component A, can play the role of a hydrogen carrier next to the terminal oxidase in a respiratory chain of the sweet potato.

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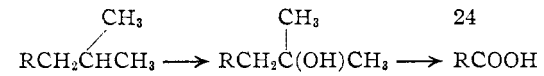
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, JEFFERSON MEDICAL COLLEGE]

The Preparation of C-27 Steroids from Bile Acids. I. Coprostanetetrol-3(α), 7(α),12(α),25

BY W. H. PEARLMAN[1]

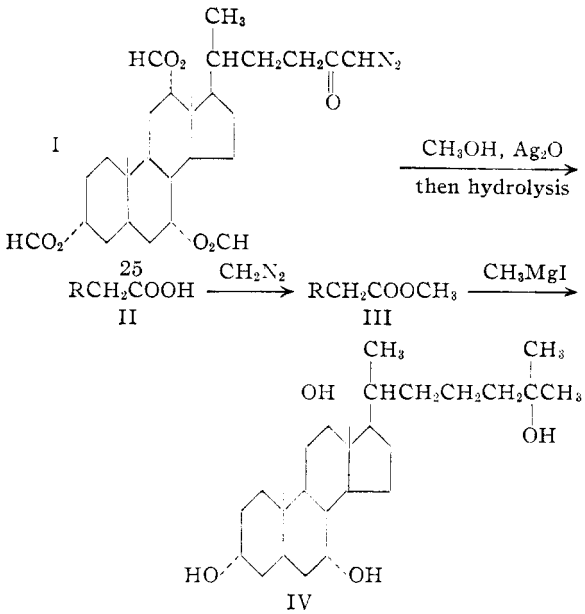
The preparation of coprostanetetrol-3(α),7(α), 12(α),25 was considered desirable for the study of the intermediary metabolism involved in the biological conversion of cholesterol to cholic acid.[2] One of the metabolic routes obviously requires the removal of the terminal isopropyl group in the side-chain of the cholesterol skeleton. On the basis of chemical analogy, the oxidative attack *in vivo* may very well begin with the introduction of oxygen on carbon atom 25 and proceed as follows



R represents the rest of the steroid molecule as *e. g.*, in cholic acid.

According to this hypothesis, an immediate metabolic precursor of cholic acid might conceivably be coprostanetetrol-3(α),7(α),12(α),25. The preparation of the latter substance from cholic acid is described in this paper. It is noteworthy that the C-27 steroid, scymnol, which like coprostanetetrol-3(α),7(α),12(α),25 possesses a nucleus identical[3] with that of cholic acid, has been isolated from shark bile.[4] Similar polyhydric alcohols of the cholestane and ergostane series have been found to occur in toad bile.[5] A search for scymnol and related products in mammalian bile has thus far been unsuccessful, however.[6]

25-Homocholic acid (II), m. p. 219.5–220°, was prepared from cholic acid by the Arndt–Eistert method; the intermediary triformyl diazoketone (I), m. p. 134.5–135°, was first described by Ruzicka, *et al.*,[7] who had prepared it from the triformyl cholyl chloride of Cortese and Baumann.[8,9] The methyl ester of 25-homocholic acid (III), m. p. 166–167° (another form melts at 150–151°) on treatment with methylmagnesium iodide yielded coprostanetetrol-3(α),7(α),12(α),25 (IV),[10] m. p. 188–189°; the tetracetyl derivative melted at 142.5°.[11] Other methods for the extension of the side-chain in bile acids have been previously reported[11,12] but these do not provide a means for the introduction of oxygen on carbon 25 in the terminal isopropyl group.



R represents the rest of the molecule as in cholic acid.

Experimental[13]

25-Homocholic Acid (II).—The triformyl derivative of 25-diazo-25-homocholanetriol-3(α),7(α),12(α)-one-24 was prepared from triformyl cholyl chloride in a manner essentially that described by Ruzicka, *et al.*[7] The product thus obtained was purified by chromatography and crystallized from ethyl acetate–petroleum ether as clusters of plates, melting at 134.5–135° with decomposition (*cf.* m. p. 128–129°).[7]

(1) Aided by a grant from the National Cancer Institute.

(2) K. Bloch, B. N. Berg and D. Rittenberg, *J. Biol. Chem.*, **149**, 511 (1943).

(3) H. Ashikari, *J. Biochem. (Japan)*, **29**, 319 (1939); W. Bergmann and Wm. T. Pace, THIS JOURNAL, **65**, 477 (1943).

(4) Cited by L. F. Fieser, "The Chemistry of Natural Compounds Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1937.

(5) Cited by H. Sobotka and E. Bloch, *Ann. Rev. Biochem.*, **2**, 45 (1943).

(6) W. H. Pearlman, THIS JOURNAL, **66**, 806 (1944).

(7) L. Ruzicka, P. A. Plattner and H. Heusser, *Helv. Chim. Acta*, **27**, 186 (1944).

(8) F. Cortese and L. Baumann, THIS JOURNAL, **57**, 1393 (1935).

(9) Melting points of 155 and 110° have been reported for the methyl ester of cholic acid in H. Sobotka, "The Chemistry of the Sterids," Williams and Wilkins Co., Baltimore, 1937, p. 372.

(10) The 12-OH group is designated α rather than β in view of the recent evidence that in desoxycholic acid this group is α: T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, **162**, 495 (1946); M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **29**, 1218 (1946).

(11) *Cf.* dimethyl carbinol of cholic acid, m. p. 177–177.5°, tetraacetate, m. p. 111.5–112°; T. Shimizu and T. Kazuno, *Z. physiol. Chem.*, **244**, 167 (1936).

(12) (a) H. Wieland and R. Jacobi, *Ber.*, **59**, 2064 (1926); (b) F. Reindel and K. Niederlander, *Ann.*, **522**, 218 (1936); (c) E. Fernholz, *Ber.*, **69**, 1792 (1936); (d) B. Riegel and I. A. Kaye, THIS JOURNAL, **66**, 723 (1944).

(13) All melting points are corrected.

Anal. Calcd. for $C_{28}H_{40}O_7N_2$: C, 65.10; H, 7.81; N, 5.42. Found: C, 65.05; H, 7.80; N, 5.01.

To 1.283 g. of this diazoketone in 22 ml. of methanol at 55–60°, a suspension of silver oxide in 8 ml. of methanol, which had been freshly prepared from 1.5 ml. of 10% aqueous silver nitrate, was added in small portions with shaking during the course of one hour; the reaction mixture was then refluxed for three hours, cooled and centrifuged. The supernatant was evaporated to dryness, and the residue dissolved in 3 ml. of benzene and passed over a short column containing 3 g. of aluminum oxide (Harshaw). Elution with 75 ml. of benzene containing 5% methanol served to remove colloidal silver from the crude reaction product which was then hydrolyzed by refluxing for one hour in 15 ml. of 10% potassium hydroxide in 90% methanol. The reaction mixture was poured into a dilute potassium carbonate solution and extracted with ether, thereby removing 24 mg. of neutral material. An ether extract of the acidified aqueous phase yielded 1.032 g. of a colorless oil, which on the addition of little acetone, gave 717 mg. of thick rectangular plates, m. p. 213–215.5°. Repeated crystallization from acetone raised the melting point to 219.5–220°.

Anal. Calcd. for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02. Found: C, 71.02; H, 9.90.

Methyl Ester of 25-Homocholic Acid (III).—This was prepared by dissolving 25-homocholic acid in little methanol and treating with ethereal diazomethane. The reaction product was repeatedly crystallized from acetone to yield needles, m. p. 166–167° (occasionally a lower-melting form was obtained, m. p. 150–151°).[9]

Anal. Calcd. for $C_{26}H_{44}O_5$: C, 71.52; H, 10.16. Found: C, 71.39; H, 10.06.

Coprostanetetrol-3(α),7(α),12(α),25 (IV).—A solution of methylmagnesium iodide, prepared from 316 mg. of magnesium and 0.8 ml. of methyl iodide in 50 ml. of absolute ether, was added to 1.062 g. of the methyl ester of 25-homocholic acid in 25 ml. of absolute benzene. The mixture was heated so that the ether distilled in the course of one hour, and was then refluxed for two hours. The product was decomposed by the addition of 50 ml. of cold saturated ammonium chloride, 50 g. of crushed ice and 15 ml. of 20% sulfuric acid. The aqueous phase was extracted repeatedly with ether; the combined benzene and ether extracts were washed with dilute hydrochloric acid, then with water, and finally evaporated. The residue was hydrolyzed by refluxing for one hour in a mixture of 15 ml. of methanol and 5 ml. of 10% potassium hydroxide, diluted with water and extracted with ether to yield 352 mg. of neutral material. The aqueous phase gave, on acidification, 581 mg. of a crystalline product, m. p. 215°, consisting of 25-homocholic acid. The neutral fraction crystallized readily from acetone to yield 252 mg., m. p. 185.5–187°; repeated crystallization from acetone raised the melting point to 188–189°. This product is readily soluble in alcohol, moderately soluble in acetone, and rather difficultly soluble in ethyl acetate; it may also be crystallized from the latter solvent. Like scymnol, it gives an intense orange-red color when dissolved in concentrated sulfuric acid at room temperature; cholic acid, when treated similarly, gives a yellow color with marked green fluorescence. The three aforementioned compounds give a positive Hammersten test.

Anal. Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 74.15; H, 11.09.

Tetraacetate of Coprostanetetrol-3(α),7(α),12(α),25.—118 mg. of the above product (IV), m. p. 188–189°, was dissolved in 1 ml. of anhydrous pyridine and 2 ml. of acetic anhydride added. The solution was heated for twenty-five hours on the steam-bath under anhydrous conditions, and then evaporated *in vacuo*. The residue was taken up in ether and washed successively with dilute hydrochloric acid, dilute sodium bicarbonate solution and water. Evaporation yielded 154 mg. of a product which on treatment with methanol gave a micro-crystalline product, m. p. 142°; recrystallization from the same solvent raised the melting point to 142.5°.

Anal. Calcd. for $C_{35}H_{56}O_8$: C, 69.50; H, 9.33. Found: C, 69.70; H, 9.15.

Acknowledgments.—The author is indebted to Mr. Irwin Schreibman and Miss Edith Goldberg for their technical assistance. Mr. James Rigas performed the microanalyses reported in this paper.

Summary

1. 25-Homocholic acid has been prepared from cholic acid by application of the Arndt–Eistert method.

2. Coprostanetetrol-3(α),7(α),12(α),25 has been prepared from 25-homocholic acid.

PHILADELPHIA, PA. RECEIVED FEBRUARY 1, 1947

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, PARKE, DAVIS AND COMPANY]

Isolation and Characterization of Vitamin Bc from Liver and Yeast.[1] Occurrence of an Acid-labile Chick Antianemia Factor in Liver

BY J. J. PFIFFNER, S. B. BINKLEY, E. S. BLOOM AND B. L. O'DELL[2]

Introduction

The chemical nature of the antianemia substances present in liver and in yeast is a problem which has attracted attention for some time. In 1925 Whipple and Robscheit-Robbins[3] demonstrated that the feeding of beef liver exerted a favorable influence on severe secondary anemia of dogs and the following year Minot and Murphy[4] reported the effectiveness of whole liver therapy in pernicious anemia. In spite of numerous attempts[5] the so-called antipernicious anemia principle has not been obtained in pure form probably because of the fact that its concentration can be

(1) Angier, *et al.* (*Science*, **103**, 667 (1946)), demonstrated by degradation and synthesis the structure of the liver *L. casei factor* to be N[4-{[(2-amino-4-hydroxy-6-pteridyl)-methyl]amino}benzoyl]-glutamic acid and named the compound pteroylglutamic acid. A sample of the synthetic compound was generously supplied us by the Lederle Laboratories. We found it to be identical with the compound which we isolated from liver and yeast and which we tentatively called vitamin Bc (*Science*, **97**, 404 (1943)) pending elucidation of its structure.

(2) Present addresses: S. B. Binkley, Bristol Laboratories, Syracuse, N. Y.; B. L. O'Dell, University of Missouri, Columbia, Mo.; E. S. Bloom, E. I. Dupont de Nemours & Co., Wilmington, Del.

(3) Whipple and Robscheit-Robbins, *Am. J. Physiol.*, **72**, 395 (1925); **72**, 419 (1925).

(4) Minot and Murphy, *J. Am. Med. Assoc.*, **87**, 470 (1926).

(5) For review of literature see Subbarow, Hastings and Elkin, in "Vitamins and Hormones," Academic Press, Inc., 1945, Vol. III, pp. 237–296.