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 ±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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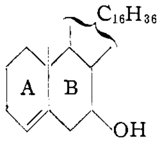
[7] Unpublished results of V. F. Raaen at this Laboratory indicate that higher cross-contamination, 2.5 to 3%, of CO_2 by the methyl carbon of acetic acid degraded by the Schmidt method occurs when gas counting is used without prior precipitation as $BaCO_3$.

Pseudocholesterol. I. Preparation and Configuration

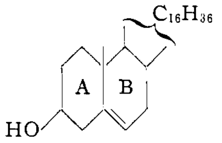
BY QUENTIN R. PETERSEN AND C. T. CHEN[1]

RECEIVED NOVEMBER 12, 1954

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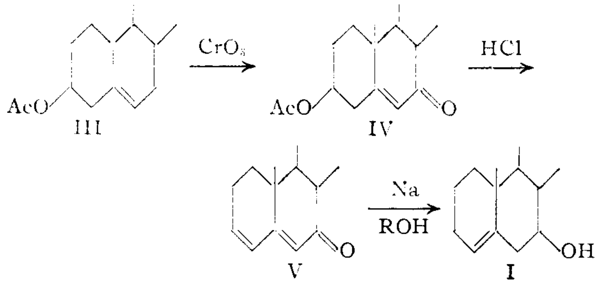
[1] Taken, in part, from the M.A. Thesis of C. T. Chen, Wesleyan University, 1954.

[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).

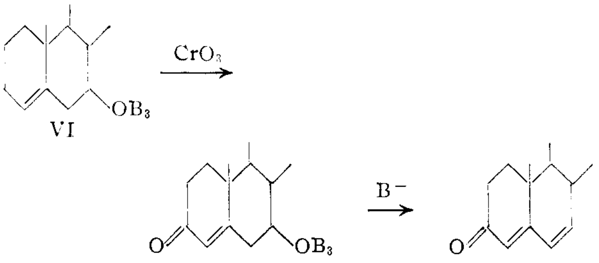


gave many fractions melting variously at 110 to 118° and having rotations of from +50 to +80°. The highest constants reported prior to this work were m.p. 116–117° and $\alpha]_D$ +67°.[4] Benzoylation of I followed by chromatography of the benzoate gave a pure ester (VI) which was hydrolyzed to pure I, m.p. 122.5–124°, $[\alpha]^{27}D$ +90°.

The position of the hydroxyl group in I is apparent from the position of the keto group from which it was derived. The location of the double bond was demonstrated by Windaus,[3] who replaced the hydroxyl group by treatment of I with phosphorus pentachloride and then reduced the resulting chloride with sodium in alcohol. From these reactions he obtained cholest-4-ene which was characterized by its dibromide.

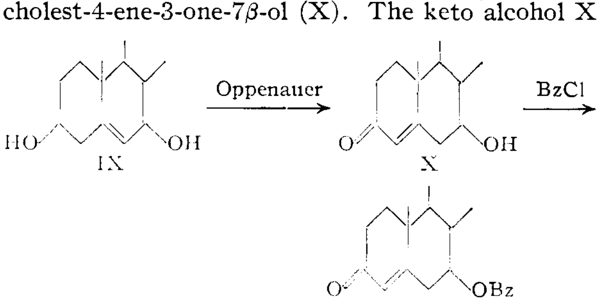
Thus the functional groups in I are seen to bear the same positional relationship to one another as they do in II, and the only problem remaining is that of the orientation of the hydroxyl group.

The approach used in the establishment of this configuration also demonstrated that reactions VI → VII → VIII, analogous to the sequence III → IV → V, could be carried out in the pseudocholesterol series.



VII, m.p. 165–167°, $[\alpha]^{26}D$ +79° VIII

The production of VII proved to be the key to the configuration of I. The keto benzoate VII, with identical properties, has been reported by Greenhalgh, *et al.*,[5] to result from the benzoylation of cholest-4-ene-3-one-7β-ol (X). The keto alcohol X was prepared by the British workers through an Oppenauer oxidation of 7β-hydroxycholesterol (IX) as shown below. The 3β,7β-configuration of the diol IX has been unequivocally demonstrated by Fieser and Heymann.[6]



VII, m.p. 165–167°,[5] $[\alpha]^{16}D$ +81°[5]

Thus VII and, by inference, I possess the 7β-configuration.[7]

We also have found that the attempted direct oxidation of 7β-benzoyloxycholesterol by the Oppenauer method resulted in the production of cholesta-4,6-dien-3-one (VIII) confirming the work of Greenhalgh.[5]

The attempted hydrolysis of VII to X was not successful, even under the mildest of basic conditions,[8] only the dienone VIII being recovered from each endeavor.

Acknowledgment.—The authors are pleased to acknowledge a Frederick Gardner Cottrell grant which supported a portion of the work described in this paper. The Schering Corp. is thanked for some of the microanalytical determinations reported.

Experimental[9]

Cholest-3,5-dien-7-one (V).—To a solution of 10.0 g. of 7-ketocholesteryl acetate[10] in 200 ml. of absolute alcohol was added 10 ml. of 6 *N* hydrochloric acid and the resulting solution refluxed. A bright yellow color was produced after about 20 minutes of reflux. After one hour, reflux was stopped and distilled water was added to the solution to the point of incipient turbidity. The solution was seeded and at once began to crystallize. Upon reaching room temperature the solid was filtered to give 8.0 g. (89%) of pale yellow crystals, m.p. 112–113°. The mother liquor produced a further 0.6 g. of the dienone. Recrystallization from 95% alcohol raised the m.p. to 113–114°, $[\alpha]^{28}D$ −305° (*c* 2.4). The reaction also was run in methanol, giving essentially similar results.

Reduction of Cholest-3,5-dien-7-one with Sodium.—Chunks of sodium metal totaling 35.0 g. were added, at one time, to a solution of 13.3 g. of cholest-3,5-dien-7-one in 400 ml. of isopropyl alcohol. As the reaction proceeded the solution changed from yellow to orange to green to very dark green. Reflux temperature was provided by the reaction for about one hour, after which heat was necessary to keep the solution refluxing and to keep the sodium isopropoxide from solidifying. After five hours a small piece of unreacted sodium was removed and the remaining solution was poured into water. The resulting yellow mixture containing a small amount of solid was acidified with 12 *N* hydrochloric acid. At neutrality a quantity of solid precipitated. The mixture was cooled and filtered to yield a crop of yellow-brown crystals which were washed well with water. After drying, the solid weighed 10.50 g. (79%) and melted at 105–115°. This material could be purified by recrystallization from acetone–water or alcohol–water to give fractions melting in two-degree ranges between 110 and

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(5) C. W. Greenhalgh, H. B. Henbest and E. R. H. Jones, *J. Chem. Soc.*, 2375 (1952).

(6) L. F. Fieser and H. Heymann, *Helv. Chim. Acta*, **35**, 631 (1952).

(7) After this manuscript had been submitted, R. J. W. Cremlyn, R. W. Rees and C. W. Shoppee, (*J. Chem. Soc.*, 3790 (1954)) described the hydrogenation of impure pseudocholesterol and subsequent isolation of the known cholestan-7β-ol. These workers therefore preceeded us in establishment of the 7β-configuration for pseudocholesterol. However, the pseudocholesterol obtained by these workers contained the levorotatory impurities common to all previous preparations. The physical properties of the free alcohol and its benzoate were identical with the properties of the compounds prepared by Windaus. The impurities present effected lowering of the m.p. of the acetate to 34° below that described in this communication. We are at present investigating the nature of the levorotatory contaminating material.

(8) Q. R. Petersen, This Journal, **77**, 1743 (1955).

(9) Melting points were taken on the hot-stage of a polarizing microscope, and are corrected. Rotations taken in chloroform. Microanalyses by The Schering Corp. and Schwarzkopf Microanalytical Laboratory.

(10) L. F. Fieser, This Journal, **71**, 2226 (1949).

118°. Optical rotation of these samples varied from +50 to +80°.

Cholest-4-ene-7β-ol Benzoate (VI).—A solution of 31 g. of the crude reduction product in a mixture of 500 ml. of pyridine and 66 ml. of benzoyl chloride was stored at 10° for 24 hours. The reaction mixture then was poured into a solution of 80 ml. of 12 *N* hydrochloric acid in 1 l. of water. The benzoate was extracted with a total of 1250 ml. of Skellysolve B and this non-aqueous extract washed with dilute hydrochloric acid, water, 10% sodium bicarbonate, and finally water. The neutral yellow hexane solution was dried over Drierite and shaken during one-half hour with 250 g. of alumina. The alumina was filtered off to give a colorless filtrate. When evaporated the filtrate deposited 24.7 g. of white solid which was crystallized from an ether–methanol pair. The product, weighing 13.5 g. (33%), consisted of glistening, thick needles, m.p. 158-160°, $[\alpha]^{26}\text{D}$ +94° (*c* 0.558).

Anal. Calcd. for $C_{34}H_{50}O_2$: C, 83.21; H, 10.27. Found: C, 83.35; H, 10.11.

Cholest-4-ene-7β-ol (I).—To 0.68 g. of the benzoate VI was added 50 ml. of a 95% ethanol solution containing 4.0 g. of potassium hydroxide. The resulting solution was refluxed for five hours. After this time water was added to the hot solution until turbidity was produced. After overnight cooling the solution had deposited 0.43 g. (86%) of small prisms, m.p. 122–124°. Recrystallization from acetone–water gave needles, m.p. 122.5-124, $[\alpha]^{26}\text{D}$ +90 (*c* 0.628).

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.89; H, 12.00. Found: C, 83.67; H, 11.78.

The acetate was prepared in the usual fashion giving mica-like plates, m.p. 96-97°, $[\alpha]^{27}\text{D}$ +78° (*c* 2.200).

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.09; H, 11.07.

The tosylate was prepared in a fashion similar to that previously described.[11] The tosylate was precipitated from the pyridine solution with water and the solid recrystallized from acetone–water. The stocky rods melted 125.5–126° dec. typical of tosylates, $[\alpha]^{27}\text{D}$ +27.6°.

Anal. Calcd. for $C_{34}H_{52}O_3S$: C, 75.51; H, 9.69. Found: C, 75.71; H, 9.58.

Cholest-4-ene-3-one-7β-ol Benzoate (VII).—During a period of 1.5 hours, 6.0 g. of chromic acid was added to a suspension of 13.4 g. of the benzoate VI in 280 ml. of glacial acetic acid. The solution was kept between 52 and 54°. Stirring at this temperature was continued for one-half hour after the addition was complete. When the solution was cooled, 8 ml. of ethanol was added to destroy the excess chromic acid and the mixture was evaporated to dryness. After washing with water, the residue was extracted with 300 ml. of ether and this ether extract washed with water, 10% sodium bicarbonate, and again water. The resulting ethereal solution was filtered through a short column of alumina and the filtrate was evaporated to a solid residue. This residue was crystallized from 95% ethanol to give 2.0 g. (14.5%) of white solid, m.p. 142–153°. Four recrystallizations from ethanol gave 1.2 g. of fine needles melting at 158–159° with partial resolidification followed by remelting at 168°. Chromatography of this material on a 6 × 1 cm. column of 60–100 mesh Florisil gave the pure keto-benzoate VII, m.p. 165–167°, $[\alpha]^{28}\text{D}$ +79° (*c* 1.368).

Anal. Calcd. for $C_{34}H_{48}O_3$: C, 80.90; H, 9.58. Found: C, 80.66; H, 4.40.

Oppenauer Oxidation of Cholest-5-en-3β,7β-diol 7-Benzoate.—A mixture of 1.0 g. of the 7-benzoate, 1.0 g. of aluminum isopropoxide, 35 ml. of dry cyclohexanone and 35 ml. of dry toluene was refluxed gently for one hour. After this time 0.3 ml. of acetic acid was added and steam was passed through the mixture for 1.5 hours. The resulting solution was extracted with ether which, after washing, was evaporated to 610 mg. of a yellow oil. Purification of this oil by chromatography produced mostly oily residue, but gave 80 mg. of cholest-5,7-diene-3-one as a crystalline material, undepressed upon admixture with authentic dienone.

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[11] R. H. Baker and Q. R. Petersen, THIS JOURNAL, **73**, 4080 (1951).

Preparation of Methylenebisamides[1]

BY CHARLES W. SAUER AND ROBERT J. BRUNI

RECEIVED JUNE 2, 1954

Knudsen[2] has reported the preparation of methylenediformamide from paraformaldehyde and formamide in 30% yield. In searching for a method to improve this yield, we investigated the use of trioxane, monochlorodimethyl ether, di-(chloromethyl) ether and hexamethylenetetramine as a source of the methylene group. The only one giving the desired product was the last of these.

The literature[3] contains numerous references to the use of hexamethylenetetramine as a source of formaldehyde. All of these reactions are reported to take place under conditions in which formaldehyde is liberated from the hexamethylenetetramine prior to formation of the final product; while it is difficult to visualize the formation of formaldehyde under the conditions of the reactions reported here. Descude[4] has reported the reaction of hexamethylenetetramine with benzamide at 200° to give azotrimethylenetribenzamide, but no product resembling the methylenedibenzamide we have obtained from hexamethylenetetramine and benzamide.

The reaction of formamide with hexamethylenetetramine gave a low (30 to 50%) yield of methylenediformamide, as had the Knudsen procedure. However, reuse of the mother liquor as solvent for succeeding runs gave yields of 70 to 100% based on added formamide and hexamethylenetetramine, which was not the case when paraformaldehyde was used instead of hexamethylenetetramine. Normally for the reaction, the mother liquor from a run was refreshed with amounts of hexamethylenetetramine and formamide equivalent to the amount of methylenediformamide obtained in the previous run. No determination was made of the optimum number of runs for reuse of the mother liquor, but it is at least 15 to 20. A typical reaction is outlined in Table I, which gives some yields obtained by this method. Other experiments reusing the mother liquor up to 15 times have been run. Infrared spectra of the products from paraformaldehyde and from hexamethylenetetramine were compared, and no significant differences were observed.

The reaction of hexamethylenetetramine with amides has been extended to the formation of methylenediacetamide, methylenebis-(malonamide), and methylenedibenzamide from acetamide, malonamide and benzamide, respectively. Their preparation is given in the Experimental section.

Experimental

Methylenediformamide A.—A mixture of 540 g. (12 moles) of formamide and 70 g. (0.5 mole) of hexamethylenetetramine is heated at a pot temperature of 140° for 5 hours. The mixture is cooled to ice temperature and the product is collected on a filter and washed with 90 g. (2 moles) of form-

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[1] This work was done under Contracts DA-19-020-ORD-12 and DA-19-020-ORD-47 with the Office of the Chief of Ordnance and has been released by the Office of Public Information, Department of Defense, for publication.

[2] P. Knudsen, *Ber.*, **47**, 2698 (1914).

[3] J. F. Walker, "Formaldehyde," 2nd Edition, Reinhold Publ. Corp., New York, N. Y., 1953, p. 416 *et seq.*

[4] M. Descude, *Ann. chim. phys.*, [7] **29**, 533 (1903).