pure state, chlorinated, and many derivatives made and reactions studied.

2. The Hofmann rearrangement of 3,3,4,4-tetramethylvaleramide has been studied and products recovered in 94% yield without rearrangement of the hexamethylethane nucleus.

3. The reaction of iodohexamethylethane with potassium hydroxide failed to give any evidence of rearrangement.

4. Twenty-two compounds containing the hexamethylethane carbon skeleton have been prepared and described. They include such difficultly obtainable materials as 2,2,3,3-tetramethyl-1-butanol, 1-amino-2,2,3,3-tetramethylbutane, 2,2,3,3-tetramethylbutanoic acid (dimethyl-*t*-butylacetic acid), 3,3,4,4-tetramethylpentanoic acid and 2,2,3,3,6,6,7,7-octamethyloctane.

STATE COLLEGE, PA. RECEIVED MARCH 29, 1941

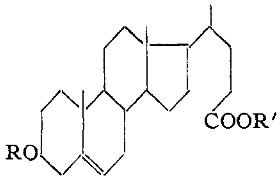
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Certain Ethers and Esters of 3-Hydroxy-5-cholenic Acid

BY BYRON RIEGEL, JAMES A. VANDERPOOL AND MELVIN F. W. DUNKER

In an effort to protect the nuclear hydroxyl group of 3-hydroxy-5-cholenic acid two labile ethers have been prepared. Because of the low solubility of the free acid, its methyl ester has been used. The usual procedure of tritylation, namely, treatment with trityl chloride in pyridine which has been applied to cholesterol by Josephson[1] gave, on modification, methyl 3-triphenylmethoxy-5-cholenate. The trityl ethers of steroids[1,2] are reported to be stable to alkaline reagents but easily hydrolyzed by mineral acids. Refluxing an acetic acid solution of methyl 3-triphenylmethoxy-5-cholenate resulted in an exchange of the trityl for the acetyl group.

Direct benzylation of hydroxylated steroids has proven difficult. The benzyl ether was prepared by heating methyl 3-*p*-toluene-sulfonoxy-5-cholenate with benzyl alcohol, analogous to the preparation[3] of benzyl cholesteryl ether. However, under the conditions of the reaction partial alcoholysis of the methyl ester took place. This fact together with the liberation of *p*-toluenesulfonic acid which probably promoted tar formation, as well as other reactions, not only militated against a good yield but also increased the difficulty of isolating a pure product. The equation for this reaction is $I + 2C_6H_5CH_2OH \xrightarrow{100^\circ} II + CH_3OH + C_7H_7SO_3H$.



I, $R = p\text{-}CH_3C_6H_4SO_2\text{—}$; $R' = CH_3$
II, $R = R' = C_6H_5CH_2\text{—}$

The benzyl 3-benzyloxy-5-cholenate exists in dimorphic forms, one melting at 87–88° and the other at 108.5–109.5°. When the lower melting form is melted, allowed to stand several months or crystallized several times, it is converted to the higher melting form.

The benzyl ester-ether was also formed in small amounts when 3-hydroxy-5-cholenic acid and benzyl alcohol were heated with a few drops of sulfuric acid. The main product from this reaction was the expected benzyl 3-hydroxy-5-cholenate.

To confirm the formula of the benzyl ester-ether, isolated in the reaction illustrated above, it was converted to the methyl 3-benzyloxy-5-cholenate. Saponification of the benzyl ester-ether gave 3-benzyloxy-5-cholenic acid which was esterified with diazomethane. The methyl ester benzyl ether melted at 99–100.5°.

The authors wish to thank the Rockefeller Foundation for aiding this research.

Experimental[4]

Methyl 3-Hydroxy-5-cholenate.—The authors wish to thank Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, New Jersey, for supplying us with generous amounts of two different materials from which the pure 3-hydroxy-5-cholenic acid was isolated. The first material was a rather crude mixture containing sodium 3-acetoxy-5-cholenate. A procedure for obtaining the pure bile acid

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(1) Josephson, *Ann.*, **493**, 174 (1932).

(2) (a) Girard and Sandulesco, French Patent 817,340; British Patent 488,801; (b) Williams, British Patent 467,161; U. S. Patent 2,180,762.

(3) Stoll, *Z. physiol. Chem.*, **207**, 147 (1932).

(4) All melting points are corrected. Microanalyses are by Dr. T. S. Ma, University of Chicago.

from the acid fraction from sterol oxidation is not well described in the literature.[5] The following method gave the best results.

The crude mixture was saponified with boiling methanolic potassium hydroxide.[5e] Any insoluble material was removed by filtration and the filtrate was vacuum concentrated. The precipitation of the potassium salt was completed by the addition of water and the alkaline suspension was extracted several times with ether to remove some of the tarry material. Many extractions of the acidified suspension with large volumes of ether did not completely dissolve the gummy solid. This solid was leached with acetone containing a few drops of hydrochloric acid and the solution combined with the ether extracts. The ether–acetone solution was washed with water, dried and concentrated until a brownish tar began to separate. Acetone was added till the solid dissolved and the solution carefully concentrated until the free acids began to crystallize. After cooling slowly, the tan crystals were filtered and washed with cold acetone. Further concentrations of the mother liquors yielded additional crops of crystals and finally a dark brown tar. The combined crystals were dissolved in hot acetone, but did not crystallize until the solution was concentrated. A 12 to 13% yield of 3-hydroxy-5-cholenic acid melting at 231° with decomposition was obtained.

The pure acid was obtained from the second material which was largely the crude acid along with sodium salts as follows. Ten grams of the finely powdered material was refluxed with 50 ml. of ether for fifteen to twenty minutes. The ether was decanted through a filter and the solid similarly treated with two small portions (50 ml.) of acetone; all filtrates were combined. These washings removed a small amount of the acid along with tarry materials. The washed solid was then refluxed with 300–400 ml. acetone for one hour and the solution filtered. The insoluble solid remaining was ground with 1–2 ml. concentrated hydrochloric acid and re-extracted with acetone till no more material dissolved. The insoluble residue weighed about 1.25 g. and was sodium chloride. The acetone extracts were concentrated and allowed to crystallize. The first crop of 3-hydroxy-5-cholenic acid was of sufficient purity for this work, m. p. about 228°. The product obtained from the mother liquors was again crystallized. About 5 g. of almost pure acid was recovered and about 2–3 g. of less pure material.

While the methyl ester[5b,e,f,g,6] has been reported previously, no yields were given and in no case was dry hydrogen chloride used as the catalyst. A solution of the acid in anhydrous methanol containing about 3% hydrogen chloride was refluxed. The practically pure ester precipitated upon vacuum concentration. Recrystallization from methanol gave 93–98% yields of the pure methyl 3-hydroxy-5-cholenate, m. p. 142–143°.

Methyl 3-*p*-Toluenesulfonoxy-5-cholenate.—A solution of 23 g. of methyl 3-hydroxy-5-cholenate and 23 g. of *p*-toluenesulfonyl chloride in 92 ml. of anhydrous pyridine was allowed to stand for twenty-four hours in a cool place, analogous to the preparation of cholesteryl *p*-toluenesulfonate.[7] The compound crystallized from acetone–petroleum ether in large, clear cyrstals melting at 120–120.6° with decomposition. The crystals cannot be dried at elevated temperatures and decompose on standing. The yields varied from 90 to 93%.

Anal. Calcd. for $C_{32}H_{46}O_5S$: C, 70.81; H, 8.54. Found: C, 70.81; H, 8.39.

Benzyl 3-Benzyloxy-5-cholenate.—A solution of 1.8 g. of the above compound in 15 ml. of freshly distilled benzyl alcohol was heated on a steam-bath for five hours and it darkened considerably. The mixture was neutralized with sodium bicarbonate and steam distilled to remove the benzyl alcohol. The reddish oil was taken up in ether, washed, dried and the solvent removed. The insolubility of the benzyl ester-ether in methanol facilitated its isolation. It was crystallized from acetone–methanol in glistening white platelets, melting 87–88° and remelting 108.5–109.5°; yields 55–61%.

Attempts to isolate methyl 3-benzyloxy-5-cholenate from the mother liquors have not, as yet, proved successful. The fused benzyl 3-benzyloxy-5-cholenate did not immediately resolidify when the melting point tube was removed from the bath, but formed a beautiful light blue fluorescent gel. The gel solidified when placed in a warm bath below the temperature of its higher melting form and then remelted at only the higher temperature. Evidence that the dual melting points are due to dimorphism was confirmed by the optical rotation of the two forms; low melting form $[\alpha]^{20}\text{D}$ $-23.9 \pm 0.2°$ (204.7 mg. in 5 ml. of chloroform α_D $-0.96°$, l 1 dm.); high melting form $[\alpha]^{22}\text{D}$ $-24.2 \pm 0.1°$ (431.2 mg. in 5 ml. of chloroform α_D $-2.08°$, l 1 dm.).

Anal. Calcd. for $C_{38}H_{50}O_3$: C, 82.26; H, 9.09. Found: C, 82.48; H, 9.35.

3-Benzyloxy-5-cholenic Acid.—The saponification of 210 mg. of benzyl 3-benzyloxy-5-cholenate with alcoholic potassium hydroxide yielded 180 mg. of the free acid. It crystallized from acetone in white needles melting 166–168°.

Anal. Calcd. for $C_{31}H_{44}O_3$: C, 80.13; H, 9.55. Found: C, 79.95; H, 9.29.

Methyl 3-Benzyloxy-5-cholenate.—Esterification of 200 mg. of 3-benzyloxy-5-cholenic acid with diazomethane in ether gave a yellow oil which on several crystallizations from acetone containing a drop of water gave tufts of white needles melting 99–100.5°. When fused, it gave a light blue fluorescent gel. A mixed melting point with benzyl 3-benzyloxy-5-cholenate (m. p. 108.5–109.5°) was 86–100°.

Anal. Calcd. for $C_{32}H_{46}O_3$: C, 80.29; H, 9.69. Found: C, 80.37; H, 9.56.

Benzyl 3-Hydroxy-5-cholenate and Benzyl 3-Benzyloxy-5-cholenate.—A mixture of 1.0 g. of 3-hydroxy-5-cholenic acid, 3 drops of concentrated sulfuric acid and 10 ml. of benzyl alcohol was heated on a steam-bath for eighteen hours. The bile acid soon dissolved and the solution slowly darkened in color. The reaction mixture was worked up as

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[5] (a) Butenandt, Dannenbaum, Hanisch and Kudszus, *Z. physiol. Chem.*, **237**, 57 (1935); (b) Wallis and Fernholz, THIS JOURNAL, **57**, 1504 (1935); (c) Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 986 (1935); (d) British Patent 453,773; (e) Haslewood, *J. Chem. Soc.*, 224 (1938); (f) Fujii and Matsukawa, *J. Pharm. Soc. Japan*, **56**, 93 (1936); (g) Kuwada and Yago, *ibid.*, **57**, 963 (1937); (h) U. S. Patent 2,180,095.

[6] Hattori, *J. Pharm. Soc. Japan*, **58**, 548 (1938); **59**, 32 (1939).

[7] Freudenberg and Hess, *Ann.*, **448**, 121 (1926).

given for the benzyl ester-ether. The residual red oil was dissolved in acetone and methanol added till a slight cloudiness persisted. On cooling, 130 mg. of crystals melting 107–108° and giving no depression of melting point with benzyl 3-benzyloxy-5-cholenate was obtained. A small amount of impure 3-hydroxy-5-cholenic acid was recovered from the sodium bicarbonate solution after the steam distillation. In three trials using 0.5 g., 1.0 g., and 2.0 g. of 3-hydroxy-5-cholenic acid and 3 drops of concentrated sulfuric acid, about 120–150 mg. of the benzyl ester-ether was obtained. In one trial in which 10 drops of sulfuric acid were used, tar formation occurred.

The mother liquor from the ester-ether slowly thickened and solidified. The solid after several crystallizations from petroleum ether melted at 81.5–82.5° and was benzyl 3-hydroxy-5-cholenate.

Anal. Calcd. for $C_{31}H_{44}O_3$: C, 80.13; H, 9.55. Found: C, 79.99; H, 9.32.

In an attempt to prepare the benzyl ether by the action of benzyl chloride on the potassium alcoholate of methyl 3-hydroxy-5-cholenate in the dimethyl ether of ethylene glycol, only 3-hydroxy-5-cholenic acid could be isolated. A similar attempt in liquid ammonia, using sodium, likewise resulted in recovery of the free acid. When methyl 3-hydroxy-5-cholenate was treated with benzyl chloride in pyridine, under conditions similar to those used for tritylation (see below), no benzyl ether was obtained.

Methyl 3-Triphenylmethoxy-5-cholenate.—To 3.0 g. of methyl 3-hydroxy-5-cholenate was added 3.0 g. of trityl chloride and 5 ml. of anhydrous pyridine in a flask previously dried by a stream of dry hot air. This reaction was quite sensitive to traces of moisture and it was found most essential to have perfectly dry reagents and reaction vessels. The mixture was heated on a steam-bath for seven to eight hours under a condenser protected by a drying tube. On cooling, crystals formed in the flask. The reaction mixture was diluted with ice and extracted with ether. The ether solution was successively washed with water, 2% hydrochloric acid, half-saturated solution of sodium bicarbonate and water, ice being used throughout the washings. The ether was dried and removed. The pale yellow sirupy residue was taken up in acetone, methanol added to cloudiness and set aside. The blunt white rods which separated weighed 3.95 g. and melted 146–147°. A second crop of crystals was obtained, giving a total yield of 4.15 g. (86%). The recrystallized product melted 147.5–149°.

Anal. Calcd. for $C_{44}H_{54}O_3$: C, 83.76; H, 8.63. Found: C, 83.36; H, 8.65.

When 0.3 g. of the above trityl ether was refluxed for two and one-half hours with 2 ml. of glacial acetic acid, 65 mg. of crystals, m. p. 151–153° and giving no depression of m. p. when mixed with methyl 3-acetoxy-5-cholenate, was obtained. Some prisms, m. p. 158–161°, and giving no depression when mixed with trityl alcohol, were also obtained from the sirupy residue.

Summary

Several new esters and ethers of 3-hydroxy-5-cholenic acid are described, among which are the labile benzyl and trityl ethers.

Evanston, Illinois Received February 24, 1941

[Contribution from the Division of Plant Nutrition, College of Agriculture, University of California]

The Molecular Constitution of Glycogen and Starch from the Seed of Sweet Corn (*Zea Mays*)

By W. Z. Hassid and R. M. McCready

Morris and Morris[1] prepared a polysaccharide from the seed of *Zea mays*, Golden Bantam variety, which had the properties commonly associated with glycogen. The polysaccharide gave a red-brown coloration with iodine, its aqueous solution was opalescent, it was resistant to the action of alkali and had a high positive rotation. Since, with the exception of giving a clear solution, the properties of dextrins are similar to those described, these authors also compared the rate of enzymic hydrolysis and the cupric chloride crystallization patterns of this polysaccharide with dextrin from glycogens from animal sources. Using those two characteristics as definite criteria for distinction of dextrin from glycogen, Morris and Morris concluded that the polysaccharide isolated from the corn was apparently identical with glycogen. Although a substance bearing at least a superficial resemblance to animal glycogen is found in some lower plants—fungi, yeasts, bacteria—it has never been reported before in any of the higher plants. This finding is unique, since glycogen is *par excellence* the reserve polysaccharide of the animal world. It was therefore of considerable interest to study further this corn polysaccharide particularly in regard to its molecular constitution.

Haworth, Hirst and their collaborators[2,3,4,5] and also other workers[6] showed that the mole-

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[1] D. L. Morris and C. T. Morris, *J. Biol. Chem.*, **130**, 535 (1939).

[2] W. N. Haworth, E. L. Hirst and M. D. Woolgar, *J. Chem. Soc.*, 177 (1935).

[3] W. N. Haworth and E. G. V. Percival, *ibid.*, 2277 (1932).

[4] E. L. Hirst and G. T. Young, *ibid.*, 951 (1939), also 1471 (1939).

[5] W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939).

[6] W. Z. Hassid and I. L. Chaikoff, *J. Biol. Chem.*, **123**, 755 (1938); D. J. Bell, *Biochem. J.*, **31**, 1683 (1937).