

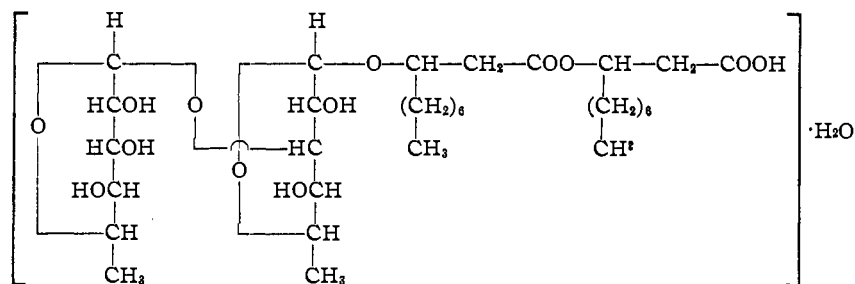
uble hydrolytic fraction could not be obtained in crystalline form. It proved to have a neutral equivalent of about 360 and yielded two moles of β -hydroxydecanoic acid upon saponification. As no such ester is formed by the conditions employed during hydrolysis, and as the proportion of this compound decreases with hydrolysis time, it was concluded that two moles of *l*- β -hydroxydecanoic acid exist in esterified form in the original acidic compound.

The following formula is presented as being most compatible with the accumulated data. The 1,3-linkage shown is, of course, largely speculative as the only pertinent data obtained were the uptake of only two atoms of oxygen per mole of compound by periodate oxidation. Assuming a pyranoside-type ring, either the 1,2- or 1,4-linkages would result in a structure which would normally take up three oxygen atoms. No direct evidence for

the type of sugar linkages involved (α or β) was obtained.

Summary

A crystalline, acidic glyco-lipide was produced



by three strains of *Pseudomonas aeruginosa* on peptone-glycerol broth.

This compound was found to contain two units each of *L*-rhamnose and normal *l*- β -hydroxydecanoic acid.

The most likely formula on the basis of the available data is proposed.

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RECEIVED JULY 18, 1949

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

The Preparation of 3,4,5- d_3 -Lithocholic Acid¹

BY W. H. PEARLMAN, M. R. J. PEARLMAN AND S. ELSEY

Schoenheimer and Berliner² described a procedure whereby deuteriolithocholic acid might be obtained by reduction of methyl $\Delta^{4,5}$ -3-ketocholenate in ethanol-ether solution in the presence of platinum oxide; the isotopically labelled bile acid should prove useful for metabolic study. However, their work was carried out with hydrogen and not with deuterium. On repeating their procedure but using deuterium, we obtained lithocholic acid containing only 0.35 atom % excess deuterium whereas the theoretical value for 3,4,5- d_3 -lithocholic acid is 7.50. This result is not altogether surprising in view of the observation by Anderson and MacNaughton³ that deuterium is exchanged for the hydroxyl hydrogen of alcohols such as isopropyl alcohol in the presence of catalysts; evidence was obtained, however, that exchange did not occur with isopropyl alcohol as long as any acetone was present. When absolute ether was substituted for ethanol-ether in the catalytic reduction, lithocholic acid was obtained containing 1.92 atom % excess deuterium. Further investigation of optimal

conditions for catalytic deuteration of methyl $\Delta^{4,5}$ -3-ketocholenate appeared to be desirable; the results of such an investigation are summarized in Table I. The most satisfactory procedure found was the following. Reduction of methyl $\Delta^{4,5}$ -3-ketocholenate in ether using a 5% palladium on charcoal catalyst gave a mixture consisting principally of methyl 3-ketocholenate and some methyl 3-ketoallocholenate; the mixture contained 3.59 atom % excess deuterium. Deuteration of this mixture in ether in the presence of platinum oxide yielded lithocholic acid with a slightly higher value, 3.72 atom % excess deuterium. Another run was made under identical conditions starting with methyl $\Delta^{4,5}$ -3-ketocholenate; lithocholic acid was obtained containing 3.89 atom % excess deuterium. Thus, it appears that two step deuteration of methyl $\Delta^{4,5}$ -3-ketocholenate furnishes lithocholic acid with twice as much deuterium as that obtained in the single step procedure; yet, theoretically three deuterium atoms should enter the steroid molecule in either procedure.⁴ By way of comparison, it is interesting that Schoenheimer,

(1) This investigation was supported by a grant-in-aid from the United States Public Health Service, under the National Cancer Institute Act.

(2) R. Schoenheimer and F. Berliner, *J. Biol. Chem.*, **115**, 19 (1936).

(3) L. C. Anderson and N. W. MacNaughton, *THIS JOURNAL*, **64**, 1456 (1942).

(4) Since there are 40 hydrogen atoms per molecule, the value corresponding to a content of three deuterium atoms is 7.5 atom% excess deuterium. A fourth deuterium atom is introduced but, being an hydroxyl deuterium, it is readily exchanged in subsequent manipulation.

et al.,⁵ obtained 4,5-*d*₂-coprostanone-3, which is identical in structure with methyl 3-ketocholanate with respect to rings A and B, by deuteration of $\Delta^{4,5}$ -cholestenone-3 in dry ether in the presence of palladium and that this product contained 3.44 atom % deuterium (theoretical value 4.35). However, the 3,4,5-*d*₃-coprosterol described by Anchel and Schoenheimer⁶ contained only 2.32 atom % deuterium (theoretical value 6.25); unfortunately, the mode of preparation of this compound was not described.

Further study of the mechanism of catalytic reduction of α,β -unsaturated ketones might afford an explanation of the results described here; Anderson and MacNaughton³ studied the mechanism of catalytic reduction of some carbonyl compounds with the aid of deuterium.

Of incidental interest is the observation that the proportion of non-digtonin precipitable products (chiefly if not entirely lithocholic acid) formed on complete reduction of methyl $\Delta^{4,5}$ -3-ketocholanate is dependent on the nature of the solvent used (see Table I). In addition to lithocholic acid and 3(β)-hydroxyallocholanolic acid pre-

viously² described, a small amount of 3(β)-hydroxycholanolic acid was isolated.

Experimental⁷

3,4,5-*d*₃-Lithocholic Ac'd.—8.56 g. of methyl $\Delta^{4,5}$ -3-ketocholanate, m. p. 124–125°, obtained by Oppenauer oxidation² of methyl $\Delta^{5,6}$ -3(β)-hydroxycholanate,⁸ was dissolved in 400 ml. of absolute ether and shaken in deuterium at atmospheric pressure at 5° in the presence of 3.5 g. of 5% palladium on charcoal catalyst (previously treated with deuterium) until there was no further uptake of gas. The reduction product appeared to consist chiefly of methyl 3-ketocholanate and some methyl-3-ketoallocholanate. This mixture was dissolved in 400 ml. of absolute ether and deuterated under the same conditions as above but in the presence of 4.0 g. of platinum oxide. The final reduction mixture was separated into digtonin and non-digtonin fractions in a manner essentially that described by Schoenheimer and Berliner.³ The latter fraction weighed 5.51 g. It was dissolved in 3 ml. of benzene plus 21 ml. of petroleum ether, b. p. 35–45°, adsorbed on a column containing 40 g. of aluminum oxide (Harshaw), and eluted with mixtures of benzene (5–100%)–petroleum ether. The eluates were worked up individually, yielding from aqueous methanol, a total of 3.51 g. of crystals with melting points ranging from 120 to 125°. This material was refluxed for two hours with 5% potassium hydroxide in 90% methanol; the solution was poured into water, acidified and extracted with ether. The product after two recrystallizations from ethyl acetate yielded 2.71 g. of lithocholic acid, m. p. 186–187°, containing 3.89 atom % excess deuterium; an additional 0.34 g. of crystals, m. p. 185–185.5° were obtained from the mother liquors.

The digtonin-precipitable material weighed 2.96 g. It yielded on treatment with ether–petroleum ether 740 mg. of crystals, m. p. 139–144°. The material in the mother liquor gave crystalline mixtures and hence was dissolved in 1.6 ml. of benzene plus 8 ml. of petroleum ether, adsorbed on a column containing 25 g. of aluminum oxide (Harshaw) and eluted with benzene (10–100%)–petroleum ether and finally with methanol. The benzene (10–20%)–petroleum ether eluates gave on repeated crystallization from aqueous methanol 380 mg., m. p. 108.5–110.5°. This material was hydrolyzed as above and crystallized from ethanol to furnish 359 mg. of 3(β)-hydroxycholanolic acid,⁹ m. p. 176–177.5°. The benzene (50–100%)–petroleum ether eluates were worked up individually furnishing, from aqueous methanol, a total of 224 mg. of crystals with melting points ranging from 141 to 148°. This material was combined with 740 mg. of the aforementioned product, m. p. 139–144°, and hydrolyzed as before; on repeated crystallization from ethanol, 604 mg. of 3(β)-hydroxyallocholanolic acid, m. p. 219–220°, was obtained.

Summary

3,4,5-*d*₃-Lithocholic acid, containing 3.89 atom % excess deuterium, has been prepared by catalytic deuteration of methyl $\Delta^{4,5}$ -3-ketocholanate in two steps. In addition, 3(β)-hydroxyallocholanolic acid and 3(β)-hydroxycholanolic acid were obtained.

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RECEIVED JUNE 9, 1949

(7) All melting points determined by the authors are corrected.

(8) Generously furnished by the Ciba Pharmaceutical Products, Inc., Summit, New Jersey, and by the Schering Corporation, Bloomfield, New Jersey.

(9) Melting points cited by H. Sobotka in "Chemistry of the Sterids," Williams and Wilkins Company, 1937, are 177–178° and 113–114.5°, 115–116° for this compound and its methyl ester respectively.

TABLE I

CATALYTIC DEUTERATION OF METHYL $\Delta^{4,5}$ -3-KETO-CHOLENATE

Reduction procedure (in D ₂ at atm. pressure)	Non-digtonin pptble. fraction (as % of total reduction products)	Reduction product analyzed	Analysis ^a (atom % excess deuterium)
PtO ₂ ; ethanol-ether; 25°	67	Lithocholic acid	0.35
PtO ₂ ; ether; 5°	67	Reduction ^b mixt.	2.07
		Lithocholic acid	1.92
PtO ₂ ; ethanol; 25°	36	Reduction mixt. ^c	0.32
PtO ₂ ; acetic acid; 25°	36	Digtonin-pptble. fraction	0.34
Pd-charcoal; ether; 5°	..	Reduction mixt.	3.59
PtO ₂ ; ether; 5° (using reduction mixture from preceding run)	66	Lithocholic acid	3.72
Pd-charcoal; ether; 5° followed by PtO ₂ ; ether; 5°	64	Lithocholic acid	3.89
Pd-ZrO ₂ ; ethyl acetate followed by PtO ₂ ; acetic acid; 25°	34	3(β)Hydroxy-cholanolic acid	3.22

^a The "falling-drop method" was employed as described by A. S. Keston, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **122**, 227 (1937-1938), and modified by M. Cohn in "Preparation and Measurement of Isotopic Tracers," J. W. Edwards, Ann Arbor, Michigan, 1947.

^b Similarly, deuteration of methyl $\Delta^{4,5}$ -3-keto,12(α)-hydroxycholanate gave a reduction mixture containing 1.80 atom % excess deuterium; 74% of this mixture was non-digtonin precipitable. ^c Similarly, deuteration of $\Delta^{4,5}$ -cholestenone-3 gave a reduction mixture containing 0.98 atom % excess deuterium.

(5) R. Schoenheimer, D. Rittenberg, B. J. Bergman and L. Rousselot, *J. Biol. Chem.*, **115**, 635 (1936).

(6) M. Anchel and R. Schoenheimer, *ibid.*, **125**, 23 (1938).