single bond due to the adjacent $>P{=}O$ bond in the formula III (as it is the case with carboxylic acid amides), it appears improbable that such a decrease in intensity of the $>C{=}O$ band occurs. Thus, this region of the spectrum suggests formula IV as the reaction product.

In the range of the H valency vibrations the reaction product has several absorption maxima. The sharp maxima at 3.51 μ and at 3.40 μ with a weak shoulder at 3.33 μ are caused by the methyl groups. However, the band at 3.28 μ can no longer be assigned to a methyl group. Because of its longer wave length and sharpness this band cannot be an O–H band. Therefore, it corresponds to the C–H valency vibration of the $>C{=}CH$ group.[9,10] Thus, an additional proof is given for formula IV.

In the spectra of isomeric O,O-dialkyl phosphates and O,O-dialkyl phosphonates,[11] the maxima of the $>P{=}O$ band are 7.8 and 8.1 μ, respectively. The $>P{=}O$ band of the phosphonates dissolved in CS is split into a doublet, whereas in the phosphates the band is slightly displaced toward a shorter wave length while its form is preserved. The $>P{=}O$ band of the reaction product is at 7.8 μ, and after dissolving this substance in CS_2 it is split into a doublet at 7.67 and 7.81 μ. Therefore, this region of the spectrum is additional evidence for structural formula IV.

This rearrangement of O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate is of considerable theoretical interest as it represents the first reported instance of such transformation of a phosphonate to a phosphate.

The phosphoric acid ester IV is considerably more toxic to rats, mice and houseflies than the phosphonate II. Thus, the LD-50 values for IV are: rat oral 25 mg./kg., mouse subcutaneous 25 mg./kg.; and the LD-100 values to the housefly were: contact 0.01 γ and oral 0.005 γ, as opposed to 0.1 γ and 0.03 γ, respectively, for II.

Experimental Part

O,O-Dimethyl 2,2,2-Trichloro-1-hydroxyethylphosphonate (II) was prepared following the procedures described by Lorenz.[3] These differ from the method later described by Barthel, *et al.*,[4] only in that a reaction temperature of 120–130° was used, and that the product was recrystallized from water.

O,O-Dimethyl O-(2,2-Dichloroethenyl) Phosphate (IV) from Ester II by Alkaline Treatment.—One hundred and twenty-nine grams (0.5 mole) of O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate is dissolved in 500 ml. of water and warmed to 30°. A solution of 20 g. (0.5 mole) of sodium hydroxide in 50 ml. of water is added dropwise with stirring. The temperature rises and O,O-dimethyl O-(2,2-dichloroethenyl) phosphate (IV) separates as a colorless oil. The oil is dissolved in benzene, washed twice with ice-water and then dried with sodium sulfate (sicc.). On fractionating the benzene solution 54 g. of IV having the boiling point 86–87° at 3 mm. are obtained; yield 58% of the theoretical; $n^{20}{}_D$ 1.4541; $d^{20}{}_4$ 1.423.

Anal. Calcd. for $C_4H_7O_4Cl_2P$: Cl, 32.09; OCH_3, 28.08. Found: Cl, 31.34; OCH_3, 28.36.

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(9) V. Z. Williams, *Rev. Sci. Instr.*, **19**, 143 (1948).

(10) W. Brügel, "Einführung in die Ultrarotspektroskopie," Verlag von Dr. Dietrich Steinkopff, Darmstadt, 1954, p. 209.

(11) A detailed report will appear in *Zeitschrift für Naturforschung.*

The Complete Degradation of Carbon-14 Labeled Succinic Acid and Succinic Anhydride by the Schmidt Reaction[1]

BY E. F. PHARES AND MARY V. LONG

RECEIVED NOVEMBER 11, 1954

Succinic acid labeled with isotopic carbon previously has been degraded completely by the use of various combinations of enzymatic and chemical steps[2] and by the Curtius reaction.[3] These methods are laborious, and the last procedure gave less than 30% yield of the methylene carbons.

A partial degradation employing the Schmidt reaction as applied to fatty acid degradation[4] has been used by Strassman and Weinhouse,[5] and at this Laboratory. A 70% yield of carbon dioxide from the carboxyl carbons is obtained by this method, but the recovery of ethylenediamine, derived from the methylene carbons, is only 10% when steam distillation is used for isolation. The same yield was reported when precipitation of the picrate derivative was employed.[6] With these low recoveries, carbon-14 specific activity values for the methylene carbons usually must be calculated by difference.[5]

The present paper presents: (a) a procedure which increases the recovery of ethylenediamine from the Schmidt reaction by vacuum distillation, and (b) an additional step involving conversion of the succinic acid to the anhydride prior to degradation, which considerably increases the yield of both products.

When vacuum distillation was used for separation of the ethylenediamine, 45% yields of products having satisfactory specific activities were obtained (Table I). Excellent agreement of melting points of dibenzenesulfonamide and dibenzamide derivatives of this product with the literature val-

TABLE I

CARBON RECOVERIES AND SPECIFIC ACTIVITIES

Starting compd.	Yield,[a] % CO_2	Yield,[a] % Ethylenediamine[e]	Relative C^{14} Specific activities[b] Carboxyl	Relative C^{14} Specific activities[b] Methylene
Sodium succinate-2-C^{14}	75	45	0.28	101.0 ± 1.6[c]
Sodium succinate-1-C^{14}	63	42	99.0	0.56
Succinic anhydride-2-C^{14}	84[d]	73	0.10	97.0
Succinic anhydride-1-C^{14}	85[d]	64	97.9	0.05
Succinic anhydride	95	71		
	91	71		
	82	78		

[a] Assayed as barium carbonate. [b] Total molecule × 2 = 100. [c] Standard deviation of six degradations. [d] Over-all yields from succinic acid. [e] Isolation by vacuum distillation.

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(1) Work performed at the Oak Ridge National Laboratory under Contract Number W-7405-Eng-26 for the Atomic Energy Commission.

(2) (a) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 253–255; (b) S. F. Carson, J. W. Foster, W. E. Jefferson, E. F. Phares and D. S. Anthony, *Arch. Biochem. Biophys.*, **33**, 448 (1951).

(3) A. A. Benson and J. A. Bassham, THIS JOURNAL, **70**, 3939 (1948).

(4) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951).

(5) M. Strassman and S. Weinhouse, THIS JOURNAL, **74**, 3457 (1952); *ibid.*, **75**, 1680 (1953).

(6) M. Oesterlin, *Z. angew. Chem.*, **45**, 536 (1932)

ues, and lack of melting point depression when mixed with authentic derivatives indicate that ethylenediamine was the only volatile amine formed in the reaction.

Considerable increases in yields of both products were obtained when succinic acid was converted to succinic anhydride prior to degradation (Table I). Data are given for the complete degradation of sodium succinate through succinic anhydride to carbon dioxide and ethylenediamine, and isotopic data for each labeled species. The specific activities agree favorably with those from the direct degradation of sodium succinate. The cross-contamination values for the anhydride degradation are considerably lower than for succinic acid, indicating a possible removal of radioactive impurities by conversion to anhydride.[7]

This procedure also has been used for the degradation of fumaric acid, after reduction to succinic acid. This latter procedure was considerably less laborious than the enzymatic degradation of fumaric acid, and the results were more clear cut than with permanganate oxidation.

Experimental

Degradation of Succinic Acid or Sodium Succinate.—A solution of 0.25 mM succinic acid or sodium succinate was evaporated to dryness in a 15-ml. pear-shaped flask and further dried in an oven at 105° for about 30 minutes. The flask was stoppered and cooled, 0.25 ml. of 38 N fuming sulfuric acid was added with shaking and warming, until the sample was nearly dissolved. The resulting sulfuric acid solution was well chilled under the tap and 50 mg. of sodium azide was added, with shaking to disperse the azide crystals. The flask was quickly attached to a train containing an acid permanganate scrubber and an alkali trap, and the tip of the flask was warmed slowly until the reaction started. The reaction usually proceeded vigorously without further application of heat. When the rapid gas evolution subsided, the flask was placed in a water-bath at 70° which was brought to boiling over a period of several minutes, following which the system was swept for 5–10 minutes with carbon dioxide-free air. The carbonate in the alkali trap was precipitated with barium chloride and filtered on a porous porcelain disc for weighing and radioactivity assay. The acid residue was cooled and completely mixed with about 1 ml. of water, whereupon the flask was chilled, and about 2 volumes of saturated sodium hydroxide for each volume of sulfuric acid were added slowly, with cooling (an excess of about 1 drop of alkali was added past the phenol red end-point). The alkaline solution was frozen in a thin layer on the side of the flask by use of liquid nitrogen, distilled *in vacuo*, and the distillate was collected in a trap immersed in liquid nitrogen. After the flask had warmed to room temperature, it was immersed in warm water and pumping was continued for 15 minutes. The distillate was then thawed and titrated to the acid end-point of chlor phenol red. Wet oxidation was carried out on the ethylenediamine with persulfate–silver nitrate mixture, and the resulting carbon dioxide was handled in the same manner as that from the carboxyl carbons. Radioactivity of samples containing more than 0.01 μc. of C^{14} was assayed by use of a G–M counter at near-infinite thickness of barium carbonate, the over-all counting accuracy being $\pm 3\%$. The very low activity samples, measuring the cross-contamination, were assayed by gas counting using a vibrating-reed electrometer.

A blank degradation gave 2 mg. of barium carbonate from CO$_2$ production and 5 mg. from ethylenediamine production and oxidation.

Procedure *via* Succinic Anhydride.—One-fourth mM of dry sodium succinate or succinic acid and 15λ (0.17 mM) of POCl$_3$ were heated in a 15-ml. pear-shaped flask, attached to a drying tube, by immersing the tip of the flask in a 200° bath for 10 minutes. The heating bath was then replaced by ice-water, and the sides of the flask were carefully heated with a small flame to drive the succinic anhydride back into the tip of the flask. The anhydride, mixed with chlorides and phosphates, was degraded by the procedure used for succinic acid. The increased reactivity of the anhydride made necessary more careful control of the first warming, as well as traps which would handle a rapid flow of gas. With quantities in excess of 0.25 mM, cooling was often required to control the reaction.

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Pseudocholesterol. I. Preparation and Configuration

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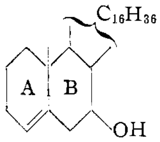
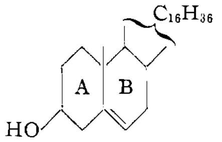
Pseudocholesterol (I) has maintained a measure of interest over a number of years largely because the positions of the functional groups in the A and B rings are the reverse of those in cholesterol (II).



I



II

As has been observed with other complex molecules, some of the generalizations of organic chemistry do not hold when applied to cholesterol and its derivatives. These physical and chemical properties, exceptional in the cholesterol system, if studied in the pseudocholesterol system, could well provide a measure of the degree to which the anomalous observations in the cholesterol series are dependent upon the relative conformation of that portion of the molecule considerably removed from the reactive centers.

Before such a study could be undertaken it was necessary to establish that compound I did, in fact, have the structure proposed. If I possessed a 7α-hydroxyl group rather than the indicated 7β-hydroxyl group, I would be an analog of epicholesterol rather than cholesterol and hence comparison of its properties with those of cholesterol would not have the significance that would obtain from a comparison of I and II.

That the unique nature of some of the cholesterol reactions is repeated in pseudocholesterol has not been demonstrated previously, although an attempt to repeat the *i*-steroid rearrangement with pseudocholesterol has been intimated.[2]

All previously reported[3,4] syntheses of I appear to have resulted in the isolation of an impure substance. Our preparation of pure I proceeded through a modification of the sequence used by the earlier investigators.

In our hands repeated recrystallization of crude I

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[7] Unpublished results of V. F. Raaen at this Laboratory indicate that higher cross-contamination, 2.5 to 3%, of CO$_2$ by the methyl carbon of acetic acid degraded by the Schmidt method occurs when gas counting is used without prior precipitation as BaCO$_3$.

[1] Taken, in part, from the M.A. Thesis of C. T. Chen, Wesleyan University, 1954.

[2] C. W. Shoppee and H. G. R. Summers, Abstracts of Papers, 120th Meeting of the American Chemical Society, New York City, 1951, p. 8L.

[3] A. Windaus and C. Resau, *Ber.*, **48**, 851 (1915).

[4] A. Ogata and I. Kawakami, *J. Pharm. Soc. Japan*, **58**, 738 (1938).