are *cis* to one another and this is consistent with the larger coupling constant assigned to J_{ab}.[4]

In the nmr spectrum of II obtained in deuteriochloroform the absorptions due to the methyl groups are partially masked by the large methylene peak. The lactone methyl absorptions in the acetate of II are shifted upfield to 1.05 ppm (superimposed doublets) when benzene is used as solvent and the integral and J values (6 cps) are easily discernible. In the nmr spectrum of the acetate of dihydrohydroxyancepsenolide obtained in benzene the methyl absorptions appear as doublets centered at δ 0.9 and 1.0 ppm with overlapping peaks at 0.95 ppm.

Experimental Section

Melting points were determined in capillary tubes with a Thomas–Hoover melting apparatus and are corrected. Ultraviolet spectra were measured in 95% ethanol on a Beckman DK-1 spectrophotometer and infrared spectra were taken with a Beckman IR-8 spectrophotometer. Nmr spectra were determined using tetramethylsilane as an internal standard with a Varian A-60 spectrometer.

Isolation of Hydroxyancepsenolide.—Collections of *Pterogorgia anceps* were made along the outside of Boca Chita Key, Miami, Fla., and in Bimini, Bahamas. The air-dried ground gorgonian material (1.9 kg) was extracted consecutively in a continuous percolator–extractor[5] with the following solvents: (1) hexane, 18 hr; (2) hexane, 96 hr; (3) hexane, 48 hr; (4) benzene, 48 hr; (5) benzene, 72 hr; (6) methanol, 28 hr; (7) methanol, 48 hr. The first hexane extract consisted of a complex lipid mixture from which ancepsenolide is isolated by chromatography over alumina[6] or silicic acid.[2] Some hydroxyancepsenolide precipitated from the second hexane extract which contained a total of 1.87 g of material. A 2.84-g sample of crude hydroxyancepsenolide was absorbed on silica gel (140 g, 35 × 653 mm) and eluted with 25% ethyl acetate in benzene (75-ml fractions). Fractions 5–18 contained 1.53 g of white solid which was recrystallized several times from isopropyl alcohol to give white platelets: mp 122.5–123.7°; $[\alpha]^{24}D$ +3.4°;[7] uv max (95% C_2H_5OH) 209 mμ (ϵ 15,800); ir ($CHCl_3$) 3600 very weak (OH), and 1750, broad (lactones); ir (KBr, concentrated) 3600 (OH), 1760, 1720 (lactone C=O's); nmr ($CDCl_3$) δ 7.0 (q, 1, vinyl hydrogen), 5.0 (complex quartet, 1, $CH_3CH(-O^-)CH=$), 4.30–4.73 (broadened q, 1, $-CH(-O^-)CHOH-$), 4.24, (dd, 1, $-CH(-O^-)CHOH-$), 1.1–3.0 ppm (31, $-(CH_2)_{12}-$, $2CH(-O^-)CH_3$, -OH).

Hydroxyancepsenolide Acetate.—Hydroxyancepsenolide (0.266 g, 0.77 mmol) was dissolved in a mixture of 10 ml of pyridine and 1 ml of acetic anhydride and the resulting solution was stirred overnight at room temperature. The reaction mixture was poured into ice water and the product was recovered by extraction into ether. The ether solution was washed with dilute hydrochloric acid, sodium bicarbonate, and water and dried ($MgSO_4$). Evaporation of the ether left 0.288 g of white solid of which 0.180 g was recrystallized four times from isopropyl alcohol, 53 mg, mp 68.3–70.3°.

Anal. Calcd for $C_{24}H_{38}O_6$: C, 68.24; H, 9.01. Found: C, 68.23; H, 9.03.

Dihydrohydroxyancepsenolide.—A solution of hydroxyancepsenolide (0.369 g, 0.972 mmol) in ethyl acetate (170 ml) was stirred under hydrogen at atmospheric pressure and room temperature in the presence of prereduced platinum oxide (0.178 g). A fine white precipitate was apparent in the reaction mixture by the time hydrogen uptake ceased after the absorption of slightly more than 1 mol equiv of gas. The catalyst was removed by filtration and washed with warm ethyl acetate to remove the precipitated product. Evaporation of the solvent left a white solid. The mixture of diastereomers expected in this reaction could not be resolved by fractional crystallization, nor could any separation of isomers be detected by tlc under the conditions employed. A sample recrystallized five times from ethyl acetate (29 mg from 148 mg) exhibited a melting point range of 119.3–133.0°. The material recovered from the mother liquors of the first recrystallization attempt had a melting point range of 118.8–125.5° after three recrystallizations from ethyl acetate: ir ($CHCl_3$) 3500, (OH) and 1765 cm^{-1} (saturated γ-lactones); ir (KBr) 3450 (OH), 1760, 1730 (C=O); nmr ($CDCl_3$, sparingly soluble) δ 4.10–4.80 (overlapping multiplets, 3, —CHOHCH(—O⁻), —CHOHCH(—O⁻), —CH_2CH(—O⁻)).

Anal. Calcd for $C_{22}H_{38}O_5$: C, 69.11; H, 9.95. Found: C, 68.90; H, 9.98.

Dihydrohydroxyancepsenolide Acetate.—Acetylation of dihydrohydroxyancepsenolide (0.104 g, 0.271 mmol), mp 117–133°, with pyridine–acetic anhydride as described above for II afforded a solid acetate (0.121 g) which was recrystallized once from isopropyl alcohol and then twice from carbon tetrachloride–hexane to give 57 mg of material: mp 71.2–72.5°; ir ($CHCl_3$) 1765 (saturated lactones) and 1740 cm^{-1} (acetate); nmr ($CDCl_3$) 5.12 (dd, 1, —CHOAcCH(—O⁻)—), 4.15–4.95 (overlapping complex quartets, 2, —CHOAcCH(—O⁻)— and —CH_2CH(—O⁻)—), 2.1 (s, 3, —OC(—O⁻)CH_3).

Dehydration of Hydroxyancepsenolide.—Hydroxyancepsenolide (0.253 g, 0.665 mmol) was stirred overnight with a mixture of 0.2 ml of phosphorus oxychloride and 11 ml of pyridine. The reaction mixture was diluted with four volumes of water and the product recovered by extraction into ether. The ether solution was washed with dilute hydrochloric acid and water and dried ($MgSO_4$). Evaporation of the solvent left a white solid (0.143 g, 60%) which tlc and nmr analysis indicated to be ancepsenolide. The crude product was filtered through silica gel and crystallized once from chloroform to give 0.103 g of ancepsenolide: mp 92.8–94.3°; no depression on admixture with authentic ancepsenolide; $[\alpha]^{24}D$ +7.7°. The infrared and mmr spectra of the dehydration product were identical with those of authentic ancepsenolide.[2]

Registry No.—II, 18634-45-2; acetate of II, 18634-46-3; dihydro derivative of II, 18634-47-4; acetate of dihydro derivative of II, 18634-48-5.

Acknowledgments.—We are pleased to acknowledge the use of the collecting facilities of the Institute of Marine Science, University of Miami, Miami, Fla., and the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, Bahamas, W. I. We wish to thank Mr. Paul Fenessey, Massachusetts Institute of Technology, and Dr. Ronald Grigsby, Continental Oil Co., Ponca City, Okla., for providing mass spectra. This work was supported by NIH Training Grant 5675 from the National Heart Institute.

(5) L. S. Ciereszko, *J. Chem. Educ.*, **43**, 252 (1966).

(6) L. S. Ciereszko, D. H. Sifford, and A. J. Weinheimer, *Ann. N. Y. Acad. Sci.*, **90**, 917 (1960).

(7) The sample used to determine the physical properties was isolated from the extracts of a batch of *Pterogorgia anceps* colonies. We have previously noted[2b] variation in the optical rotation of samples of ancepsenolide isolated from different batches of dried animal. We have since observed that a sample of ancepsenolide isolated from a single animal colony of *Pterogorgia anceps* exhibited a rotation of +12.03° (+13.2° originally reported[2b]), while a sample of ancepsenolide isolated from a single colony of another species of this same genus, *Pterogorgia guadalupensis*, exhibited a rotation of +47.9°. All of the above samples appeared to be homogeneous as judged by tlc and nmr, and were found to be identical by virtue of mixture melting points as well as ir, uv and nmr spectral comparisons. Thus the correct value for the optical rotation of ancepsenolide and hydroxyancepsenolide remains uncertain. More individual colonies will be examined in the hope of clarifying this question.

Synthesis and Characterization of Cholesterol β-D-Glucuronide and Derivatives[1]

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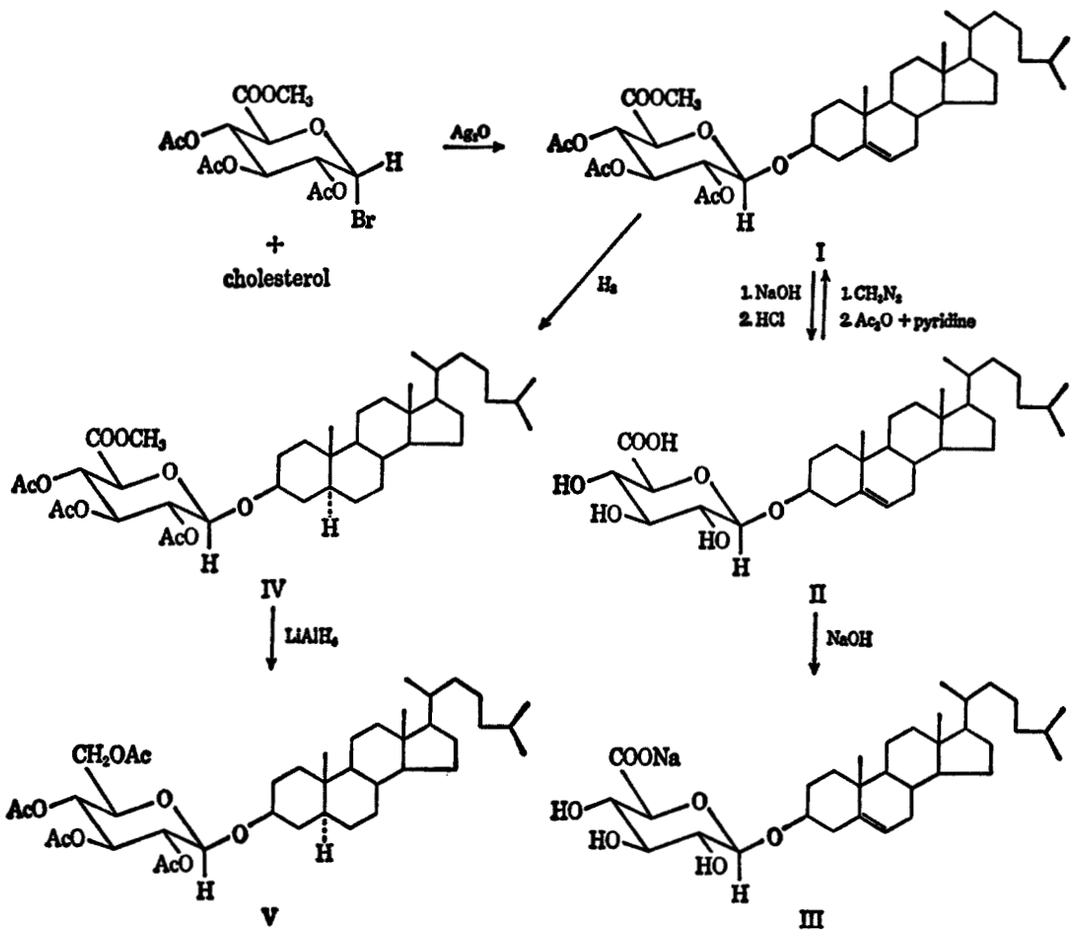
The recent isolation of cholesterol sulfate from human blood plasma[2] and from the urine of normal men[3] has

(1) This work was supported by a research grant, AM 01255, from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

(2) N. M. Drayer and S. Lieberman, *Biochem. Biophys. Res. Commun.*, **18**, 126 (1965).

(3) J. S. D. Winter and A. M. Bongiovanni, *J. Clin. Endocrinol. Metab.*, **28**, 927 (1968).

SCHEME I



led us to consider the possibility that this sterol also is conjugated with glucuronic acid in man. As a preliminary to biochemical studies, it was essential to prepare cholesterol β-D-glucuronide[4] in pure form. This report presents an efficient synthesis of this conjugate *via* its triacetyl methyl ester. Earlier preparative attempts (see Experimental Section) gave in all cases an inadequately characterized product in low yield.

The reactions involved in the synthesis and characterization of cholesterol β-D-glucuronide are outlined in Scheme I. Condensation of methyl 1-bromo-2,3,4-tri-*O*-acetyl-1-deoxy-α-D-glucuronate with cholesterol (the familiar Koenigs–Knorr reaction[5]) was carried out under mild conditions. A benzene solution of the brom methyl ester and cholesterol, in a 3:1 molar ratio, was shaken with silver oxide for 24 hr at room temperature. Following column chromatography on silica gel, methyl [cholest-5-en-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (I) was recovered in a yield of 86% based on the amount of cholesterol used. It was characterized by its elemental analysis and the usual physical constants and, particularly, in terms of its nuclear magnetic resonance and mass spectra.

The chief difficulty in effecting hydrolysis of the triacetyl methyl ester (I) to the trihydroxy acid (II) centered on the very low solubility of the former in alcoholic systems. This difficulty was circumvented by first removing the acetoxyl groups by methanolysis in an anhydrous alkaline methanol–methylene dichloride system, followed by hydrolysis of the carbomethoxyl group in an aqueous alkaline methanol–tetrahydrofuran system. It was found convenient to recover the product as the free acid (II) which, although amorphous rather than crystalline, was easily filtered, washed with water, and dried. Following conversion of acid II into its sodium salt (III), the latter was crystallized from aqueous ethanol. The yield of the twice-crystallized salt was 83% of theory, based on the amount of I employed.

It was not possible to crystallize samples of acid II regenerated from the twice-crystallized sodium salt (III), nor did the triacetoxy acid prepared from II show any inclination to assume crystalline form. The trihydroxy methyl ester, prepared by treating acid II with diazomethane, also proved an unsuitable derivative since it could be crystallized only with difficulty and in low yield, but either acetylation of the crude trihydroxy methyl ester or treatment of the purified acid (II) successively with diazomethane and acetic anhydride–pyridine gave a crystalline product indistinguishable from the triacetyl methyl ester (I) thus serving to interrelate I, II, and III.

Conversion of the primary product (I) into a known compound without rupture of the glycosidic link was achieved by catalytic reduction of I to methyl [5α-chloestan-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (IV) which, on further reduction with

(4) Systematic designations for compounds named trivially in the text are cholesterol, cholest-5-en-3β-ol; cholestanol, 5α-cholestan-3β-ol; cholesterol β-D-glucuronide, cholest-5-en-3β-yl-β-D-glucopyranosiduronic acid; triacetoxy acid, cholest-5-en-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosiduronic acid; trihydroxy methyl ester, methyl [cholest-5-en-3β-yl-β-D-glucopyranosid]uronate. Where required, numbers referring to the carbohydrate moiety are distinguished by priming.

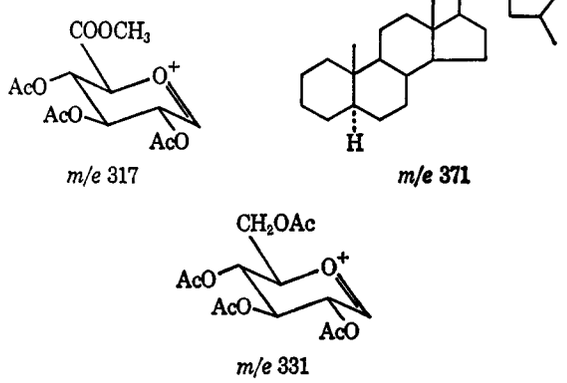
(5) W. Koenigs and E. Knorr, *Ber.*, **34**, 957 (1901).

lithium aluminum hydride followed by reacetylation, furnished 5α-chloestan-3β-yl-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (V), originally prepared by Linstead.[6]

Nuclear Magnetic Resonance Spectrum of I.—The configuration at the anomeric carbon atom was confirmed by examining a deuteriochloroform solution of the triacetyl methyl ester (I) at 100 and 220 MHz. The sugar moiety attached to C-3 could have only one of two configurations, namely α-D-*C1* or β-D-*C1*, since β-L-*1C* (indistinguishable from β-D-*C1*) is excluded by the nature of the reagent, and α-D-*1C* would be too unstable to exist under normal conditions. If the glucuronide exists in the α-D-*C1* form, the anomeric hydrogen would be equatorially oriented and the rest of the ring protons would be axially situated. Under these conditions the signal due to H-1′ would present a rather narrow pattern since the spin coupling between an equatorial H-1′ and an axial H-2′ would be in the range 2–5 Hz. However, in a β-D sugar, where all the ring hydrogens are in the axial configuration, the H-1′ resonance doublet would be wider because of the greater coupling between two axially oriented protons. Examination of the data obtained at both 100 and 220 MHz shows the presence of doublets at δ 4.64 and 4.00 having coupling constants of 8 and 10 Hz, respectively. These are assigned to the axially oriented protons at C-1′ and C-5′, respectively, on the basis of their chemical shifts. (The deshielding effect of the two oxygen atoms exceeds that due to one oxygen atom and the carbomethoxyl function.)

Mass Spectroscopy Studies.—Analysis of the fragmentation patterns of derivatives I, IV, and V may be summarized as follows. The triacetyl methyl ester (I) furnished a very low intensity molecular (M^+) ion, *m/e* 702. The fragment pair *m/e* 670 and *m/e* 32 (methanol, *via* fission and H transfer) indicates the presence of the methyl ester grouping. The sterol part of the molecule yielded free cholesterol (*m/e* 386),[7] cholesta-3,5-diene (*m/e* 368), and the expected fragments derived from them. The sugar moiety was detected intact as the *m/e* 317 pyronium ion (Scheme II), and also was represented by the ion pair *m/e* 257 and *m/e* 60 (acetic acid or methyl formate). The saturated triacetyl methyl ester (IV) gave an easily detected molecular ion, *m/e* 704. Its fragmentation pattern otherwise differed from that of I only to the extent that the sterol moiety was represented chiefly by the *m/e* 371 neutral fragment. The saturated tetraacetyl glucoside (V) furnished the most intense molecular ion, *m/e* 718. The presence of the acetylated primary hydroxyl group at C-6′ was indicated by the ion pair *m/e* 645 and *m/e* 73 ($CH_3COOCH_2^+$). The cholestanol part of the molecule again yielded the prominent *m/e* 371 neutral fragment. The sugar moiety was represented intact as the *m/e* 331 pyronium ion which is analogous to the *m/e* 317 ion derived from I and IV. Further fragmentation of the *m/e* 331 ion furnished the ion pair *m/e* 257 and *m/e* 74 (methyl acetate, *via* fission and H transfer).

SCHEME II



m/e 317 *m/e* 371 *m/e* 331

(6) R. P. Linstead, *J. Amer. Chem. Soc.*, **62**, 1766 (1940).

(7) The cholesterol ion is regarded a normal fragment ion and not due to contamination of the sample with the free sterol. The cholesterol peak remained constant relative to the higher mass peaks over the entire temperature range. If it was present as an impurity, one would expect to see its spectrum in the absence of higher mass peaks since cholesterol is probably more volatile than the sample itself.

Experimental Section[8]

Synthesis of Methyl [Cholest-5-en-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (I).—To a solution of 4.77 g (12 mmol) of methyl 1-bromo-2,3,4-tri-*O*-acetyl-1-deoxy-α-D-glucuronate and 1.54 g (4 mmol) of cholesterol (purified *via* the dibromide[9]) in 70 ml of benzene (stored over and distilled from phosphorus pentoxide), 1.85 g (8 mmol) of freshly prepared and dried silver oxide was added. The suspension was shaken with a vigorous rotary motion at room temperature in the dark for 24 hr. After the addition of Celite, the suspension was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed on a 46 × 960 mm column of silica gel (Davison, grade 923) prepared and developed with a system consisting of ethyl acetate, 200 ml, diluted to 1000 ml with isooctane. Fractions (10 ml) of effluent were collected at a rate of six per hour. Following the emergence of the unreacted cholesterol and traces of the anomer of I, the contents of tubes 451–667 were pooled and evaporated to dryness as above. Two crystallizations from ethyl acetate–methanol gave 2.42 g (86%) of I with the following constants: mp 165–165.5°; $[\alpha]_D$ −33°; ν_{max} 1755 (acetate), 1470, 1438, 1375, 1250–1210 cm^{-1} (acetate) [lit. mp 162–164.5° (7% yield),[10] 188–190° (44% yield),[11] 176–178° (yield unstated)[12]].

Anal. Calcd for $C_{40}H_{62}O_{10}$: C, 68.35; H, 8.89; CH_3CO, 18.37; OCH_3, 4.41. Found: C, 68.22; H, 8.86; CH_3CO, 18.76; OCH_3, 4.80.

Saponification of I to Sodium Cholest-5-en-3β-yl-β-D-glucopyranosiduronate (III).—To a solution of 421 mg (0.6 mmol) of methyl [cholest-5-en-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (I) in 6 ml each of anhydrous methanol and methylene dichloride, 1.2 ml of 0.1 *N* sodium hydroxide in anhydrous methanol (0.12 mmol) was added. After 3 hr at room temperature, 40 ml of tetrahydrofuran was added and stirring was initiated. Aqueous sodium hydroxide (1 *N*, 12 ml) was added in one portion followed by 35 ml of methanol and, gradually, a total of 75 ml of water. Some material separated at this point but returned to solution within 1 hr. After stirring for a total of 4 hr at room temperature, the pH of the solution was adjusted to around 3 by the addition of dilute hydrochloric acid, *t*-butyl alcohol was added to suppress foaming, and the

(8) Melting points were determined with a Fisher-Johns apparatus and are reported uncorrected. Optical rotations were obtained in chloroform solution, at a concentration of around 1%, and at a temperature of 25 ± 2° in a Zeiss 0.005° photoelectric polarimeter. Infrared spectra were determined in KBr dispersion with a Beckman IR-8 instrument. The nuclear magnetic resonance spectra were obtained with Varian HA-100 or HR-220 instruments, using tetramethylsilane as internal standards of reference. Mass spectra were determined by Dr. Robert Schaffer of the Morgan-Schaffer Corp., Montreal, Canada, using a Hitachi–Perkin-Elmer RMU-6D instrument with direct introduction of sample. Elemental analyses were those of Aug. Peisker–Ritter, Brugg, Switzerland. All samples were dried to constant weight at 80–100° under high vacuum over phosphorus pentoxide prior to analysis. The sodium salts were combusted in the presence of vanadium pentoxide.

(9) L. F. Fieser, *J. Amer. Chem. Soc.*, **75**, 5421 (1953).

(10) E. Shapiro, *Biochem. J.*, **33**, 385 (1939).

(11) H. Pelzer, *Z. Physiol. Chem.*, **314**, 234 (1959).

(12) F. Nagayama, A. Saito, and D. R. Idler, *Can. J. Biochem.*, **44**, 1109 (1966).

solution was concentrated under reduced pressure to a volume of around 20 ml. The suspended acid was recovered by filtration, washed with water, and dried *in vacuo* over anhydrous calcium chloride to give a white powder weighing 344 mg.

The crude acid was suspended in warm aqueous ethanol, and sufficient aqueous sodium hydroxide was added to provide a pH of around 7 at a point where all the acid had dissolved. Additional ethanol was added, and the solution was filtered and concentrated by warming to a volume slightly greater than that which would induce spontaneous crystallization. The sodium salt (III) was recovered as two crops of needles. These were washed with ethanol, and recrystallized from aqueous ethanol to yield a total of 298 mg (83%) of colorless needles: mp 286–287° dec; ν_{max} 3650–3100 (hydroxyl), 1610 cm^{-1} (carboxylate).

Anal. Calcd for $C_{33}H_{53}O_7Na$: C, 67.78; H, 9.14. Found: C, 67.62; H, 9.11.

A 20-mg sample of sodium cholest-5-en-3β-yl-β-D-glucopyranosiduronate (III) in 250 ml of dilute acetate buffer (pH 5, containing 10% ethanol) was incubated for 72 hr at 38° with 100,000 units of β-glucuronidase derived from beef liver. Extraction with chloroform, followed by two crystallizations of the recovered free sterol from ether–methanol, gave 3.2 mg of plates, mp 149–150°. The melting point was unchanged on admixture with an authentic preparation of cholesterol, and the ir spectra of the recovered and reference sterols were identical.

Preparation of Cholest-5-en-3β-yl-β-D-glucopyranosiduronic Acid (II).—Solution of a sample of the twice-crystallized sodium salt (III) in aqueous ethanol, followed by acidification and concentration under reduced pressure, provided a suspension of acid II. The product was recovered by filtration, washed with water, and dried *in vacuo* over anhydrous calcium chloride: mp 232–233° dec; ν_{max} 3600–3100 (hydroxyl), 1735 cm^{-1} (carboxyl).

Anal. Calcd for $C_{33}H_{54}O_7$: C, 70.43; H, 9.67; COOH, 7.99. Found: C, 70.19; H, 9.63; COOH, 7.84.

A sample of purified acid II in tetrahydrofuran was treated with excess ethereal diazomethane. Acetylation of the dried residue or of the crystallized methyl ester (needles, from aqueous tetrahydrofuran) gave, from ethyl acetate–methanol, needles melting at 164–165°. The melting point was unchanged on admixture with an authentic sample of methyl [cholest-5-en-3β-yl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (I), and their ir spectra were identical.

Methyl [5α-Cholestan-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (IV) from I.—A solution of 500 mg of methyl [cholest-5-en-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (I) in 25 ml of ethyl acetate was shaken for 3 hr in a hydrogen atmosphere in the presence of a 5% palladium-on-carbon catalyst (Engelhard Industries). After removal of the catalyst by filtration and the solvent by evaporation in a stream of nitrogen, the residue was crystallized from ethyl acetate–methanol to furnish 427 mg of needles: mp 180–181°; $[\alpha]_D$ −5°; ν_{max} 1755 (acetate), 1470, 1440, 1370, 1250–1210 cm^{-1} (acetate).

Anal. Calcd for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15; CH_3CO, 18.32; OCH_3, 4.40. Found: C, 68.18; H, 9.14; CH_3CO, 18.07; OCH_3, 4.42.

Saponification of 212 mg (0.3 mmol) of methyl [5α-cholestan-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (IV), as in the preparation of III from I, gave 148 mg of **sodium 5α-cholestan-3β-yl-β-D-glucopyranosiduronate** as needles from aqueous ethanol: mp 286–287° dec; ν_{max} 3650–3100 (hydroxyl), 1610 cm^{-1} (carboxylate).

Anal. Calcd for $C_{33}H_{55}O_7Na$: C, 67.55; H, 9.45. Found: C, 67.40; H, 9.51.

Incubation of a 20-mg sample of sodium 5α-cholestan-3β-yl-β-D-glucopyranosiduronate with β-glucuronidase, as in the previous example, gave 5.0 mg of leaflets from methanol, mp 141–142°. The melting point was unchanged on admixture with an authentic preparation of cholestanol, and the ir spectra of the isolated and reference sterols were identical.[13]

(13) The object of these hydrolyses was to obtain samples of the free sterols for formal identification, but it was apparent from the low recovery of the sterols that both sterol glucuronides are resistant to hydrolysis by β-glucuronidase of hepatic origin. It was reported earlier [K. D. Voigt, M. Lemmer, and J. Tamm, *Biochem. Z.*, **332**, 550 (1960)] that a preparation of cholesterol β-D-glucuronide (supplied by Professor Rudolph Tscheche but not described in the literature) was not hydrolyzed by the same enzyme preparation. The low rate of hydrolysis of cholesterol β-D-glucuronide by β-glucuronidase of limpet origin is evident from the data of Nagayama, *et al.*,[12] who did not, however, comment on the point.

5α-Cholestan-3β-yl-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (V) from IV.—To a solution of 200 mg of methyl [5α-cholestan-3β-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (IV) in 25 ml of dry ether, 300 mg of lithium aluminum hydride was added. After refluxing for 3 hr, excess reagent was decomposed by the successive addition of ethyl acetate and water. The solution was further diluted with ethyl acetate, washed with acidic and neutral brine, dried with anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. Following reacetylation, the product was crystallized from ethanol, furnishing 95 mg of needles: mp 174.5–175.5°; $[\alpha]_D$ +3°; ν_{max} 1750 (acetate), 1468, 1440, 1365, 1250–1210 cm^{-1} (acetate) [lit. for 5α-cholestan-3β-yl-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (V) mp 175°; $[\alpha]_D$ +5° ($CHCl_3$)[6]].

Anal. Calcd for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25; CH_3CO, 23.95. Found: C, 68.40; H, 9.23; CH_3CO, 23.07.

Registry No.—II, 17435-78-8; III, 19459-08-6; IV, 19459-09-7; V, 19459-10-0; sodium 5α-cholestan-3β-yl-β-D-glucopyranosiduronate, 19459-11-1.

Acknowledgments.—The two-stage methanolysis–saponification of the triacetyl methyl ester (I) to the sodium salt (III) is based on a technique devised earlier by Dr. Vernon Mattox for similar derivatives. We wish to thank him for offering the method to us prior to its publication. We express also our gratitude to our associate, Dr. Marvin Lewbart, who pointed out the feasibility of reducing the saturated triacetyl methyl ester (IV) to the tetraacetyl glucoside (V) with lithium aluminum hydride. The nuclear magnetic resonance spectra were obtained with instruments in the laboratories of Varian Associates whom we wish to thank for this courtesy.

A New Dimer of Pyridoxol (Vitamin B_6)

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A number of syntheses of pyridoxol (**5**) by Diels–Alder condensations of a variety of dienophiles with 5-ethoxy-4-methyloxazole (**1**) have been described.[1] While examining the conversion of adduct **3** of this oxazole and *cis*-1,4-diacetoxybutene-2 (**2**) to pyridoxol in moist acetic acid solvent (Scheme I), we have observed that a high yield of product is obtained from dilute solutions of adduct, but that the yield falls off rapidly as the initial concentration of adduct is increased. However, the apparent yield when measured by the intensity of the pyridoxol chromophore in the total reaction mixture appears essentially independent of concentration (Table I). The bulk of this difference can be accounted for by the presence of a new dimer **6**, N-(5-desoxypyridoxolyl)pyridoxol, isolated from the reaction mixture by ion-exchange chromatography.

(1) E. E. Harris, R. A. Firestone, K. Pfister, 3rd, R. R. Boettcher, F. J. Cross, R. B. Currie, M. Monaco, E. R. Peterson, and W. Reuter, *J. Org. Chem.*, **27**, 2705 (1962); W. Kimel and W. Leimgruber, U. S. Patent 3,250,778 (1966); T. Naito and T. Yoshikawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 918 (1966); R. A. Firestone, E. E. Harris, and W. Reuter, *Tetrahedron*, **23**, 943 (1967).