methylcyclohexanol in the presence of aluminum chloride.[18]

The structures of several of the remaining polynuclear compounds were determined by dehydrogenation to known crystalline aromatic hydrocarbons using a platinum–alumina catalyst at 250–350°.

The **phenyldecahydronaphthalene V** yielded product, m.p. 92–95°, which on recrystallization from alcohol melted at 100–101°. 2-Phenylnaphthalene is reported to melt at 100–102°, 1-phenylnaphthalene at 84–86°.[19] Attempts to isolate product melting at about 85° by fractional crystallization were unsuccessful. Hence, V was apparently principally 2-phenyldecahydronaphthalene.

Compound VIII yielded yellow needles melting at 205–207° which did not depress the melting point of anthracene. Since analysis indicated that the formula of VIII was $C_{14}H_{18}$, it may be concluded that it was an octahydroanthracene.

The lower boiling **tetrahydronaphthyltetrahydronaphthalene (IXa)** gave yellow crystals, m.p. 73–74°. The higher boiling material (IXb) yielded product of m.p. 180–181°. The approximate melting points reported in the literature[20] for the binaphthlyls are: 1,1′-, 156°; 1,2′-, 76°; and 2,2′-, 187°. Fractional crystallization of the products failed to yield any material melting near 156°. It may be concluded that IX consists of 6-(1,2,3,4-tetrahydro-2-naphthyl)-1,2,3,4-tetrahydronaphthalene and either or both 6-(1,2,3,4-tetrahydro-1-naphthyl)- and 5-(1,2,3,4-tetrahydro-2-naphthyl)-1,2,3,4-tetrahydronaphthalene. While the relative amounts of these isomers were not determined, it appeared from the fractionation data that the 6-(1,2,3,4-tetrahydro-2-naphthyl)- isomer was present in largest amount.

The phenylbicyclohexyl (Xa) liquid fraction yielded a crystalline product which on fractional crystallization gave 2 parts of white flakes, m.p. 205–206°, and 8 parts of white needles, m.p. 83–85°. Dehydrogenation of the crystalline phenylbicyclohexyl Xb yielded product, m.p. 204–205°. The literature[21] values for the melting points of 1,2-, 1,3- and 1,4 diphenylbenzene are, respectively, 55–58°, 84–89° and 205–214°. Hence, X was a mixture of 3- and 4-phenylbicyclohexyl, the latter being a crystalline compound, Xb.

(18) L. Schmerling, unpublished results.

(19) G. Egloff, "Physical Constants of Hydrocarbons," Vol. IV, Reinhold Publishing Corp., New York, N. Y., 1947, p. 226.

(20) G. Egloff, ref. 19, Vol. IV, p. 317.

(21) G. Egloff, ref. 19, Vol. III, p. 473.

DES PLAINES, ILLINOIS

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Structure of Chloretyl, the Product of the Reaction between Chloral and Biacetyl

BY NELSON J. LEONARD, JOHN C. LITTLE[1] AND A. JERRY KRESGE[2]

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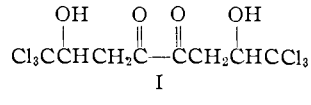
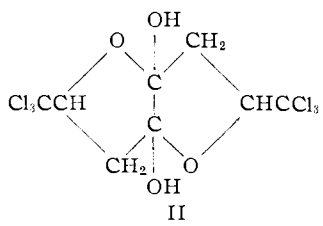
Spectral and chemical evidence has been presented to establish the structure of chloretyl, the product of the reaction between chloral and biacetyl, as 3,7-di-(trichloromethyl)-2,6-dioxabicyclo[3.3.0]octane-1,5-diol (II). The stereochemical structures of the α- and β-isomers of chloretyl (racemates) have been tentatively assigned as *cis–cis–cis* (VI) and *cis–cis–trans* (VII).

In 1952, a product of molecular formula $C_8H_8Cl_6O_4$ was obtained by Schlenk[3] from the reaction of chloral with biacetyl, in 2:1 molar proportion, in the presence of piperidinium acetate. The structure of the colorless solid, m.p. 199–201° dec., was not established, and the suggested formulations of the compound as a diketone dihydrate or a monohydrate of a hydroxyketone were not fully satisfactory. The property of the compound that was difficult to accommodate in postulated structures was the absence of absorption in the ultraviolet and visible regions. We reasoned that if the initial reaction product were conceived as normal (I), simple conversion to a bis-hemiketal would provide a structural expression II, 3,7-di-(trichloromethyl) - 2,6 - dioxabicyclo[3.3.0]octane - 1,5 - diol, which would satisfy the data presented by Schlenk for the $C_8H_8Cl_6O_4$ compound. Our postulate has been confirmed by infrared absorption and nuclear magnetic resonance studies and by additional chemical evidence. Moreover, we are in a position to consider the stereochemistry of the predominant product and its isomers.



I



II

We used conditions for the reaction between chloral and biacetyl similar to those of Schlenk. However, we were able to isolate two isomeric compounds, $C_8H_8Cl_6O_4$: an α-isomer capable of existing in readily interconvertible dimorphic forms, m.p. *ca.* 206° dec. and *ca.* 175° dec., and a β-isomer, m.p. 175–177°, in lower yield. As a convenience, we wish to use the name "chloretyl" (klôrĕtĕl) for these products, or specifically, "α-chloretyl" and "β-chloretyl."

The infrared absorption spectra of the dimorphic forms of α-chloretyl were virtually identical as Nujol mulls and identical as 1% solutions in benzene. The spectrum of the β-isomer was similar but not identical to the α-chloretyl spectrum. The spectra of both isomers confirmed the absence of olefinic and carbonyl unsaturation and indicated the presence of hydroxyl. The spectrum of solid α-chloretyl showed a sharp hydroxyl band at 3480 cm.$^{-1}$ and that of β-chloretyl at 3450 cm.$^{-1}$ (Nujol mull). There were also present in the 9–11 μ region absorption bands resembling those exhibited by tetrahydrofuran. The most logical structure for chloretyl, consistent with the precursors in synthesis and the ana-

(1) National Science Foundation Fellow, 1954–1957.

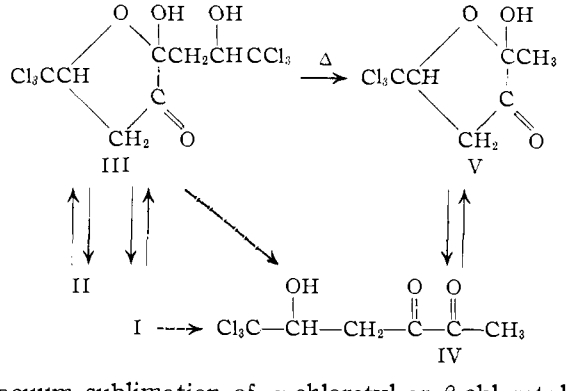
(2) Eli Lilly and Co. Fellow, 1952–1953.

(3) H. Schlenk, *Ber.*, **85**, 901 (1952). We wish to record our appreciation for recent conversations with Dr. Schlenk concerning the structure and chemistry of this product.

lytical and spectral data, is given by II. As further physical confirmation of this structure, the NMR spectrum, determined in dioxane solution,[4] showed clearly (Fig. 1) the ratio of 1:1:2 for the different types of hydrogen in α-chloretyl, *i.e.*, 2 hydroxyl hydrogens: 2 CH–CCl_3 hydrogens: 4 methylene hydrogens.

Chemical confirmation of structure II was found in the positive identification of the hemiketal hydroxyl function. Although the usual acetylation methods failed,[3] it was possible to obtain the monoacetate by prolonged heating of α-chloretyl with isopropenyl acetate in benzene solution in the presence of *p*-toluenesulfonic acid. The high frequency of the C=O stretching band (1765 $cm.^{-1}$ in the infrared spectrum of α-chloretyl monoacetate was indicative of the $-O-\overset{|}{\underset{|}{C}}-O-\overset{O}{\overset{\|}{C}}-CH_3$ system. This argument is based upon the general finding that an electronegative substituent on the α-carbon of the alcohol portion of an ester increases the carbonyl absorption frequency and the specific observation that the model compound 1,1-diacetoxypropane has a carbonyl absorption maximum at 1761 $cm.^{-1}$.[5] The mono-3,5-dinitrobenzoate of α-chloretyl also exhibited ester carbonyl absorption in this region, at 1764 $cm.^{-1}$. Attempts to combine both hemiketal hydroxyls of II in ketal formation with methanol, ethylene glycol, acetone or cyclopentanone were not successful.

The presence of an easily accessible α-diketone grouping in α-chloretyl was demonstrated in the reactions with *o*-phenylenediamine, hydroxylamine and phenylhydrazine. The quinoxaline, dioxime and osazone formed were derivatives of 6,6,6-trichlorohexan-5-ol-2,3-dione (IV). Under the usual reaction conditions chloretyl apparently reverts to the intermediate IV by losing the chloral moiety from either the acyclic I or monocyclic III form. The hemiketal form V of IV was obtained in the



vacuum sublimation of α-chloretyl or β-chloretyl. The suggested structure, 2-methyl-5-trichloromethyl-1-oxacyclopentan-2-ol-3-one (V), is based mainly on analysis and spectrum. Oxidation of chloretyl with hydrogen peroxide in acetic acid has been found to give 4,4,4-trichloro-3-hydroxybutyric acid in low yield,[3] consistent with any sequence leading to III, I or IV, followed by oxidation. The isomeric relation of α-chloretyl and β-chloretyl was shown by the production of the same quinoxaline (of IV) and the same monocyclic compound (V) from either precursor.

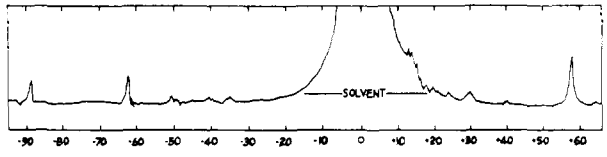
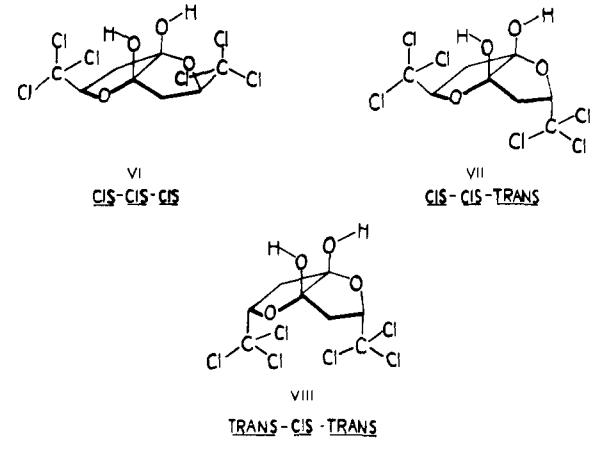


Fig. 1.—Nuclear magnetic resonance shift relative to reference (parts per million): $(H - H_r/H_t)$; frequency 30 mc.; zero of reference, dioxane.

In considering the possible stereochemical structural assignments of α- and β-chloretyl, if it is recognized that in the synthetic procedure an energetically favorable ring closure completes the formation of the bicyclic system (*e.g.*, I or III → II), it follows that forms of II having a *trans* ring juncture can be excluded on the basis of analogy with the greater stability of *cis*-bicyclo[3.3.0]octane over the *trans* isomer.[6,7] Among the possible representations of chloretyl having a *cis* ring juncture (VI, VII, VIII; one form used to designate each racemate), the *trans–cis–trans* structure VIII is the least favored, since it is the only one in which the trichloromethyl groups are in steric apposition. Moreover,



the infrared O–H stretching frequency exhibited by a structure like VIII should be in the normal free hydroxyl range, whereas both isomers of chloretyl which have been isolated had infrared hydroxyl bands at lower than normal frequency, suggestive of the proximity of chlorine to hydroxyl hydrogen,

(4) By Dr. James N. Shoolery and staff of Varian Associates, Palo Alto, Calif., on the commercial Varian instrument. We are grateful to Dr. Shoolery for his interest and his help. It is possible that dioxane might conceal some peaks of the compound, so that reliance must be placed on other information available as well.

(5) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949).

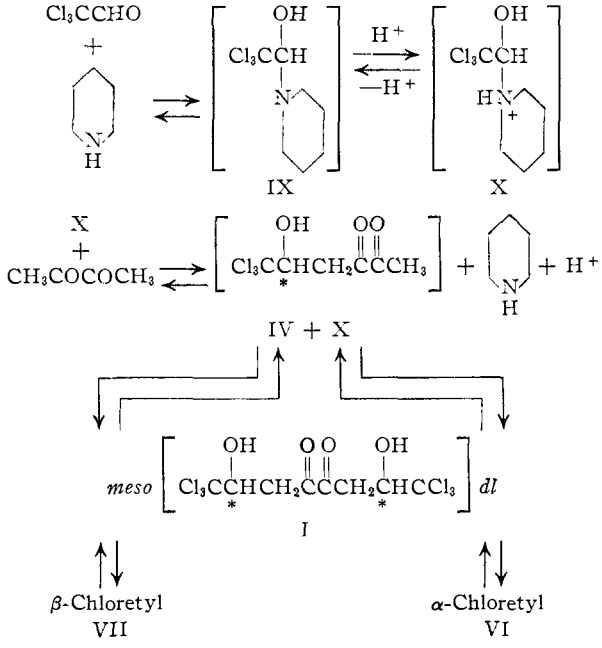
(6) J. W. Barrett and R. P. Linstead, *J. Chem. Soc.*, 611 (1936).

(7) For a discussion of the relatively greater stability of the *cis* form in another model ring system, 3,7-dioxabicyclo[3.3.0]octane, a moiety of sesamin and asarinin, see M. Beroza and M. S. Schechter, THIS JOURNAL, **78**, 1242 (1956); *cf.* A. Michael and J. Ross, *ibid.*, **55**, 3684 (1933). A. C. Cope and T. Y. Shen, *ibid.*, **78**, 5916 (1956), have recently demonstrated highly stereospecific ring closures to *cis*-2,6-dioxabicyclo[3.3.0]octanes.

as in the examples of the chloroalcohols[8-10] and chlorophenols.[11,12]

On the basis of the foregoing arguments, the remaining problem lies in the assignment of the proper geometrically-isomeric structures, VI and VII, to α- and β-chloretyl. In our tentative assignment, reliance has been placed again upon the infrared spectral effect of an adjacent chlorine atom, which is to lower the frequency of the free hydroxyl O–H stretching vibration,[10,12] as exemplified by the following series: $CH_3CH_2CH_2OH$, 3636 cm.$^{-1}$; CCl_3CH_2OH, 3597 cm.$^{-1}$; and $(CCl_3)_2CHOH$, 3584, 3534 cm.$^{-1}$; $CCl_3(C_2Cl_5)CHOH$, 3571, 3509 cm.$^{-1}$ (in carbon tetrachloride solution).[8] Dilute solutions of the two chloretyl isomers in benzene were examined in the 3 μ region, and maxima were observed at 3550 cm.$^{-1}$ for β-chloretyl and 3510 cm.$^{-1}$ for α-chloretyl. No splitting of either band was observed. The difference is considered real and suggests that in α-chloretyl the equivalent hydroxyls are in a more electronegative environment than they are in β-chloretyl. This situation would be satisfied if α-chloretyl were assigned the *cis–cis–cis* structure (VI), with effectively two chlorines adjacent to the hydroxyls, and β-chloretyl, the *cis–cis–trans* structure (VII), with one chlorine exerting attraction for the hydroxylic hydrogens. Consistent with the infrared findings is the qualitative observation that α-chloretyl appears to be the thermodynamically more stable isomer. Thus, reactions of chloral with biacetyl which were allowed to run for 36 hr. gave approximately equal quantities, although in low yield, of α- and β-chloretyl, while reactions which were allowed to proceed to near completion (11 days) produced the α-isomer almost exclusively, in yields approaching 80%. These rough observations suggest that a rate *vs.* equilibrium process is occurring, in which the α- and β-isomers are formed randomly at first and a conversion of β- to α-chloretyl takes place subsequently and more slowly. Since a reversible process exists whereby the γ-isomer VIII could revert to VI, it is logical that VIII would not survive even if it were formed initially. We found no evidence of the presence of a third isomer in the crude reaction products.

Of the several reaction schemes which can be postulated for the formation of chloretyl, the formula diagram representation (I to X) accounts satisfactorily for the presently available facts. The intermediate IX is similar to the benzylidene-bis-piperidine intermediate proposed in condensations involving benzaldehyde and a piperidine catalyst.[13] The equilibrium between the hydroxyamine IX and the corresponding bis-amine probably lies predominantly on the side of the hydroxyamine because of the great stability of the hydroxyl group in this system, as in chloral hydrate, chloral hemiacetals and other examples.[14-20] The reaction of chloral with piperidine (or morpholine) at about 50° is vigorous and leads to the formation of the N-formyl derivative,[21] probably by elimination of



chloroform from IX. This possible side reaction in the chloretyl preparation was repressed by chilling the reaction mixture prior to the addition of piperidine. Participation of the protonated intermediate X in the aldol condensation with biacetyl is envisaged with the loss of piperidine and the formation of IV. The key step in the reaction sequence then becomes the condensation of IV with a second chloral intermediate to form either *dl*- or *meso*-I. The *meso* form is a precursor of β-chloretyl, the racemate in which the methinyl carbons are of opposite configuration, and the *dl* form is a precursor of α-chloretyl, one of the racemates (the other is the non-isolable γ-isomer) in which the methinyl carbons have the same configuration. Accumulation of α-chloretyl because of slightly greater stability would be possible in the reversible scheme which has been pictured.

Biological Activity[22]

A. Insecticidal.—α-Chloretyl and its monoacetate were found to induce instantaneous reversible paralysis upon injection (dioxane solution) into houseflies. Dosages of as low as 0.01 μg. per fly produced immediate paralysis lasting at least 45

(8) R. N. Haszeldine, *J. Chem. Soc.*, 1757 (1953).

(9) O. Bastiansen, *Acta Chim. Scand.*, **3**, 415 (1949).

(10) L. R. Zumwalt and R. M. Badger, THIS JOURNAL, **62**, 305 (1940).

(11) O. R. Wulf, U. Liddel and S. B. Hendricks, *ibid.*, **58**, 2287 (1936).

(12) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1939, pp. 300 ff.

(13) W. Dilthey and B. Stallmann, *Ber.*, **62**, 1603 (1929), and work in progress in this Laboratory.

(14) F. Caujolle, P. Couturier and C. Dulaurens, *Bull. soc. chim. France*, [5] **17**, 19 (1950).

(15) F. Caujolle, P. Couturier and M. Doumerc, *ibid.*, [5] **17**, 22 (1950).

(16) P. Couturier, *ibid.*, [5] **17**, 25 (1950).

(17) M. Ikawa and K. P. Link, THIS JOURNAL, **72**, 4373 (1950).

(18) H. Gault and G. Mennicken, *Compt. rend.*, **229**, 1239 (1949).

(19) K. Matsumura and M. Ito, THIS JOURNAL, **77**, 6671 (1955).

(20) J. Décombe, *Compt. rend.*, **237**, 336 (1953).

(21) G. B. L. Smith, M. Silver and E. I. Becker, THIS JOURNAL, **70**, 4254 (1948); E. Merck, German Patent 334,555.

(22) We wish to thank Professor C. W. Kearns, Department of Entomology, University of Illinois, for making the results of the entomological tests available to us, and Dr. K. K. Chen, The Lilly Research Laboratories, Eli Lilly and Co., for the pharmacological results.

minutes. By contrast, α-chloretyl mono-3,5-dinitrobenzoate and β-chloretyl were inactive.

B. Pharmacological. Anticonvulsant.—α-Chloretyl showed 40% protection at 400 mg./kg. by the Electroshock method and 25% protection by the Metrazol method (same dosage) upon oral administration to rats. **Hypnotic.**—Oral administration of from 400 to 900 mg./kg. to rats produced no hypnosis.

Experimental[23]

Condensation of Chloral with Biacetyl.[24]—To a chilled solution of 20.0 g. (0.233 mole) of biacetyl, 70 g. (0.48 mole) of chloral and 9.0 g. (0.15 mole) of glacial acetic acid in 70 ml. of anhydrous benzene, 8.4 g. (0.10 mole) of piperidine was added dropwise. The system was swept with nitrogen and stirred at 25° for 10 days. The precipitate was collected by filtration and washed with hexane. The crude chloretyl (37.2 g.) was dissolved in 120 ml. of warm dioxane, and the solution was diluted with water. The mixture was chilled with vigorous shaking until the chloretyl crystallized. The solid was broken into fine particles, and the mixture was filtered. After washing with cold 50% aqueous methanol and drying, the **α-chloretyl** weighed 36.5 g. (41%), m.p. *ca.* 203° dec. An analytical sample was prepared by recrystallization from benzene–hexane as colorless needles, m.p. *ca.* 206° dec. from a hot or acidic (with a trace of acetic acid) solution or m.p. *ca.* 175° dec. from a cool basic (a trace of pyridine) solution.

Anal. Calcd. for $C_8H_8Cl_6O_4$: C, 25.23; H, 2.12. Found: C, 25.24; H, 2.14.

The infrared absorption spectrum showed maxima (Nujol mull) at 3480 (OH); 1292, 1149, 1095, 1080, 1048, 1013; 814, 790 cm.$^{-1}$ (CCl_3); (1% solution in benzene) at 3510 (OH); 1392, 1294, 1250, 1135, 1106, 950, 910, 836; 812, 801 cm.$^{-1}$ (CCl_3). The compound in cyclohexane solution was transparent in the ultraviolet region, 215–400 mμ.

The mother liquor and washings from the α-chloretyl isolation were diluted with benzene. The combined liquid was washed thoroughly with water, followed by a saturated aqueous sodium chloride solution. The organic layer was dried over anhydrous magnesium sulfate and treated with decolorizing carbon. Filtration yielded a light yellow-orange solution from which the solvent was removed in vacuum to yield an orange solid. The solid was suspended in boiling hexane, and the mixture was filtered. After washing and drying, the solid obtained (14.6 g., 17%) was found to be a mixture of approximately 90% α- and 10% β-chloretyl. The latter was separated and purified by fractional crystallization from benzene–hexane, with treatment with decolorizing carbon. An analytical sample of **β-chloretyl** was prepared by recrystallization from benzene–hexane as colorless needles, m.p. 175–177°, different from the isomorphic form of α-chloretyl, m.p. 175° dec.

Anal. Calcd. for $C_8H_8Cl_6O_4$: C, 25.23; H, 2.12. Found: C, 25.39; H, 2.18.

The infrared absorption spectrum showed maxima (Nujol mull) at 3450 (OH); 1288, 1264, 1192, 1173, 1142, 1130, 1110–1085, 1044, 1018, 925, 903, 864, 840; 810–790 cm.$^{-1}$ (CCl_3); (1% solution in benzene) at 3550 (OH); 1395, 1368, 1320, 1270, 1205, 1145, 935, 903, 868, 837; 809 cm.$^{-1}$ (CCl_3).

Both isomers of chloretyl were instantly soluble in cold, dilute sodium hydroxide,[25] soluble in cold methanol and ethanol, somewhat soluble in benzene and insoluble in hexane and water. Chloretyl decomposed upon prolonged heating in hydroxylic solvents but appeared to be stable in crystalline form when pure.

Quinoxaline Formation.—A mixture of 0.50 g. (1.31 mmoles) of α-chloretyl and 0.16 g. (1.48 mmoles) of *o*-phenylenediamine in 15 ml. of absolute ethanol was heated under reflux for 8 hr. and then allowed to stand overnight. Dilution with water yielded 0.39 g. (97%) of red-brown platelets, m.p. 147–148°. Sublimation or recrystallization from aqueous ethanol gave ivory platelets, m.p. 148–149°.

Anal. Calcd. for $C_{12}H_{11}Cl_3N_2O$: C, 47.16; H, 3.63; N, 9.17; Cl, 34.80. Found: C, 47.22; H, 3.61; N, 9.10; Cl, 34.72.

The analysis and infrared spectrum (including maxima at 3200, 1104 and 794–810 cm.$^{-1}$) were consistent with the formulation of this product as **2-[1′-(2′-hydroxy-3′,3′,3′-trichloropropyl)]-3-methylquinoxaline.**

β-Chloretyl was converted to the quinoxaline by heating 85 mg. (2.24 mmoles) of this isomer with 27 mg. (2.5 mmoles) of *o*-phenylenediamine in 4 ml. of absolute ethanol for 6 hr. Dilution with 4 ml. of water and cooling caused the separation of 47 mg. (69%) of ivory platelets, which were recrystallized from aqueous ethanol, m.p. 147–148°, identical by the usual criteria with the product described above.

Oxime Formation.—A mixture of 1.00 g. (2.63 mmoles) of α-chloretyl, 0.42 g. (6.0 mmoles) of hydroxylamine hydrochloride, 5 ml. of absolute ethanol and 5 ml. of pyridine was heated under reflux for 12 hr. Evaporation of solvent yielded a brown residue which upon trituration with water formed heavy granular crystals, m.p. 186–187° dec. Sublimation yielded colorless crystals, m.p. 188–189° dec.

Anal. Calcd. for $C_6H_9Cl_3N_2O_3$: C, 27.35; H, 3.44; N, 10.63. Found: C, 27.93; H, 3.57; N, 10.38.

The analysis and infrared spectrum (including maxima at 3560, 3210, 3070 and 1090 cm.$^{-1}$) were consistent with the formulation of the product as **6,6,6-trichlorohexan-5-ol-2,3-dione dioxime.**

Osazone Formation.—A mixture of 0.50 g. (1.31 mmoles) of α-chloretyl, 0.35 g. (3.24 mmoles) of phenylhydrazine and a few crystals of sodium acetate in 15 ml. of 95% ethanol was heated under reflux for 3 hr., diluted with water and allowed to stand at 5° overnight. The solid which separated was recrystallized from aqueous ethanol as small orange needles, m.p. 164–165°. The infrared absorption spectrum (Nujol mull) exhibited maxima at 3300, 1608, 1575 and 1100 cm.$^{-1}$ among others.

Anal. Calcd. for $C_{18}H_{19}Cl_3N_4O$: C, 52.25; H, 4.63; N, 13.56. Found: C, 52.26; H, 4.59; N, 13.30.

On the basis of analysis and infrared spectrum, this product can be assigned the structure **6,6,6-trichlorohexan-5-ol-2,3-dione bis-phenylhydrazone.**

α-Chloretyl Monoacetate.—A mixture of 8.05 g. (21.1 mmoles) of α-chloretyl, 5.00 g. (50.0 mmoles) of isopropenyl acetate and a few crystals of *p*-toluenesulfonic acid in 50 ml. of benzene was heated under reflux for 20 hr. The solvent was removed *in vacuo*, and the residue was dissolved in 95% ethanol. The solution was treated with decolorizing carbon, filtered, and the filtrate was diluted with water. The solid α-chloretyl monoacetate was collected and dried, m.p. 91–94°, yield 6.19 g. (73%). Purification by sublimation or recrystallization from aqueous ethanol resulted in colorless crystals, m.p. 93–94°.

Anal. Calcd. for $C_{10}H_{10}Cl_6O_5$: C, 28.40; H, 2.38; Cl, 50.30; mol. wt., 422.9. Found: C, 28.55; H, 2.56; Cl, 50.88; mol. wt., 423, 444 (Rast, camphor).

The infrared absorption spectrum showed maxima (Nujol mull) at 3040 (OH); 1765 (C=O); 1255, 1210, 1184, 1125, 1085, 1053, 1016, 965, 935, 912, 897, 871, 840; 814, 805 cm.$^{-1}$ (CCl_3).

α-Chloretyl Mono-3,5-dinitrobenzoate.[26]—To 4 ml. of pyridine was added 0.22 g. (1.04 mmoles) of 3,5-dinitrobenzoic acid and 0.41 g. (2.16 mmoles) of *p*-toluenesulfonyl chloride. The mixture was cooled to 0°, and 0.20 g. (0.53 mmole) of α-chloretyl was added. The mixture was kept at 0° for 8 hr. and was then diluted with ice-water. The solid which separated was recrystallized from aqueous ethanol as a light tan powder, m.p. *ca.* 208° dec., yield 0.12 g. (30%). Repeated recrystallization from dioxane–ethanol–water yielded nearly colorless platelets, m.p. 215° dec.

(23) Melting points are corrected and boiling points are uncorrected. We are indebted to Mrs. Lucy Chang, Mrs. Ruby Ju, Mrs. R. Maria Benassi, Miss Claire Higham and Mr. Joseph Nemeth for the microanalyses and to Mrs. Louise Griffing, Mr. James Brader and Mr. Cy Portnow for determination of the infrared spectra, using a Perkin–Elmer automatic recording infrared spectrometer, model 21.

(24) All reagents were purified.

(25) Equilibration with the open form(s), I and/or III, would liberate the trichloromethylcarbinol group, which is acidic. A pK'_a value of 11.8 has been reported for trichloroethanol.[8] The cause of the solubility may also reside in a hydrolysis reaction of the trichloromethyl group, since Schlenk found chloride ion to be liberated when α-chloretyl was treated with aqueous sodium carbonate.[3]

(26) J. H. Brewster and C. J. Ciotti, Jr., THIS JOURNAL, **77**, 6214 (1955).

Anal. Calcd. for $C_{15}H_{10}Cl_6N_2O_9$: C, 31.33; H, 1.75; N, 4.87; Cl, 37.00. Found: C, 31.92; H, 1.83; N, 4.68; Cl, 36.40.

The infrared spectrum showed maxima (5% in chloroform) at 1764 (C=O); 1560, 1351 (NO_2); 1274, 1161, 1130, 1082, 1020, 966; 815 cm.$^{-1}$ (CCl_3).

Decomposition of Chloretyl on Heating in Vacuum.—When either α- or β-chloretyl was sublimed at 135–140° (0.2 mm.), a small amount of unstable colorless compound, m.p. 102° dec., was obtained as the more volatile fraction. This substance was tentatively assigned the structure V, **2-methyl-5-trichloromethyl-1-oxacyclopentan-2-ol-3-one**, on the basis of analysis and infrared absorption spectrum: ν_{max}^{Nujol} 3355 (OH); 1751 (C=O); 1185, 1156, 1139, 1099, 995, 936, 911, 869; 799 cm.$^{-1}$ (CCl_3).

Anal. Calcd. for $C_6H_7Cl_3O_3$: C, 30.86; H, 3.02. Found: C, 30.89; H, 2.82.

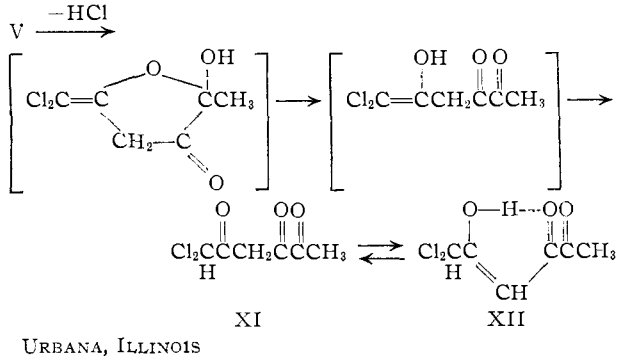
This material soon decomposed on standing at 25° to a yellow oil, with apparent loss of HCl. The infrared spectrum of the oil is consistent with the structure XI, **6,6-dichlorohexane-2,3,5-trione**, which probably exists largely as the enol XII.[27] The infrared spectrum showed maxima (5% in chloroform) at 3600–3000 (broad absorption with much fine structure, characteristic of bonded OH); 1720 (C=O); 1642 (conj. C=O); 1602 (C=C); 1423–1416, 1365, 1320, 1240–1213, 1115, 1098, 977, 840 cm.$^{-1}$.

Preparation of 2,2,2-Trichloroethyl 3,5-Dinitrobenzoate.[26]—To a cold solution of 1.42 g. (6.7 mmoles) of 3,5-dinitrobenzoic acid and 2.54 g. (13.6 mmoles) of *p*-toluenesulfonyl chloride in 20 ml. of cold dry pyridine was added 1.0 g. (6.7 mmoles) of 2,2,2-trichloroethanol. The mixture was allowed to stand at 0° for 1.5 hr. and was poured into 300 ml. of ice-water with vigorous stirring. The resultant peach-colored solid was collected by filtration and recrystallized from 95% ethanol to yield 1.55 g. (68%) of ivory platelets, m.p. 128–131°. Treatment with decolorizing carbon and repeated recrystallization from ethanol yielded an analytical sample of white platelets, m.p. 140–141°.

Anal. Calcd. for $C_9H_6Cl_3N_2O_6$: C, 31.45; H, 1.48; N, 8.15. Found: C, 31.70; H, 1.58; N, 8.33.

The infrared spectrum (5% in chloroform) showed maxima at 1755 (C=O); 1634, 1603 (aromatic); 1554, 1351 (NO_2); 1274, 1163 (C–O); 835 cm.$^{-1}$ (non-enhanced CCl_3). It will be noted that the position of the C=O maximum is at lower frequency than that exhibited by α-chloretyl mono-3,5-dinitrobenzoate.

(27) The formation of XI may be conceived as



URBANA, ILLINOIS

COMMUNICATIONS TO THE EDITOR

THE ENZYMATIC CONVERSION OF MEVALONIC ACID TO SQUALENE

Sir:

The recent isolation and identification of mevalonic acid (DL-β,δ-dihydroxy-β-methylvaleric acid, MVA) a new precursor of cholesterol[1–3] constitute an important advance in the understanding of terpene and steroid biogenesis. In this communication, we wish to report the transformation of mevalonic acid to squalene by soluble yeast enzymes and to describe experiments bearing on the mechanism of MVA utilization. Particle-free extracts of dried baker's yeast have been prepared which catalyze the conversion of DL-2-C^{14}-MVA to a radioactive lipid, shown to be squalene by alumina chromatography and by preparation of the thiourea adduct[4] and of the crystalline hexahydrochloride.[5] No sterols are synthesized from MVA under these conditions. Dialysis and treatment with charcoal to remove cofactors render the yeast extract completely inactive. Activity is restored by addition of Mn^{++}, ATP[6] and pyridine nucleotide (Table I). The reaction proceeds anaerobically as well as in air. Experiments bearing on the mechanism of the condensation process have been carried out with 2-C^{14},5-di-T-mevalonic acid. Tritium was introduced by partial reduction of β-hydroxy-β-methylglutaric acid with $LiAlT_4$. The purified product, when combined with authentic DL-2-C^{14}-MVA and recrystallized several times as the dibenzylethylenediamine salt or chromatographed on paper, showed no change in the T:C^{14} ratio. Squalene synthesized by yeast extracts from the doubly labeled MVA contained T and C^{14} in the same ratio as the precursor (Table II). From the work of Tavormina and Gibbs,[7] it is known that C_1 of mevalonic acid is lost during the conversion to cholesterol and hence the isoprenoid chain must be formed by condensations linking C_5 of one MVA residue (or a derivative thereof) to C_2 of another. The retention of T during squalene formation clearly rules out a preliminary oxidation of mevalonic acid to β-hydroxy-β-methylglutaric acid (or a derivative). Though the unchanged T:C^{14} ratio suggests that both hydrogen atoms at C_5 (δ-carbon) of MVA are

(1) D. E. Wolf, C. H. Hoffman, P. E. Aldrich, H. R. Skeggs, L. D. Wright and K. Folkers, THIS JOURNAL, **78**, 4499 (1956).

(2) P. A. Tavormina, M. H. Gibbs and J. W. Huff, *ibid.*, **78**, 4498 (1956).

(3) L. D. Wright, *Federation Proc.*, **16**, 274 (1957).

(4) N. Nicolaides and F. Eaves, THIS JOURNAL, **76**, 2590 (1954).

(5) I. M. Heilbron, E. D. Kamm and W. M. Owens, *J. Chem. Soc.*, 1630 (1926).

(6) The following abbreviations are used: DPN and TPN, di- and tri-phosphopyridine nucleotide; ATP, adenosine triphosphate.

(7) P. A. Tavormina and M. H. Gibbs, THIS JOURNAL, **78**, 6210 (1956).