols have been prepared.[2-6] The object of the present work was the preparation of dialkylaminoalkanol derivatives of cyclic 1,3-diones, and we describe below the synthesis of 5-(*p*-hydroxyphenyl)-4,6-dicarboxy-2-(β-diethylaminopropanol)-cyclohexanedione-1,3 and a derivative.

Compound I, prepared by acylation of 5-(*p*-acetoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 with β-chloropropionyl chloride followed by treatment with potassium iodide, was transformed into II by the action of diethylamine. On reduction by the Meerwein–Ponndorf–Verley method, II yielded the expected secondary alcohol III and a further substance $C_{21}H_{25}O_7N$, assumed to be a lactone.

Physiological properties of these compounds will be examined and reported later.



I, R = I
II, R = $N(C_2H_5)_2$



III

Experimental

5-(*p*-Acetoxyphenyl)-4,6-dicarbethoxy-2-(β-chloropropionyl)-cyclohexanedione-1,3.—To a solution of 230 mg. of sodium in methanol, 3.90 g. (0.01 mole) of 5-(*p*-acetoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3[7] was added and the mixture refluxed for one hour. The methanol was distilled and the residue dried under vacuum. To the dry material absolute ether and one ml. of freshly distilled β-chloropropionyl chloride was added and the mixture was

(1) Based on a paper submitted to THIS JOURNAL, Dec. 17, 1951.

(2) H. King and T. S. Work, *J. Chem. Soc.*, 1307 (1940).

(3) E. L. May and E. Mosettig, *J. Org. Chem.*, **11**, 1, 105, 296, 429, 631 (1946).

(4) R. C. Elderfield and co-workers, *ibid.*, **11**, 123, 143, 247 (1946).

(5) T. L. Jacobs and co-workers, *ibid.*, **11**, 21, 150, 215 (1946).

(6) R. E. Lutz and co-workers, *ibid.*, **12**, 617 (1947).

(7) P. E. Papadakis, THIS JOURNAL, **67**, 1799 (1945).

refluxed on a water-bath for 21 hours. The ether was removed and the residue treated with water, filtered, dried and washed with ether twice. The ether-insoluble portion, m.p. 149°, is soluble in alcohol and gives a yellow color with ferric chloride; yield 95%.

Anal. Calcd. for $C_{23}H_{25}O_9Cl$: C, 57.44; H, 5.24. Found: C, 57.62; H, 5.47.

5-(*p*-Acetoxyphenyl)-4,6-dicarbethoxy-2-(β-iodopropionyl)-cyclohexanedione-1,3 (I).—The procedure was similar to the above with the exception that to the dry sodio derivative and β-chloropropionyl chloride in dry ether two grams of dry potassium iodide was added and the mixture refluxed as described. The product melted at 145° and gave a yellow color with ferric chloride; yield almost quantitative. This compound was prepared first by Greenfield.[8]

Anal. Calcd. for $C_{23}H_{25}O_9I$: C, 48.44; H, 4.45. Found: C, 48.95; H, 4.89.

5-(*p*-Acetoxyphenyl)-4,6-dicarbethoxy-2-(β-diethylaminopropionyl)-cyclohexanedione-1,3 (II).—5-(*p*-Acetoxyphenyl)-4,6-dicarbethoxy-2-(β-chloropropionyl)-cyclohexanedione-1,3 (4.8 g.) and 2.0 ml. diethylamine in dry ether were mixed, allowed to stand in ice-bath for 1 hour and then refluxed for 5 hours. The ether was removed and the residue was treated with chloroform. The undissolved part was washed with chloroform, then dissolved in a mixture of methyl alcohol and chloroform. The solution was filtered and to the cooled filtrate ether was added. A precipitate formed, m.p. 201–202°, yield 87%. The product gave a yellow color with the ferric chloride test.

Anal. Calcd. for $C_{27}H_{35}O_9N$: C, 62.65; H, 6.81. Found: C, 62.37; H, 7.24.

5-(*p*-Hydroxyphenyl)-4,6-dicarboxy-2-(3-diethylamino-1-hydroxypropyl)-cyclohexanedione-1,3 (III).—Compound II was converted to III by the Meerwein–Ponndorf–Verley reduction. The procedure was similar to that described by Wilds.[9] In a 500-cc. round-bottomed flask were placed 4 g. of II (0.0076 mole), 3.5 g. of aluminum isopropoxide (0.0156 mole) and 250 ml. of dry isopropyl alcohol. A small fractionating column was attached to the flask with a water condenser set for distillation. After six hours of slow distillation, the distillate showed a negative acetone test. The remaining isopropyl alcohol was removed and the residue was dried under reduced pressure. Benzene was added to the residue to dissolve any unreacted aluminum isopropoxide. The residue was then treated with cold water and ammonium hydroxide was added to the mixture with mechanical stirring. After removal of the aluminum hydroxide, the filtrate was cooled, acidified with cold hydrochloric acid and allowed to stand in the refrigerator several hours. The precipitate which formed was collected, washed with distilled water, dried, and crystallized from alcohol. The first crop of crystals had m.p. 209° (IV).

Anal. Calcd. for $C_{21}H_{25}O_7N$: C, 62.52; H, 6.23. Found: C, 62.72; H, 5.97.

Distilled water was added to the filtrate and the precipitate formed was dissolved in alcohol and reprecipitated with distilled water, collected and dried, m.p. 193° (III). It gave an orange color with ferric chloride.

Anal. Calcd. for $C_{21}H_{27}O_6N$ (III): C, 59.84; H, 6.46. Found: C, 60.06; H, 6.57.

(8) L. Greenfield, Creighton University M.S. Thesis, 1948.

(9) A. L. Wilds, "Organic Reactions," Editors Roger Adams. W. E. Bachmann, L. F. Fieser, J. R. Johnson, H. R. Snyder, Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 200–204.

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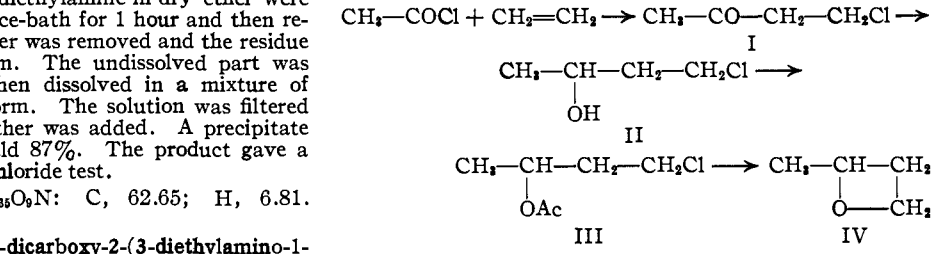
A Synthesis of 1,3-Butylene Oxide

BY FRANZ SONDHEIMER[1] AND R. B. WOODWARD

RECEIVED FEBRUARY 23, 1953

1,3-Butylene oxide (1,3-epoxybutane) (IV) was required for synthetic purposes. The previously described[2] method of preparation of this substance proceeded only in poor yield, and was not particularly suitable for laboratory scale production. For these reasons a convenient new synthesis of IV was developed.

The first step, the condensation of ethylene with acetyl chloride to give 4-chloro-2-butanone (I), has been described previously.[3] The use of excess acetyl chloride and the absence of solvent were recommended, and I was obtained in 40% yield. We have found that these precautions are unnecessary, and by applying the conditions used with propionyl chloride,[4] I could be isolated in 61% yield. The reduction of this chloroketone with lithium aluminum hydride proceeded smoothly to yield the corresponding alcohol II, which was acetylated to III.



The procedure for carrying out the last step, heating to 140° with *ca.* 90% aqueous potassium hydroxide, was based on that employed with the lower homolog, 3-chloropropyl acetate.[5] It seems however that the secondary nature of the acetoxy group in III favors this type of reaction, for a 66% yield of the required 1,3-butylene oxide (IV) was realized as compared with the 42–44% yield of 1,3-propylene oxide obtained from the above mentioned lower homolog containing a primary acetoxy group. The over-all yield in the present process is 30%.

Experimental[6]

4-Chloro-2-butanone (I).—Acetyl chloride (510 g., 6.50 moles) was added during 20 minutes to a mixture of aluminum chloride (910 g., 6.82 moles) and chloroform (2 l.) with stirring and ice–salt cooling. At the end of the addition the temperature had risen to *ca.* 25°, and cooling was continued until it had fallen to 0°. Ethylene was then bubbled into the stirred mixture at such a rate that all was absorbed, the internal temperature being kept between 5 and 10° by continued ice–salt cooling. Gas started escaping after *ca.* 2 hours, and after another 30 minutes the reaction mixture was poured into a mixture of 1 l. of concentrated hydrochloric acid and 5 kg. of ice. The organic layer was washed with dilute hydrochloric acid, sodium bicarbonate and water, and was then dried and slowly evaporated through a 25-cm. Vigreux column. Distillation of the residue through the same column yielded the β-chloroketone I as a mobile liquid, b.p. 47° (16 mm.), $n^{27}D$ 1.4299 (reported[3] b.p. 48° (15 mm.)). The yield was 421 g. (3.95 moles), or 61%.

4-Chloro-2-butanol (II).—The chloroketone (388 g.) in dry ether (400 cc.) was added during 1 hour to a stirred solution of 50 g. of lithium aluminum hydride in 1.5 l. of ether, so as to maintain gentle reflux. After stirring for another 30 minutes, water was added dropwise to decompose excess

(1) Syntex, S. A., Laguna Mayran 413, Mexico, D. F.

(2) Celanese Corp. of America, British Patent 585,245 (*C. A.*, **41**, 4167 (1947)).

(3) *Inter al.*, J. R. Catch, D. F. Elliott, D. H. Hey and E. R. H. Jones, *J. Chem. Soc.*, 278 (1948).

(4) E. M. McMahon, J. N. Roper, W. P. Utermohlen, R. H. Hasek, R. C. Harris and J. H. Brandt, THIS JOURNAL, **70**, 2971 (1948); R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, *ibid.*, **74**, 4223 (1952).

(5) C. R. Noller, *Org. Syntheses*, **29**, 92 (1949).

(6) Boiling points are uncorrected.

reagent, then *ca.* 1.5 l. of 10% sulfuric acid was added and the aqueous layer was re-extracted several times with ether. The combined organic layers were washed with sodium carbonate solution and water, dried and evaporated through a 25-cm. Vigreux column. Distillation of the residue through the same column furnished 313 g. (79%) of the chlorocarbinol, b.p. 67° (20 mm.), $n^{25}D$ 1.4408 (this compound has previously been made by the reaction of β-chloropropionaldehyde with methylmagnesium iodide[7]; reported[7a] b.p. *ca.* 70° (13 mm.)). In addition a lower boiling fraction was obtained.

4-Chloro-2-butyl Acetate (III).—A solution of the chlorocarbinol (300 g.) in 300 cc. of ether and 360 cc. of pyridine was cooled in ice and 330 g. of acetyl chloride was added dropwise during 90 minutes with stirring and continued cooling. Stirring was continued for 5 hours at room temperature and the mixture was then set aside overnight. Ice and ether were added, the organic layer was washed with water, dilute hydrochloric acid, sodium bicarbonate and water, dried and distilled through the Vigreux column. The acetate had b.p. 70° (16 mm.), $n^{25}D$ 1.4260, and weighed 395 g. (95%).

1,3-Butylene Oxide (IV).—An apparatus as described by Noller[5] incorporating a 25-cm. Vigreux column (ref. 5, Note 2) was used and the receiver was cooled in ice–salt. A mixture of 437 g. of solid potassium hydroxide and 40 cc. of water was placed in the reaction flask, which was heated to 150° in an oil-bath, and a few cc. of the acetate was added. Thereupon the mixture became liquid, and stirring was commenced. The acetate (total of 389 g.) was then added dropwise with vigorous stirring at such a rate as to keep the inside temperature at 140–150°, and that at the distillation head at 70–85°. The reaction was quite exothermic and the inside temperature was maintained by keeping the oil-bath at 120–140°. The distillate passed over at a rate of *ca.* 1 drop/second. After 3 hours the addition was complete, and the oil-bath was heated to 160° for a further 30 minutes. The distillate (186.5 g.) was dried over 40 g. of solid potassium hydroxide, whereupon a lower aqueous layer separated. The upper layer was dried over a further 20 g. of potassium hydroxide, decanted and distilled through the 25-cm. Vigreux column. The fraction (134 g.) with b.p. 55–70° was refluxed with 15 g. of sodium hydride for 1 hour to remove impurities, and was then refractionated. The oxide was obtained as a pleasant smelling liquid, b.p. 60–61° (762 mm.), $n^{25}D$ 1.3894, and weighed 122 g. (66%) (reported[2] b.p. 59.4–59.7° (736 mm.), $n^{20}D$ 1.3889).

(7) (a) E. Fourneau and P. Ramart-Lucas, *Bull. soc. chim.*, [4] **25**, 364 (1919); (b) H. J. Backer and C. C. Bolt, *Rec. trav. chim.*, **54**, 68 (1935); (c) R. C. Elderfield, *et al.*, THIS JOURNAL, **68**, 1516 (1946).

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The Reaction of Tolylene Diisocyanate Dimer with Dibutylamine

BY J. H. SAUNDERS AND EDGAR E. HARDY

RECEIVED JULY 2, 1953

Isocyanate dimers are of interest principally because they may exhibit isocyanate activity only at elevated temperatures, and because they may occur as impurities in isocyanates, especially in polyisocyanates. The reactions of phenyl isocyanate dimer have been studied qualitatively, as by Hofmann,[1] and Bayer[2] has reported that the dimer of tolylene diisocyanate (I) dissociates to the monomer at 150°. This dimer has been observed in this Laboratory to react with more than two equivalents of dibutylamine in refluxing toluene, in a modification of an analytical procedure for isocyanates.[3] To clarify further the chemistry of this dimer its reaction with di-*n*-butylamine in *o*-dichlorobenzene was studied briefly at 50–180°.

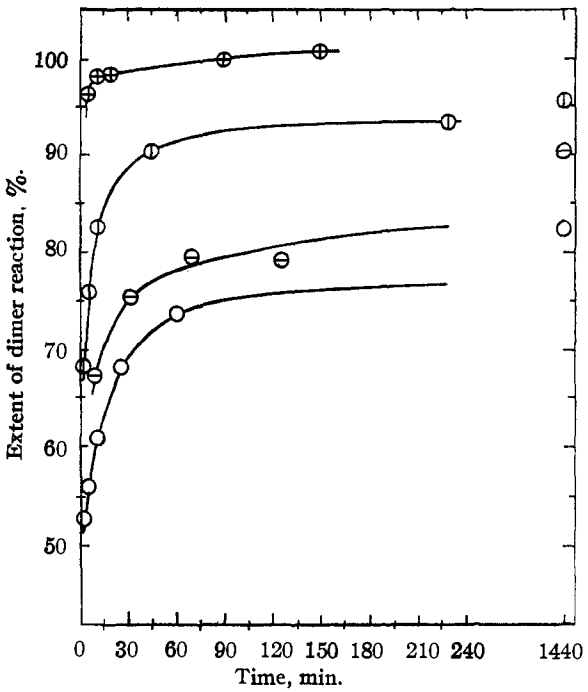
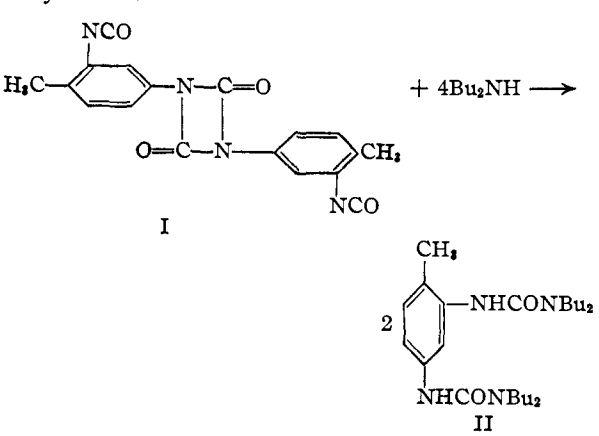


Fig. 1.—The reaction of tolylene diisocyanate dimer with dibutylamine: O, reaction at 50°, 4:1 amine:dimer molar ratio; ⊖, 70°, 4:1 ratio; ⊙, 130°, 4:1 ratio, ⊕, 170–180°, 8:1 ratio.

The results are shown in Fig. 1. It is assumed that the first 50% of the reaction, which was very fast, corresponded to the reaction of the free isocyanate groups, and that further reaction took place at the dimerized groups. The dimer ring reacted at an appreciable rate, even at 50°, and reacted completely with 100% excess of amine in 1.5 hours at 170–180°. The reaction product gave an analysis in agreement with structure (II), and showed no depression of melting point with the reaction product from monomeric tolylene diisocyanate and dibutylamine.



These results show that isocyanate polymers may exhibit considerable activity in the analysis for isocyanates, and that dimers will not be completely unreactive at moderate temperatures in systems containing amines. It is likely that dimers will

(1) A. W. Hofmann, *Ber.*, **4**, 246 (1871).
(2) O. Bayer, British Intelligence Objectives Subcommittee Final Report No. 719, July, 1946.
(3) S. Siggia and J. G. Hanna, *Anal. Chem.*, **20**, 1084 (1948).

be more stable in the presence of less reactive groups, such as hydroxyl.

Experimental

The dimer of 2,4-tolylene diisocyanate was prepared by mixing 1350 g. of dry pyridine and 1590 g. of the diisocyanate with stirring.[2] Some cooling was used to keep the temperature below 40°. After standing overnight the solid reaction mass was diluted with 3.5 liters of dry carbon tetrachloride, pulverized, filtered and dried in a vacuum desiccator. The dimer was recrystallized from chloroform, m.p. 155.4–155.7°, corrected.[4]

Anal. Calcd. for $C_{18}H_{12}O_4N_4$: C, 62.06; H, 3.47; N, 16.09. Found: C, 62.50; H, 3.75; N, 16.16.

The dimer was fairly stable toward atmospheric moisture.[2] After 48 hours exposure to the air the melting point was 4° lower than initially.

The reactions between the dimer and dibutylamine were performed in a dry 1-liter, 3-necked flask equipped with a sealed stirrer, thermometer and reflux condenser which was protected by a calcium chloride tube. The flask was immersed in a constant-temperature oil-bath. Redistilled technical *o*-dichlorobenzene which had been dried over calcium hydride was used as the solvent. The solvent (500 g). was added to the flask and was permitted to come to the bath temperature, when 25.0 g. (0.072 mole) of dimer was added. At zero time 37.6 g. (0.287 mole) of redistilled di-*n*-butylamine, neutralization equivalent 131, was added from a pipet. At intervals a sample of the reaction mixture was withdrawn and added to 25 ml. of 1.06 *N* hydrochloric acid, the mixture shaken, and the sample weight determined. Titration of the excess acid with 0.50 *N* sodium hydroxide solution to a brom phenol blue end-point permitted calculation of unreacted amine, and hence of unreacted dimer.

In the experiments at 50° 850 g. of solvent was required to dissolve the dimer. One experiment was performed in refluxing solvent (170–180°) with amine:dimer molar ratio of 8:1 to find the time required for complete reaction.

A blank experiment showed that dibutylamine did not react appreciably with the solvent during 2.25 hours refluxing. It was also found that no hydrolysis of the dimer occurred during analysis. A mixture of 50 ml. of solvent and 1 g. of dimer was refluxed and added quickly to 25 ml. of acid. Titration after 25 minutes showed that no amine had been liberated by hydrolysis of the dimer.

The results at 0–240 min. and at 1440 min. are summarized in Fig. 1.

The product from a reaction between the dimer and dibutylamine in refluxing dichlorobenzene was obtained by vacuum distillation of the solvent. Repeated recrystallizations from hexane gave a white solid, m.p. 111.1–111.7°, corrected.

Anal. Calcd. for $C_{26}H_{44}O_2N_4$: C, 69.40; H, 10.25; N, 12.95. Found: C, 69.70; H, 10.37; N, 12.74.

From a reaction between monomeric tolylene diisocyanate and dibutylamine in benzene there was obtained a white solid, m.p. 111.1–111.6°, corrected. This solid showed no depression of melting point when mixed with the reaction product from the dimer and the amine, thus confirming structure II.

(4) W. Siefken, *Ann.*, **562**, 75 (1949).

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Physical-chemical Studies on the Interaction of Surface-active Agents with Nucleoproteins. II[1]

BY MARY W. RENOLL AND QUENTIN VAN WINKLE

RECEIVED JUNE 3, 1953

The ultracentrifuge studies of the Santomerse D–calf thymus nucleohistone system[2] have been extended to provide more information on the nature of complex formation in this system. The behavior of the components of the nucleohistone molecule, histone and nucleic acid, in Santomerse D solution, in the ultracentrifuge is reported here.

Experimental and Results

The nucleohistone, histone hydrochloride and tetrasodium nucleate were prepared as previously described.[2] All solutions containing Santomerse D were used for ultracentrifuge, viscosity and diffusion measurements immediately after dialysis was completed. All solvents contained 0.02 ionic strength phosphate buffer.

Ultracentrifuge Studies.—The experimental results with the customary optical rotor are summarized in Table I. "Schlieren" patterns of tetrasodium nucleate and histone hydrochloride sedimenting in buffer and in buffered Santomerse D solution are shown in Fig. 1.

TABLE I

SEDIMENTATION VELOCITY OF TETRASODIUM NUCLEATE AND HISTONE HYDROCHLORIDE

Solute	Concn., %	Solvent	s_{20}[a]
Tetrasodium nucleate	0.31	Water	4.23
	.155	Water	5.29
	.103	Water	6.34
	.000	Water	8.33[c]
	.31	0.35% Santomerse D	4.36
	.155	.35% Santomerse D	5.53
	.103	.35% Santomerse D	6.24
	.000	.35% Santomerse D	8.00[c]
Histone hydrochloride	0.22	Water	21.2,[b] 12.8[b]
	.32	0.35% Santomerse D	3.38
	.157	.35% Santomerse D	2.45
	.078	.35% Santomerse D	2.13
	.000	.35% Santomerse D	1.90[c]
Santomerse D	0.35	Water	0.89[d]

[a] Expressed in Svedberg units. [b] Fast moving components. The sedimentation constant of the slow moving component was too low for measurement. [c] Extrapolated value. [d] Obtained with Spinco Model E ultracentrifuge at 59,780 r.p.m.

To measure the amount of Santomerse D actually involved in complex formation, the experiments with the optical rotor were parallelled with a quantity type rotor[3]. The rotor was fitted with two plastic tube liners, each containing a stainless steel thimble which extended about half way to the bottom of the tube. The perforated bottom of the

TABLE II

BINDING OF SANTOMERSE D BY NUCLEOHISTONE, TETRASODIUM NUCLEATE AND HISTONE HYDROCHLORIDE FROM QUANTITY TYPE ROTOR EXPERIMENTS

Run	System[a]	Time, hr.	RCF, g's	Santomerse D in supernatant, %	Wt. fraction Santomerse D in complex
1	Santomerse D–nucleohistone	6	104,000	0.25	0.45[b]
2	Santomerse D–tetrasodium nucleate	6	104,000	.35	.00
3	Santomerse D–histone hydrochloride	12	129,000	.07	.64
4	Santomerse D	12	100,000	.16	

[a] Contains 0.35% Santomerse D with 0.02 ionic strength phosphate buffer, and 0.16% of second component listed. [b] Corrected for 0.03% nucleohistone remaining in the supernatant, as determined by micro-Kjeldahl nitrogen analysis. This result is in agreement with the previously reported[2] value obtained from area measurements.

(1) Based on research carried out under contract between the Office of Naval Research and The Ohio State University Research Foundation.

(2) M. W. Renoll and Q. Van Winkle, THIS JOURNAL, **73**, 2504 (1951).

(3) R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instr.*, **8**, 427 (1937).

thimble was covered by a disc of hardened filter paper. The tubes were filled with solution to within about an inch from the top of the thimble (7–10 ml.) and that remaining above the disc removed for analysis at the completion of the run. Sedimentation was allowed to continue for 6–12 hours at 100,000–129,000 times gravity (40,000–50,000 r.p.m.). The amount of Santomerse D present in the supernatant liquid removed was determined by comparison of the surface tension of a water dilution with a previously prepared curve relating surface tension to per cent. Santomerse D. A du Noüy tensiometer was used. The results are summarized in Table II.

Run 2 above was repeated at 100,000 times gravity for 9 hours and similar results obtained. The nitrogen content of the supernatant was measured by the micro-Kjeldahl method and essentially complete sedimentation of nucleic acid was found.

The concentration of Santomerse D sedimenting in micelle form in the ultracentrifuge with the optical rotor (see Table I) was calculated from area measurements of the "schlieren" patterns as 0.16%

Viscosity Measurements.—The results of viscosity measurements[2] on tetrasodium nucleate are summarized in Table III. Since the change in viscosity with dilution for histone hydrochloride in water solution and in Santomerse D is very small, no calculation of $[\eta]$ was made.

TABLE III

VISCOSITY OF TETRASODIUM NUCLEATE AT 20°

Concn. of tetrasodium nuclate, %	Solvent	η, cp.	η_{sp}/c	$[\eta]$
0.31	Water	15.353	45.87	
.232	Water	8.338	31.32	
.155	Water	4.163	20.17	
.103	Water	2.702	16.30	
.000	Water			12.5[a]
.31	0.35% Santomerse D	15.082	44.63	
.155	.35% Santomerse D	4.256	20.55	
.103	.35% Santomerse D	2.676	15.84	
.000	.35% Santomerse D			12.5[a]

[a] From $\eta_{sp}/c/c^2$.

Diffusion Measurements.—The diffusion constants[2] of histone hydrochloride in 0.35% Santomerse D diffusing into Santomerse D solution were essentially constant in the range of 0.32, 0.24 and 0.16%, respectively, histone hydrochloride concentration and the average values of $D_{0.6}$, 2.51×10^{-7} and D_{20}, 4.68×10^{-7} were obtained.

Molecular Weights.—The molecular weight of tetrasodium nucleate in water and in Santomerse D solution was calculated[2] from s_{20} and η. The results were 6.4×10^5 and 6.2×10^5, respectively, which are considered significant on a relative basis only. Since the slow moving component, which comprised the larger part of the histone hydrochloride sample in water solution, had a sedimentation rate too low for measurement in the ultracentrifuge its molecular weight of 18,000 was obtained for measurement of the osmotic pressure in 0.02 ionic strength phosphate buffer, pH 6.87, by the capillary rise method in an osmometer with stainless steel cell.[4] The histone hydrochloride concentrations used in the osmotic pressure measurements were 0.073, 0.11 and 0.22%. The molecular weight of histone hydrochloride in Santomerse D solution was calculated[2] from s_{20} and D_{20}. The value of 0.729 for the partial specific volume of histone hydrochloride[5] was used. The density of 0.35% Santomerse D solution at 20° was measured with a pycnometer of approximately 20-ml. capacity[6] and was found to be 1.00018 g./ml. The apparent specific volume of Santomerse D was calculated from the equation[7]

$$V_1^* = V - w_2 V_0 / w_1$$

where V and V_0 are the specific volumes (*i.e.*, $1/\rho$, where ρ is the density) of solution and solvent, respectively, and w_1 and w_2 are the concentrations of solute and solvent, respectively, in g./g. of solution. It was found to be 0.77. The apparent specific volume of histone hydrochloride in Santomerse D solution was calculated as 0.75 from weight fractions of 0.64 for Santomerse D and 0.36 for histone hydrochloride obtained in the quantity type rotor experiment of Table II. Because the dissociation tendency of the complex caused a decrease of s_{20} with decreasing histone hydrochloride concentration, the value of s_{20} for 0.157% protein concentration was used to calculate the molecular weight of 5.1×10^4. On the basis of a complex containing one histone molecule per molecule of complex, this corresponds to a weight fraction of 0.65 for Santomerse D. Area measurements of the "schlieren" pattern, Fig. 1, I, for the system containing 0.157% histone hydrochloride gave weight frac-

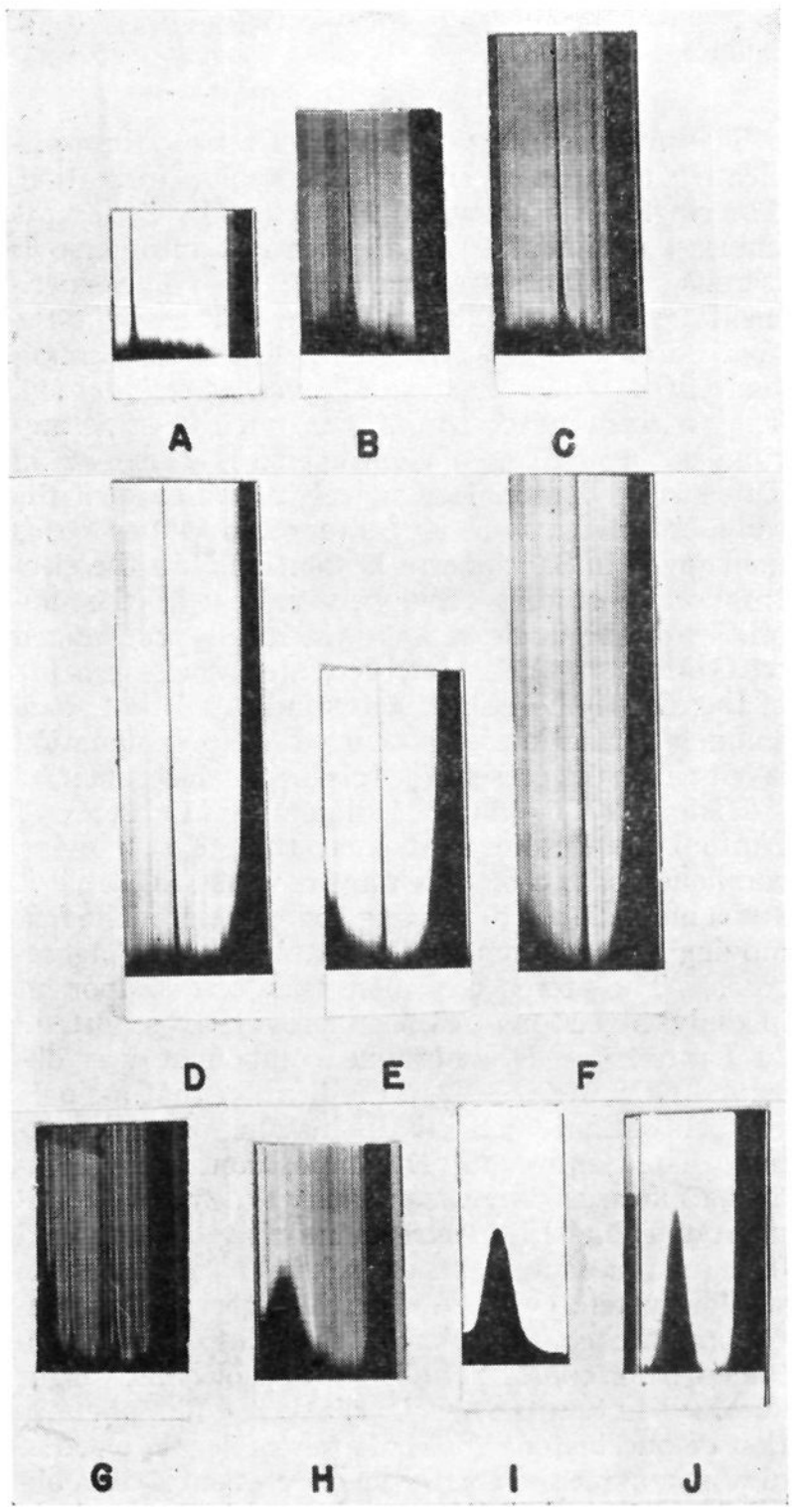

Fig. 1.—Ultracentrifuge "schlieren" scanning patterns of the sedimenting components in the tetrasodium nucleate and histone hydrochloride systems. A, B and C represent tetrasodium nucleate at percentages of 0.103, 0.155 and 0.31, respectively; D, E and F tetrasodium nucleate with 0.35% Santomerse D at nucleate percentages of 0.103, 0.155 and 0.31, respectively; G, 0.22% histone hydrochloride; H, I, J histone hydrochloride with 0.35% Santomerse D at histone hydrochloride percentages of 0.078, 0.157 and 0.32, respectively; direction of sedimentation, left to right.

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(5) L. Ahlström, *Arkiv Kemi, Mineral. Geol.*, **A24**, No. 31 (1947).

(6) M. R. Lipkin, J. A. Davison, W. T. Harvey and S. S. Kurtz, *Ind. Eng. Chem., Anal. Ed.*, **16**, 55 (1944).

(7) T. Svedberg and K. O. Pederson, "The Ultracentrifuge," Clarendon Press, Oxford, 1940, p. 59.

tions of 0.65 and 0.35 for Santomerse D and histone hydrochloride, respectively.

Discussion of Results

The ultracentrifuge behavior of tetrasodium nucleate shows no evidence of complex formation. The single peak shown in Fig. 1 (A, B, C) for the nucleate sedimenting in absence of Santomerse D is unchanged in its presence (D, E, F). The experiments with the quantity type rotor confirm the absence of complex formation. Under the conditions used no Santomerse D micelles sedimented. The intrinsic viscosity of the nucleate was unchanged in presence of Santomerse D, as shown in Table III. The molecular weight, which is of the same order of magnitude as reported values[8,9] was unchanged in Santomerse D solution. In the electrophoretic studies previously reported,[2] the mobilities of the nucleate were essentially unchanged by Santomerse D. However, area measurements of the electrophoretic patterns indicated that some complex formation had occurred. No explanation is offered at the present for this lack of agreement.

Histone hydrochloride sedimenting in absence of Santomerse D shows at least three fast moving components, two of which are reported in Table I, and a main slow sedimenting component. The fast moving components are probably histone aggregates. The presence of more than one component in dialyzed histone has been previously reported.[5] In Santomerse D, only one component was observed (Fig. 1, H, I, J), an indication that a single complex of Santomerse D and histone was sedimenting. The Santomerse D composition of the complex, as measured by the quantity type rotor experiment of Table II is in agreement with that obtained from area measurements of "schlieren" patterns of a similar system (Fig. 1). Further information concerning the composition of the complex was obtained from consideration of the molecular weight obtained in Santomerse D solution. The assumption of one histone molecule per molecule of complex is in agreement with the experimental data obtained in the present work.

A proposed structure for the histone hydrochloride–Santomerse D complex[10] assumes the binding of a monomolecular layer of detergent to the histone molecule by means of a primary ionic interaction, followed by binding of additional detergent molecules to the complex through attractive forces between the hydrocarbon chains of the detergent. The existence of a double layer of Santomerse D on the histone molecule would be consistent with the reversal of the charge by Santomerse D in electrophoresis,[2] the quantity type rotor experiments, and the results of area measurements of ultracentrifuge "schlieren" patterns.

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(8) H. G. Tennant and C. F. Vilbrandt, THIS JOURNAL, **65**, 424 (1943).

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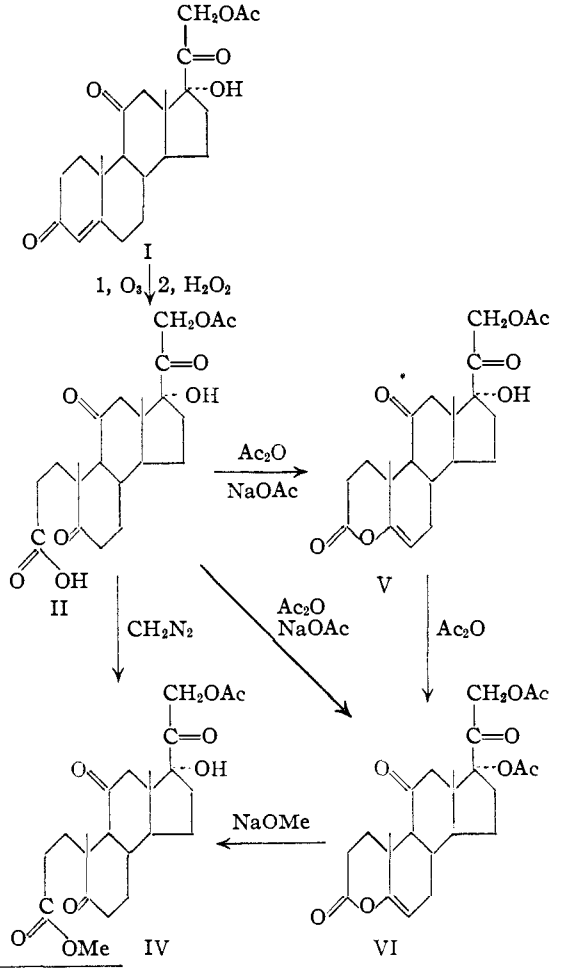
The Preparation and Properties of 4-Oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione[1]

BY A. H. SOLOWAY[2] AND D. K. FUKUSHIMA

RECEIVED JULY 2, 1953

In the course of investigations designed to introduce carbon-14 into the ring system of cortisone, the preparation of 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) was undertaken. Ozonolysis of cortisone acetate (I) followed by oxidation with hydrogen peroxide[3] yielded an acid, 3,5-seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnane-3-oic acid (II). The acid II was converted to an enol lactone in refluxing acetic anhydride in the presence of a small amount of sodium acetate. The product obtained proved to be 4-oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI).[4] With less prolonged heating, the 17α-hydroxyl group was not acetylated and 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) was produced. By heating with acetic anhy-



(1) This investigation was supported by grants from the Anna Fuller Fund, the Lillia Babbitt Hyde Foundation, the Teagle Foundation and the National Cancer Institute, United States Public Health Service.

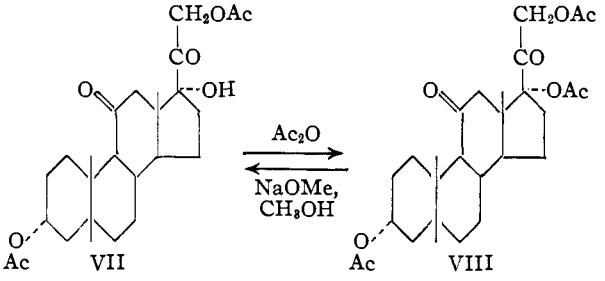
(2) Post-doctorate Fellow of the National Cancer Institute, United States Public Health Service.

(3) R. B. Turner, THIS JOURNAL, **72**, 579 (1950); C. C. Bolt, *Rec. trav. chim.*, **57**, 905 (1938).

(4) We wish to express our gratitude to Dr. George I. Fujimoto, University of Utah, Salt Lake City, Utah, for a sample of this compound.

dride as described by Turner[5] and Huang-Minlon, *et al.*,[6] V was converted to the diacetate VI. That no fundamental alteration of the molecule occurred under these conditions was demonstrated by conversion of VI to the methyl ester IV, with one mole of sodium methoxide in methanol.

This selective removal of the 17α-acetoxy group is a general reaction as evidenced by the ready conversion of 3α,17α,21-triacetoxypregnane-11,20-dione (VII) to 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione (VIII) under the same conditions without any indication for the formation of either the 17α,21-dihydroxy or 3,17,21-trihydroxy compound.



Experimental[7]

3,5-Seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnane-3-oic Acid (II).—A stream of approximately 6% ozone was passed through a solution of 3.0 g. of cortisone acetate in 400 ml. of ethyl acetate cooled in a Dry Ice–methanol bath. When the ozonolysis was complete, as evidenced by the oxidation of potassium iodide in a trap through which the effluent gases were passed, the reaction was discontinued and a solution of 4 ml. of 30% hydrogen peroxide and 4 ml. of methanol was added to the ethyl acetate solution. After standing at room temperature for 16 hours, the solvent was removed under reduced pressure. The oily solid was recrystallized from acetone–petroleum ether yielding 1.62 g. of rhombohedral crystals, m.p. 140–144°. Difficulty in recrystallization of the free acid prompted preparation of the hydrate which was obtained in long white needles from either aqueous methanol or aqueous acetone, m.p. 118–121°, $[\alpha]^{24}D$ +91.6° (ethanol). Both products had identical spectra in the region from 1150–850 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{30}O_8 \cdot H_2O$: C, 59.98; H, 7.31. Found: C, 59.98; H, 7.17.

The methyl ester of the acid was prepared with an excess of ethereal diazomethane and was recrystallized with difficulty from methanol. After recrystallization, long white needles, m.p. 154.5–156.5°, $[\alpha]^{23}D$ +99.1° (chloroform) were obtained. The hydrate likewise yielded this same methyl ester.

Anal. Calcd. for $C_{23}H_{32}O_8 \cdot {}^1/_2CH_3OH$: C, 62.37; H, 7.57. Found: C, 62.34; H, 7.43.

4-Oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI).—A slurry of 209 mg. of II in 15 ml. of acetic anhydride containing 53 mg. of anhydrous sodium acetate was heated to boiling under reflux for 1 hour. The product went into solution during heating and at the end of the reaction the acetic anhydride was removed by distillation under diminished pressure. The oily solid was dissolved in ether–ethyl acetate and extracted several times with water followed by dilute sodium bicarbonate solution and again with water, dried over anhydrous sodium sulfate, and the solvent was removed. The residue, 214 mg. of yellow oil, was chromatographed upon silica gel, and 95 mg., m.p. 200–220°, was obtained. After successive recrystallizations from acetone–petroleum ether 4-oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI) melted at 231–234°, $[\alpha]^{25}D$ −24° (chloroform).

Anal. Calcd. for $C_{24}H_{30}O_8$: C, 64.57; H, 6.77. Found: C, 64.97; H, 6.73.

4-Oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V).—A slurry of 2.01 g. of the keto acid II in 80 ml. of acetic anhydride containing 240 mg. of anhydrous sodium acetate was heated under reflux for 15 to 20 minutes. The acetic anhydride was removed under diminished pressure and the product was isolated as in the preceding section. After crystallization from acetone–petroleum ether 986 mg. of product, m.p. 200–220°, was obtained; recrystallization from the same solvents afforded prisms of 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V), m.p. 247–253°, $[\alpha]^{23}D$ +30° (chloroform).

Anal. Calcd. for $C_{22}H_{28}O_7$: C, 65.32; H, 6.73. Found: C, 64.95; H, 6.77.

4-Oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI) from V.—A solution of 107 mg. of 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) in 10 ml. of acetic anhydride was heated under reflux for 16 hours, and at the end of this time the acetic anhydride was removed under diminished pressure. The residual yellow oil after chromatography upon silica gel was recrystallized from acetone–petroleum ether to yield 99 mg. of VI, m.p. 231–234°, identical in all respects including infrared spectrum with the product prepared from the keto acid.

A solution of 25 mg. of the enol lactone V in 2 ml. of glacial acetic acid and 2 ml. of acetic anhydride containing 26 mg. of *p*-toluenesulfonic acid monohydrate was allowed to stand at room temperature for 60 hours. The solution was diluted with ethyl acetate, extracted with water, 10% sodium bicarbonate solution and again with water and dried over anhydrous sodium sulfate. The solvent was removed and the product crystallized from acetone–petroleum ether to yield 19 mg. of 4-oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI) identical in all respects including infrared spectrum with the product obtained in the preceding reaction.

Methyl 3,5-Seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnanoate (IV) from VI.—A stream of nitrogen was passed through a solution of 30 mg. of 4-oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione in 3 ml. of methanol, and to this was added 1 ml. of 0.67 *M* sodium methoxide in methanol (1 equivalent). The solution was stirred for 5 minutes by means of the nitrogen stream and at that point, 1 ml. of water was added. After an additional 3 minutes, 1 ml. of glacial acetic acid was added and the solution was extracted with ether. The ether solution was washed with water, dilute sodium bicarbonate and water and dried over anhydrous sodium sulfate. The solvent was removed and after chromatography on silica gel followed by recrystallization from methanol, 21 mg. of methyl 3,5-seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnanoate (IV) was obtained, m.p. 154.5–156.5°. The product was identical in all respects with that obtained directly from the esterification of the keto acid II.

3α,21-Diacetoxy-17α-hydroxypregnane-11,20-dione (VII) from 3α,17α,21-Triacetoxypregnane-11,20-dione (VIII).—In the manner described in the preceding experiment, 49 mg. of 3α,17α,21-triacetoxypregnane-11,20-dione was converted with sodium methoxide to 16 mg. of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione, m.p. 228–232°. The mother liquors contained the triacetate VIII as shown by the infrared spectra but there was no indication of any 3α-acetoxy-17α,21-dihydroxypregnane-11,20-dione.

Acknowledgments.—We wish to thank Dr. T. F. Gallagher for his valuable suggestions and discussion of this work. We are indebted to Friederike Herling for the determination and interpretation of infrared spectra.

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NEW YORK, NEW YORK

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(7) All melting points are corrected.

The Use of the Schlieren Optical System for Sampling after Preparative Angle Ultracentrifugation[1]

BY SAM SOROF

RECEIVED JUNE 22, 1953

The present communication demonstrates that

(1) Supported by a grant from the Damon Runyon Memorial Fund for Cancer Research, and an institutional grant from the American Cancer Society.

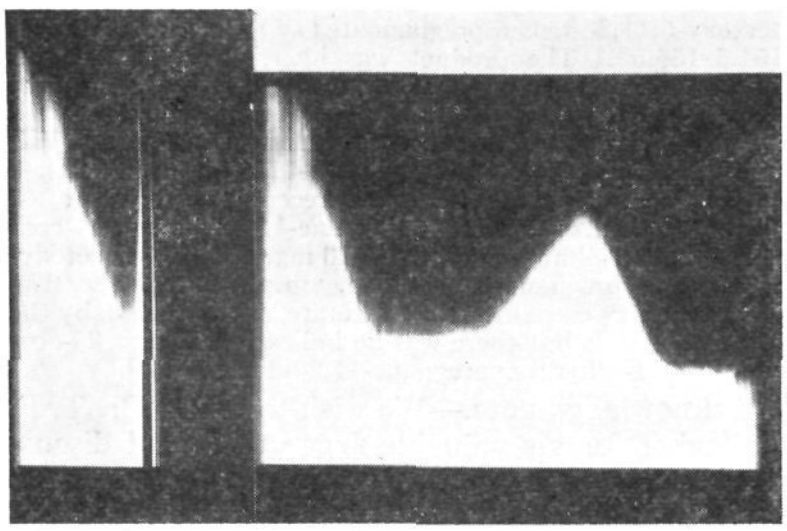
the schlieren optical system of Philpot[2] and Svensson[3] may be used successfully for the optically controlled sampling of partly sedimented proteins from lusteroid tubes after preparative angle ultracentrifugation. The method is of general applicability to proteins irrespective of their color or biological activity. It has yielded reproducible protein fractions of high ultracentrifugal purity when applied to the isolation of the slowest sedimenting constituent from a mixture of proteins.

Previous investigators have demonstrated that relatively sharp, partly sedimented boundaries of proteins can be obtained in the preparative tubes after angle ultracentrifugation. This fact has been demonstrated by subsequent chemical or biological analyses on samples arbitrarily removed from successive levels of solution in the preparative tubes (Hughes, Pickels and Horsfall[4]), and also by the absorption of light by the blue protein, hemocyanin (Pickels[5]). The position of the partly sedimented boundary in the angle ultracentrifuge tube may be used to calculate an approximate sedimentation rate.

The present study serves to demonstrate the general application of the schlieren optical system in sampling after preparative ultracentrifugation. The example chosen is the isolation in high ultracentrifugal purity of the A component,[6] consisting chiefly of albumin, from human plasma.

Experimental

Prior to angle ultracentrifugation in the Spinco Ultracentrifuge, Model E,[7] each lusteroid preparative tube was tested individually for the absence of optical imperfections and for the presence of a straight baseline in the schlieren optical system of Philpot and Svensson of the Perkin–Elmer electrophoresis apparatus, model 38.[8] The water-filled Spinco tube, $^5/_8$ inch diameter by 3 inches long, capped, and fastened in the preparative tube clamp, was placed in a preset vertical position in optical alignment with the vertical slit at the first schlieren lens. The slit width used was $^5/_8$ inch, *i.e.*, equal to the width of the preparative tube. The tube was examined in the schlieren optical system throughout a rotation of 90°, and the best orientation so determined was marked on the tube 90° to the pathway of light. Only tubes with good optical characteristics were used subsequently.



b a

Fig. 1.—(a) Cylindrical lens schlieren pattern of diluted human plasma in lusteroid tubes after preparative angle ultracentrifugation (see text). (b) Same as a, after removal of the trailing portion of the partly sedimented proteins of the A component.

Irradiated human plasma,[9] which had been stored at −17°, was diluted with two volumes of cold 0.15 *M* sodium chloride containing 0.02 *M* sodium phosphate at *p*H 7.40. Analytical ultracentrifugation of a dialyzed aliquot of the diluted plasma displayed the presence of the A, G and 20-components[6] (3.20 mg. nitrogen per ml. in 0.15 *M* sodium chloride containing 0.02 *M* sodium phosphate at *p*H 7.40). Subsequently, preparative lusteroid tubes were filled with more of the undialyzed diluted plasma, capped, and then oriented in a cold 26° angle preparative rotor A so that the above mentioned marks on the tubes were closest to the axis of the rotor. This was done in order to minimize any optical effect of deformity of the tubes resulting from ultracentrifugal stresses. The solution was then spun for 7.5 hours in an evacuated and refrigerated chamber at 50,740 r.p.m. with a relative centrifugal force in the center of the tubes equal to 152,000 × *g*. Subsequently, the rotor was slowly decelerated and the rotor and tubes carefully handled to minimize disturbance of the protein boundary. The tubes were placed one at a time into the pre-set position in the bath of the electrophoresis apparatus, examined, and recorded photographically as in Fig. 1a. A well-resolved, single peak, corresponding to the slowest sedimenting A component observed earlier in the ultracentrifugal analysis, is seen. The protein "pile-up" gradient in the lower part of the tube is shown at the left end of the pattern of Fig. 1a. A needle ($3^1/_2$" #18), attached to a syringe through a metallic three-way stopcock adapter,[10] was then entered slowly through the hole of the cap to a depth in the solution just above the "pile-up" gradient without any visible disturbance to the boundary. The syringe was mounted on a holder containing a rack and pinion for fine vertical motion of the needle. Removal of the solution above the needle tip was accomplished by means of the movement of a fine screw attached to the head of the plunger of the syringe. Figure 1b shows the resultant pattern after removal of all the solution above the tip of the needle. (Sufficient light to be photographed does not penetrate the tube above the level of the meniscus.) That extremely little, if any, protein was sucked up from below the needle tip is attested

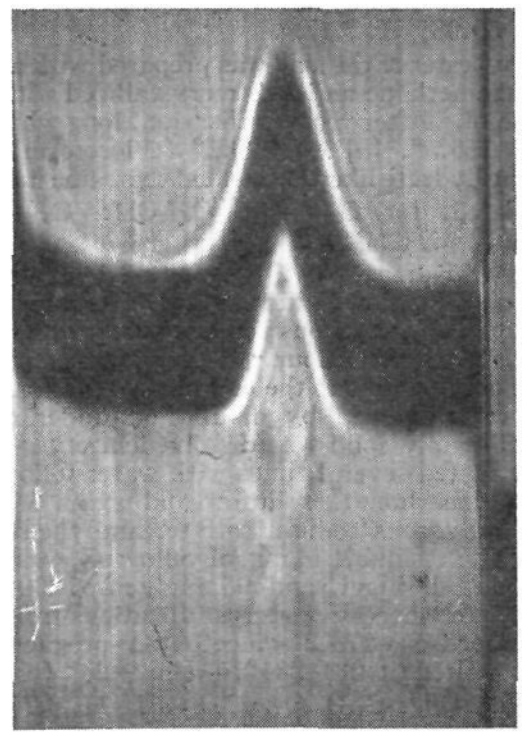


Fig. 2.—Analytical ultracentrifuge pattern of isolated A component: 0.95 mg. nitrogen per ml., 81 minutes at 59,780 r.p.m., 32° bar angle.

(2) J. S. L. Philpot, *Nature*, **141**, 283 (1938).

(3) H. Svensson, *Kolloid-Z.*, **87**, 181 (1939); **90**, 141 (1940).

(4) T. P. Hughes, E. G. Pickels and F. L. Horsfall, *J. Exp. Med.*, **67**, 941 (1938).

(5) E. G. Pickels, *J. Gen. Physiol.*, **26**, 341 (1943).

(6) K. O. Pedersen, "Ultracentrifugal Studies on Serum and Serum Fractions," Almqvist and Wiksells Boktryckeri AB, Uppsala, 1945.

(7) Manufactured by the Specialized Instruments Corp., Belmont, Calif.

(8) Manufactured by the Perkin-Elmer Corp., Norwalk, Conn.

(9) Obtained through the kindness of Sharp and Dohme, Philadelphia, Pa., Lot No. 32335.

(10) Manufactured by Becton, Dickinson and Co., Rutherford, N. J.

by the unchanged nature of the "pile-up" gradient after withdrawal.

In order to determine the purity of the isolated slow sedimenting fraction, a sample of the removed solution was dialyzed against 0.15 *M* NaCl containing 0.02 *M* sodium phosphate at *p*H 7.40, and then examined in the analytical rotor. The resultant pattern, shown in Fig. 2, displayed the A component and a barely perceptible trace of the faster G component. The former exhibited a corrected sedimentation constant, $s^0_{20} = 4.09\ S$, which agrees with the literature.[6,11]

We have found this technique to be reproducible with respect to the nature of the schlieren patterns of the protein solutions obtained in the preparative tubes, the sampling of the fractions, and the analytical ultracentrifugal purity of the proteins isolated. In addition, such use of the schlieren optical system permits the determination of the location of partly sedimented protein boundaries after preparative angle ultracentrifugation without resort to chemical or biological analysis of isolated fractions. In summary, sampling controlled by observation in the schlieren optical system has a general applicability to colorless as well as colored proteins, and when applied to the slowest sedimenting component of a mixture, is capable of reproducibly yielding isolated proteins of high ultracentrifugal purity by mild physical means.

Acknowledgment.—It is a pleasure to acknowledge the asistance of Mr. Richard H. Golder and Mrs. Marilyn G. Ott in this work.

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(11) See: G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951); G. L. Miller and R. H. Golder, *Arch. Biochem. and Biophys.*, **36**, 249 (1952); J. F. Taylor, *ibid.*, **36**, 357 (1952).

COMMUNICATIONS TO THE EDITOR

THE ENZYMIC SYNTHESIS OF TREHALOSE PHOSPHATE[1]

Sir:

Uridine diphosphate glucose (UDPG)[2] has been found to disappear when incubated with a yeast extract and glucose monophosphate. This disappearance may be measured by estimating UDPG by its coenzymatic activity[3] and also as a decrease in acid-labile glucose. During the reaction UDP is formed and the reducing power of the mixture decreases. As shown in Table I, these changes are equivalent and do not take place when any one of the reactants is added at the end of the incubation period.

TABLE I

ANALYTICAL CHANGES PRODUCED BY THE ENZYME

Incubation of 0.4 μmole of glucose-6-phosphate, 0.6 μmole of UDPG and 0.02 ml. of enzyme in 0.14 *M* tris-(hydroxymethyl)-aminomethane buffer of *p*H 7 during 100 minutes at 37°; total volume, 0.1 ml.; results expressed in μmoles. The enzyme was obtained by disintegrating brewer's yeast cells with sand in a 50 cycles per second oscillator. After centrifuging the supernatant was made 0.5 saturated with ammonium sulfate and the precipitate was dialyzed.

Sample	Substance omitted during incubation[a]	Δ Reducing power[b]	Δ Labile glucose[c]	Δ UDP[d]
1	Glucose-6-phosphate	0	−0.04	+0.02
2	UDPG	0	0	0
3	None	−0.13	−0.14	+0.14

[a] The substance omitted was added at the end of the incubation period. The Δ values represent the difference with sample 2. [b] Calculated as glucose. [c] Hydrolyzed 10 minutes at pH 2 followed by precipitation with zinc sulfate and barium hydroxide. Practically all the glucose liberated under these conditions is that of UDPG. [d] Estimated by a method based on the reaction: phosphopyruvate + UDP → pyruvate + UTP (A. Kornberg, in "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 392). Pyruvate measured colorimetrically.

Samples equal to those shown in Table I were submitted to fractionation of the barium salts. The water-soluble, alcohol-insoluble fractions were used for paper electrophoresis with borate buffer[4] and the phosphate containing compounds were subsequently developed with a molybdate spray reagent.[5] The experiment showed that sample 3, but not samples 1 or 2, contained a phosphate compound which migrated at 60% the rate of glucose-6-phosphate. Dephosphorylation of this compound with kidney phosphatase produced a substance which gave the same R_f value as trehalose when chromatographed on paper.

In other experiments the reaction products were deproteinized by heating, treated with charcoal in order to remove the nucleotides and submitted to the action of phosphatase. When chromatographed on paper a substance migrating like trehalose was found to be present in sample 3 but not in the others. The substance extracted from the paper was hydrolyzed in 1 *N* acid during 3 hours at 100° and compared chromatographically with trehalose treated in the same manner. In both cases a glucose and a trehalose spot were obtained.

The solvent used for paper chromatography was pyridine–ethyl acetate–water[6] with which trehalose, saccharose, maltose and lactose can be separated and the developer was an alkaline silver reagent[7] which reacts slowly with non-reducing disaccharides. Furthermore, reducing from non-reducing sugars can be distinguished because only the latter give color with the aniline-phthalate spray reagent.[8] Thus the ester appears to be a phosphate of trehalose which is presumably identical to that iso-

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(2) These abbreviations will be used: UDPG for uridine diphosphate glucose, UDP for uridine diphosphate, and UTP for uridine triphosphate.

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