for 2 hr. Acetic acid (0.5 ml.) was added to decompose any excess diazomethane. Distillation yielded 52% of the methyl ester of *exo*-3-*t*-butylnorbornane-2-carboxylic acid, b.p. 60–64° (0.3 mm.), single peak in v.p.c.

Anal. Calcd. for $C_{13}H_{22}O_2$: C, 74.24; H, 10.55. Found: C, 74.04; H, 10.32.

The methyl ester (2.5 g., 0.012 mole) was heated under reflux for 25 hr. in a solution of sodium methoxide (0.024 mole) in 13 ml. of absolute methanol. Methanol (12 ml.) was removed by distillation and the remaining solution was poured onto 20 ml. of ice–water. The ester was extracted with three 20-ml. portions of diethyl ether, and the ether extract was washed with two 10-ml. portions of saturated aqueous sodium chloride solution. The organic layer was dried over magnesium sulfate and, after removal of diethyl ether, the remaining oil gave a single peak in v.p.c. with a retention time identical with that of the starting ester.

Addition of *t*-Butyllithium to Vinyltrimethylsilane.—*t*-Butyllithium (0.13 mole) in 70 ml. of pentane was added over a period of 5 min. to a stirred solution of 10 g. (0.10 mole) of vinyltrimethylsilane in 250 ml. of diethyl ether at −40°. The reaction mixture was stirred at −30 to −50° for 24.5 hr. Carbonation by decantation and work-up in the manner described above gave 25% of 2-trimethylsilyl-4,4-dimethylpentanoic acid, m.p. 93–96°. After three recrystallizations from hexane, the acid had m.p. 98.5–99.5°; n.m.r. (20% in $CDCl_3$) τ 7.97, 8.03, and 8.13 (relative area 3.0), 9.13 (relative area 9.0), and 9.92 (relative area 9.0).

Anal. Calcd. for $C_{10}H_{22}O_2Si$: C, 59.35; H, 10.96; Si, 13.88; neut. equiv., 202. Found: C, 59.54; H, 10.81; Si, 14.00; neut. equiv., 201.

Acknowledgment.—We thank the Socony-Mobil Oil Company for a grant in support of this work.

C-19 Functional Steroids. VIII.[1a,b] Studies in the Synthesis of the A/B Ring System of Sarmentosigenin E[1c]

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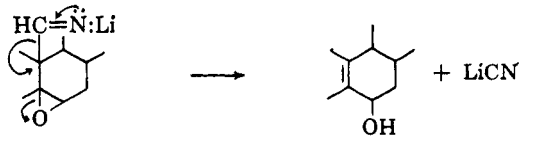
Received September 18, 1964

Oxidation of 5α-chloro-*syn*-19-oximinocholestane-3β,6β-diol 3-acetate gave 5α-chloro-3β-hydroxy-6-oxocholestane-19-nitrile 3-acetate which was allowed to react with alcoholic potassium hydroxide to form 3β,5β-dihydroxy-6-oxocholestane-19-nitrile. This diol was reduced with sodium borohydride to afford 3β,5β,6β-trihydroxycholestane-19-nitrile which on treatment with methanolic hydrogen chloride followed by hydrolysis furnished 3β,5β,6β-trihydroxycholestan-19-oic acid 6,19-lactone embodying the A/B ring system of sarmentosigenin E. Reduction of 5β,6β-epoxy-19-oximinocholestan-3β-ol or the corresponding 19-nitrile with lithium aluminum hydride afforded 19-norcholest-5(10)-ene-3β,6β-diol *via* a fragmentation reaction.

Cardiac glycosides are of major importance in drug therapy and methods for their synthesis not only are of interest *per se*, but also as a means for obtaining analogs of potential pharmacological importance. Syntheses of digitoxigenin[2] and periplogenin[3] have been disclosed recently. Neither of these aglycones has a functional group at C-19, and analysis[4,5] of the relationship between chemical constitution and biological activity in the cardiac glycosides indicates that concomitant oxygenation at C-19 and C-5 enhances cardiotonic action.

This article describes the synthesis of the A/B ring system of sarmentosigenin E (3β,5,6β,14-tetrahydroxy-5β-card-20(22)-enolide-19-oic acid 6,19-lactone),[6] utilizing the 19-oximino-5α-chloro-6β-hydroxy steroid intermediates which have been prepared in this laboratory.[7–9] These intermediates possess functionality at both C-5 and C-19, and are readily available from conventional steroids.

5β,6β-Epoxy steroids, which have the desired 5β-oxygen linkage, are available from 5α-chloro-6β-hydroxy steroids, and in principle it would be possible to reduce a 5β,6β-epoxide to the desired 5β-ol with lithium aluminum hydride.[10] Treatment of I with alkali gave the epoxide III which on lithium aluminum hydride reduction gave the 19-norsteroid VII. Moreover, the nitrile IV, prepared from I by successive treatment with hot acetic anhydride and alkali, on treatment with lithium aluminum hydride also gave VII. Presumably, in both cases this fragmentation[11] is owing to formation of an intermediate imine–metal complex, in which the electron deficiency resulting from ionization of the 5β-bond is neutralized by heterolysis of the 10β-bond, and by donation of the lone pair of electrons on the nitrogen.



After these experiments were completed, the formation of VII by treatment of V with alkali was described.[12]

(1) (a) From the undergraduate and graduate research of J. A. Muñoz. This research was supported by University funds and by a Public Health Service research grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. The n.m.r. spectrometer used in this study was provided by a grant (NSF-G-21268) from the National Science Foundation. (b) Paper VII: M. E. Wolff and W. Ho, *J. Med. Chem.*, **7**, 681 (1964). (c) A preliminary account of portions of this work has been presented: M. E. Wolff and J. A. Muñoz, *Chem. Ind.* (London), 1312 (1964).

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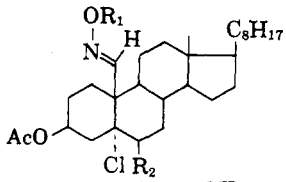
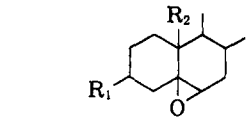
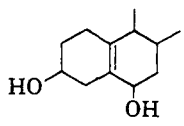


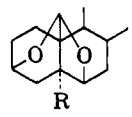
I, R_1 = H; R_2 = OH
II, R_1 = Ac; R_2 = OAc



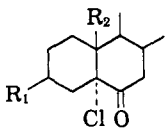
III, R_1 = OH; R_2 = CHNOH
IV, R_1 = OH; R_2 = CN
V, R_1 = OH; R_2 = CHO
VI, R_1 = OAc; R_2 = CHO



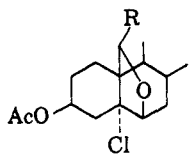
VII



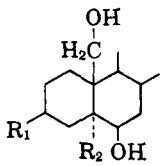
VIII, R = Cl
IX, R = OH



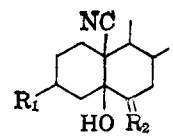
X, R_1 = OAc; R_2 = CHNOH
XI, R_1 = OH; R_2 = CHNOH
XII, R_1 = OH; R_2 = $CHNOCH_3$
XIII, R_1 = OAc; R_2 = CN



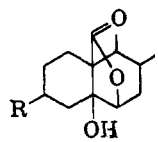
XIV, R = OAc
XV, R = OH
XVI, R = O



XVII, R_1 = OH; R_2 = H
XVIII, R_1 = OAc; R_2 = Cl

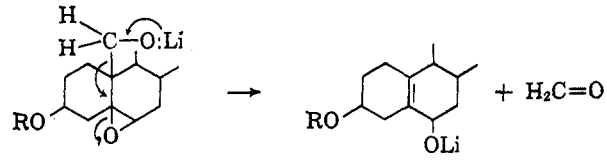


XXII, R = OBz
XXIII, R = OH



XIX, R_1 = OBz; R_2 = O
XX, R_1 = OAc; R_2 = O
XXI, R_1 = OBz; R_2 = 

Efforts were then made to degrade the oxime group in I prior to further work. Treatment of I with hydrochloric acid in methanol gave only the acetal VIII[13] and none of the intermediate aldehyde. The ketone XI was prepared by oxidation of I with N-bromoacetamide in ether, followed by saponification of the intermediate acetate X. On treatment of XI with ethanol, formaldehyde, and hydrochloric acid, no reaction took place, indicating the importance of the 6β-hydroxyl function for the hydrolysis at C-19. Compound I with sodium nitrite and acetic acid[14] gave predominantly XIV together with some XV. It was not possible to hydrolyze selectively the 19-acetate function in XIV to obtain XV; XIV with methanolic ammonia at pH 9 gave only the epoxide VI, whereas attempted hydrolysis in 50% acetic acid gave acetal IX. The formation of IX probably occurs *via* either V or VI; the epoxide ring is opened by the aqueous acetic acid to form a 5α,6β-diol intermediate which then cyclizes. An attempt to reduce directly the hemiacetal acetate XIV to a 19-hydroxyl derivative with lithium aluminum hydride in ether gave mainly the triol XVII, presumably *via* either V or VI. That the position of the hydroxyl function is 6β rather than 5β was shown by its ease of acetylation. A minor product from the reduction was the fragmentation product VII, which presumably also arose *via* V or VI through loss of formaldehyde.



Rowland[15] has shown that 6-oxo-5α-bromo steroids are converted readily to 6-oxo-5β-hydroxy steroids because of a neighboring group effect. No reaction was observed upon treatment of XI or XII with alcoholic potassium hydroxide. Since the oxime function at C-19 evidently interferes with the reaction, attempts were made to secure a 6-ketone having an aldehyde function at C-19. Reduction of XV with sodium borohydride gave XVIII. In *t*-butyl alcohol, the reaction of XVIII with N-bromosuccinimide, a reagent known to oxidize secondary alcohols selectively,[16] gave only XVI. Again oxidation of XV itself with N-bromosuccinimide in *t*-butyl alcohol gave only XVI, whereas in ether no reaction was observed.

Compound XXIII, embodying the A/B ring system of sarmentosigenin E, ultimately was obtained in the following way. Oxidation of I with chromic acid in pyridine solution[17] proceeded with concomitant dehydration of the oxime function[18] to afford XIII. Compound XIII was changed readily by alcoholic alkali to a gelatinous diol, which on benzoylation gave crystalline XIX. In harmony with the hindered 5β-configuration of the tertiary hydroxyl group, acetylation of the gelatinous diol, even under conditions known to affect tertiary alcohols,[19] gave only the monoacetate XX.

Reduction of XIX with sodium borohydride and subsequent acetylation gave XXI. The 6β-configuration of the hydroxyl group in XXI was established by its conversion to XXII *via* the corresponding imino ether hydrochloride. Hydrolysis of the imino ether hydrochloride for 2 hr. in 5% alcoholic potassium hydroxide solution at 27° gave only the diol lactone XXIII. The lability of the benzoate group is owing to the 1,3-diaxial relationship of the 3β,5β-diol system,[20] and is confirmatory evidence for this stereochemical assignment.

The extension of these studies to the synthesis of cardiac aglycones is being completed.

(13) *Cf.* D. H. R. Barton, J. M. Beaton, L. E. Geller, and M. M. Pechet, *J. Am. Chem. Soc.*, **83**, 4076 (1961), and ref. 12.

(14) D. H. R. Barton and J. M. Beaton, *ibid.*, **82**, 2641 (1960); **83**, 4083 (1961).

(15) A. T. Rowland, *J. Org. Chem.*, **27**, 1135 (1962).

(16) For a review, consult R. Filler, *Chem. Rev.*, **63**, 21 (1963).

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Experimental[21]

5α-Chlorocholestane-3β,6β-diol.—A solution of 5α-chlorocholestane-3β,6β-diol 3-acetate[22] in methanol containing 1% concentrated hydrochloric acid was kept at 27° for 18 hr. and concentrated. The resulting precipitate was recrystallized from methanol to give colorless needles: m.p. 164–166°; λ_{max}^{KBr} 2.9, 9.6 μ; $[\alpha]^{28}D$ −12° (*c* 0.44, $CHCl_3$).

Anal. Calcd. for $C_{27}H_{47}ClO_2$: C, 73.85; H, 10.69. Found: C, 73.55; H, 10.56.

5α-Chlorocholestane-3β,6β-diol 3,6-Diacetate.—A mixture of 10.0 g. of 5α-chlorocholestane-3β,6β-diol 3-acetate,[22] 1.0 g. of *p*-toluenesulfonic acid, and 20 ml. of acetic anhydride was shaken until a clear solution was obtained, kept for 5 hr. at 27°, and diluted with 60 ml. of water added in small portions. The resulting precipitate was removed and recrystallized from ethanol to afford 9.0 g. (83%) of colorless needles: m.p. 108–110°; $[\alpha]^{25}D$ −47° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 5.7, 8.0 μ.

Anal. Calcd. for $C_{31}H_{51}ClO_4$: C, 71.16; H, 9.83; Cl, 6.78. Found: C, 71.01; H, 9.68; Cl, 7.01.

19-Acetoxyimino-5α-chlorocholestane-3β,6β-diol Diacetate (II).—A solution of 1.0 g. of I[9] and 0.10 g. of *p*-toluenesulfonic acid in 30 ml. of glacial acetic acid containing 5 ml. of acetic anhydride was kept for 8 hr. at 27° and poured into water. The product was extracted with ether and the ether extract was washed with water and dried (Na_2SO_4) and evaporated. The product was obtained from methanol as colorless needles: m.p. 133–134°; λ_{max}^{KBr} 5.62, 5.75 μ; $[\alpha]^{28}D$ −27° (*c* 1.02, $CHCl_3$).

Anal. Calcd. for $C_{33}H_{52}ClNO_6$: C, 66.70; H, 8.82. Found: C, 66.09; H, 8.94.

5β,6β-Epoxy-19-oximinocholestan-3β-ol (III).—A solution of 1.6 g. of II and 5 g. of potassium hydroxide in 40 ml. of methanol was heated under reflux for 24 hr., cooled, poured into water, and neutralized carefully with 5% hydrochloric acid. The resulting precipitate was removed and recrystallized from methanol to give 1.1 g. of colorless needles: m.p. 229–231°, $[\alpha]^{28}D$ −56° (*c* 0.98, $CHCl_3$).

Anal. Calcd. for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51; N, 3.24. Found: C, 75.24; H, 10.33; N, 3.51.

5β,6β-Epoxy-3β-hydroxycholestane-19-nitrile (IV).—A solution of 2.0 g. of I in 15 ml. of acetic anhydride was heated under reflux for 3 hr., cooled, and diluted with water. The product was extracted with ether, and the ether solution was washed with water, dried (Na_2SO_4), and evaporated to give a gum which resisted crystallization. A solution of the gum in 25 ml. of 5% potassium hydroxide in methanol solution was kept for 18 hr. at 27°, poured into water, and extracted with ether. Evaporation of the washed and dried (Na_2SO_4) ether extract gave a residue which was recrystallized from methanol to furnish 0.75 g. of colorless needles: m.p. 195–196°, $[\alpha]^{28}D$ −14° (*c* 1.24, $CHCl_3$). Analysis showed this material to be a methanolate.

Anal. Calcd. for $C_{27}H_{43}NO_2 \cdot CH_3OH$: C, 75.46; H, 10.63. Found: C, 75.80; H, 10.44.

The solvent-free compound, m.p. 194–196°, was obtained by drying the methanolate at 185° at 0.01 mm.

Anal. Calcd. for $C_{27}H_{43}NO_2$: C, 78.40; H, 10.38; N, 3.39. Found: C, 78.30; H, 10.10; N, 3.62.

5β,6β-Epoxy-19-oxocholestan-3β-ol (V).—A solution of 0.50 g. of XIV in 40 ml. of 5% methanolic KOH was kept at 27° for 4 hr. and diluted with ether and brine. The ether layer was separated, washed again with brine, dried (Na_2SO_4), and evaporated. The residue was recrystallized from methanol to give 0.35 g. of colorless needles: m.p. 135–137°; λ_{max}^{KBr} 3.00, 5.82 μ; $[\alpha]^{25}D$ −31° (*c* 1, $CHCl_3$); lit.[12] m.p. 138–140°, $[\alpha]D$ −10°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.84; H, 10.64. Found: C, 77.56; H, 10.68.

5β,6β-Epoxy-19-oxocholestan-3β-ol Acetate (VI).—A solution of 1.00 g. of XIV in 100 ml. of methanol at 40° was treated with gaseous ammonia to pH 9, kept at 27° for 1 hr., neutralized with glacial acetic acid, and poured into water. The resulting precipitate was filtered and recrystallized from aqueous methanol to give 0.50 g. of colorless needles: m.p. 139–141°; λ_{max}^{KBr} 3.70, 5.75, 8.00, 12.30 μ; $[\alpha]^{25}D$ −48° (*c* 1, chloroform); lit.[12] m.p. 138–140°, $[\alpha]D$ +34°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.79; H, 9.87.

19-Norcholest-5(10)-ene-3β,6β-diol (VII). **A. From III.**—A solution of 0.10 g. of III and 0.10 g. of lithium aluminum hydride in 20 ml. of tetrahydrofuran was heated under reflux for 18 hr., cooled, and treated with excess ethyl acetate. The mixture was poured into 100 ml. of 5% acetic acid and the product was extracted with ether. The ether layer was washed with 5% sodium bicarbonate solution and water, dried (Na_2SO_4), and evaporated. The residue was recrystallized from methanol to give 0.04 g. of colorless crystals: m.p. 175–176°; $[\alpha]^{28}D$ +103° (*c* 1.32, $CHCl_3$); n.m.r.: 0.71 (C-18 methyl), 0.81, 0.91 (C-21, C-26, C-27 methyls), 3.82 (doublet) (6α-H), 4.08 (multiplet) (3α-H) p.p.m.; lit.[12] m.p. 165–168°, $[\alpha]D$ +98°.

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 79.94; H, 11.12; (N, 0.0).

B. From IV.—A solution of 0.50 g. of IV and 0.50 g. of lithium aluminum hydride in 15 ml. of tetrahydrofuran was heated under reflux for 18 hr., cooled, decomposed with ethyl acetate, acidified with 5% acetic acid, and extracted with ether. The washed and dried ether extract was evaporated to give 0.06 g. of colorless crystals of VII, identical with the previous preparation.

C. From XIV.—The benzene filtrate from the preparation of XVII was evaporated and the residue was recrystallized from acetonitrile to give 0.04 g. of the product.

5α-Chloro-3β,19-6β,19-diepoxycholestane (VIII).—A solution of 0.50 g. of I in 25 ml. of methanol was acidified to pH 1 with 2 *N* hydrochloric acid and heated under reflux for 18 hr. The prepitate which was obtained on cooling was filtered and recrystallized from methanol to afford 0.05 g. of colorless plates: m.p. 137–138°; $[\alpha]^{28}D$ +39° (*c* 1.80, $CHCl_3$); n.m.r.: 0.72 (C-18 methyl), 0.82, 0.95 (C-21, C-26, C-27 methyls), 3.97 (3α-H, 6α-H), 5.20 (C-19 H) p.p.m.

Anal. Calcd. for $C_{27}H_{43}ClO_2$: C, 74.53; H, 9.96. Found: 74.35; H, 9.91.

The reaction mixture filtrate was made alkaline with 20% sodium hydroxide and the product was extracted into ether. The washed and dried ether extract was evaporated, and the residue was recrystallized from methanol to give 0.10 g. of IV, m.p. 194–195°.

3β,19-6β,19-Diepoxycholestan-5α-ol (IX).—A solution of 0.10 g. of XIV in 30 ml. of 50% aqueous acetic acid was heated under reflux for 18 hr. and cooled. The crystalline precipitate was filtered to give 0.06 g. of VIII, m.p. 137–138° after recrystallization from acetonitrile. The filtrate was diluted with water and extracted with ether, and the ether was washed with 5% sodium bicarbonate solution and water. The dried (Na_2SO_4), filtered solution was evaporated and the residue was recrystallized from acetonitrile to give 0.02 g. of halogen-free colorless needles: m.p. 241–243°; λ_{max}^{KBr} 3.00 μ; n.m.r.: 0.72 (C-18 methyl), 0.85, 0.95 (C-21, C-26, C-27 methyls), 3.85 (doublet) (6α-H), 4.10 (3α-H), 5.32 (C-19 H) p.p.m.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.84; H, 10.64. Found: C, 77.93; H, 10.50.

5α-Chloro-3β-hydroxy-19-oximinocholestan-6-one Acetate (X).—To a solution of 1.0 g. of I in 100 ml. of ether there was added a suspension of 0.50 g. of N-bromoacetamide in 20 ml. of 70% methanol. After 5 min., the mixture turned yellow. After 15 min., the solution was washed with water and 5% sodium bicarbonate solution and dried (Na_2SO_4). The filtered solution was evaporated and the residue solidified on treatment with methanol. Recrystallization from acetonitrile gave 0.80 g. (80%) of colorless plates: m.p. 161–163°; $[\alpha]^{25}D$ −15° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 3.0, 5.8, 5.9, 7.9, 8.1 μ.

Anal. Calcd. for $C_{29}H_{46}ClNO_4$: C, 68.54; H, 9.12. Found: C, 68.43; H, 9.12.

5α-Chloro-3β-hydroxy-19-oximinocholestan-6-one (XI).—A solution of 0.50 g. of X in 25 ml. of absolute ethanol containing 5% potassium hydroxide was kept at 27° for 5 hr. and diluted with 150 ml. of ether. The resulting solution was washed with brine and dried over sodium sulfate. The residue obtained from evaporation of the ether was washed with petroleum ether and recrystallized from methanol to afford 0.45 g. (98%) of colorless

(21) Melting points were determined with a Thomas–Hoover apparatus and are corrected. Infrared spectra were obtained with a Beckman IR-5 instrument. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Optical rotations were obtained in a 0.5-dm. tube with a Rudolph photoelectric polarimeter. N.m.r. spectra were obtained at a field strength of 60 Mc./sec. on samples in deuteriochloroform solution on a Varian A-60 instrument using tetramethylsilane as internal standard. Resonance positions are reported in δ (p.p.m.) values where possible; unresolved humps are described in c.p.s. units (60 Mc./sec.). It is a pleasure to thank Mr. H. Rolewicz for excellent technical assistance.

(22) S. Mori, *J. Chem. Soc. Japan*, **64**, 981 (1943).

needles: m.p. 236–237°; $[\alpha]^{25}D$ −17° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 2.9–3.1, 5.9 μ.

Anal. Calcd. for $C_{27}H_{44}ClNO_3$: C, 69.57; H, 9.54. Found: C, 69.43; H, 9.73.

5α-Chloro-19-O-methyloximino-6-oxocholestan-3β-ol Acetate (XII). A. Using Diazomethane.—To an ice-cold solution of 3.0 g. of X in 100 ml. of anhydrous ether containing 1.0 ml. of boron trifluoride etherate, there was added excess ethereal diazomethane, and the mixture was kept in an ice bath for 3 hr. A small amount of precipitate was removed by filtration and the clear filtrate was allowed to evaporate. The residue was recrystallized from methanol to furnish 1.60 g. of colorless crystals: m.p. 121–123°; λ_{max}^{KBr} 5.75, 5.98, 8.05, 9.55 μ; $[\alpha]^{25}D$ +7° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{30}H_{48}ClNO_4$: C, 69.00; H, 9.27. Found: C, 68.98; H, 9.05.

B. Using Methyl Iodide.—A stirred solution of 1.00 g. of X in 15 ml. of purified dimethylformamide was kept at 27° and treated in five equal portions with 5.0 ml. of methyl iodide and 5.0 g. of silver oxide during 30 min. The mixture was stirred for 24 hr., diluted with 150 ml. of ether, filtered, and evaporated *in vacuo*. The residue was recrystallized from methanol to afford 0.65 g. of the product, m.p. 121–123°.

5α-Chloro-3β-hydroxy-6-oxocholestane-19-nitrile Acetate (XIII).—A solution of 10.0 g. of I in 100 ml. of pyridine was added to a stirred suspension prepared[17] by adding 10.0 g. of chromic acid to 100 ml. of pyridine. After 48 hr. the mixture was poured onto a mixture of crushed ice and 200 ml. of concentrated hydrochloric acid. The resulting suspension was shaken with ether and the resulting mixture was filtered through glass wool. The layers were separated and the ether phase was washed with water, dried over sodium sulfate, filtered, and evaporated. The residue was first washed with methanol and then dissolved in hot acetonitrile. The acetonitrile solution was filtered through alumina and concentrated to give 4.4 g. (46%) of colorless crystals: m.p. 210–211°; $[\alpha]^{25}D$ −86° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 4.5, 5.8, 7.9, 8.1 μ.

Anal. Calcd. for $C_{29}H_{44}ClNO_3$: C, 71.06; H, 9.05. Found: C, 71.47; H, 9.45.

5α-Chloro-6β,19-epoxycholestan-3β,19-diol Diacetate (XIV).—To a warm solution of 10.0 g. of I in 700 ml. of glacial acetic acid there was added a solution of 15 g. of sodium nitrite in 40 ml. of water. The resulting solution was kept for 30 min. at 50° and poured into ice water. The product was extracted with ether and the ether extract was washed with 5% sodium bicarbonate solution and water and dried (Na_2SO_4). Evaporation of the filtered solution gave a residue which was recrystallized from aqueous 2-propanol and methanol to afford 5.2 g. of colorless needles: m.p. 155–157; $[\alpha]^{27}D$ +31° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 5.77, 8.05–8.15 μ; n.m.r.: 0.71 (C-18 methyl), 0.81, 0.91 (C-21, C-26, C-27 methyl), 2.05 (C-3 acetate methyl), 4.30 (doublet) (6α-H), 5.00–5.60 (low hump) (3α-H), 6.30 (C-19 H) p.p.m.

Anal. Calcd. for $C_{31}H_{49}ClO_5$: C, 69.31; H, 9.19. Found: C, 69.10; H, 9.23.

5α-Chloro-6β,19-epoxycholestane-3β,19-diol 3-Acetate (XV).—The 2-propanol mother liquor from the recrystallization of XIV was diluted with water and extracted with ether. The ether extract was washed with water, dried, and evaporated and the residue was crystallized from alcohol and then methanol to give 1.2 g. of colorless crystals, m.p. 143–146°. The analytical sample, obtained from methanol, had m.p. 150–152°; λ_{max}^{KBr} 3.00, 5.75, 8.05 μ; n.m.r.: 0.67 (C-18 methyl), 0.81, 0.91 (C-21, C-26, C-27 methyls), 2.02 (acetate methyl), 3.30 (6α-H), 4.18 (3α-H) p.p.m.; $[\alpha]^{28}D$ +8° (*c* 1.17, $CHCl_3$).

Anal. Calcd. for $C_{29}H_{47}ClO_4$: C, 70.34; H, 9.57. Found: C, 69.68; H, 9.25.

5α-Chloro-3β,6β-dihydroxycholestan-19-oic Acid 6,19-Lactone Acetate (XVI).—A solution of 0.10 g. of XV in 20 ml. of acetone was treated dropwise with 8 *N* chromic acid solution[23] until a brown color persisted. The excess oxidant was decomposed by addition of 2-propanol and the reaction mixture was filtered through a cotton pledget and evaporated. The residue was recrystallized from acetonitrile to afford 0.07 g. of colorless plates: m.p. 178–180°; λ_{max}^{KBr} 5.60, 5.75, 7.85 μ; $[\alpha]^{26}D$ +3° (*c* 1, $CHCl_3$).

(23) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

Anal. Calcd. for $C_{29}H_{45}ClO_4$: C, 70.63; H, 9.20. Found: C, 70.86; H, 8.95.

The same product was obtained on oxidation of 0.10 g. of XV with 0.10 g. of N-bromosuccinimide in 5 ml. of *t*-butyl alcohol at 95° for 1 hr. When 0.10 g. of XV in 50 ml. of ether was treated with 0.10 g. of N-bromosuccinimide in 10 ml. of aqueous methanol and kept at 27° for 1 hr., only the starting material was recovered.

5α-Cholestane-3β,6β,19-triol (XVII).—A solution of 0.90 g. of XIV and 3.0 g. of lithium aluminum hydride in 100 ml. of anhydrous ether was stirred at 27° for 4 hr. and decomposed with ethyl acetate. It was washed with 5% hydrochloric acid solution and water, dried (Na_2SO_4), and evaporated. The residue consisted of two main fractions, as shown by thin layer chromatography. It was slurried with benzene, and the benzene-insoluble material was recrystallized from methanol to give 0.40 g. of halogen-free colorless needles: m.p. 229–231°; λ_{max}^{KBr} 3.00, 3.15 μ; $[\alpha]^{28}D$ +23° (*c* 0.052, tetrahydrofuran).

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.09; H, 11.50. Found: C, 76.92; H, 11.24.

Acetylation of the triol with acetic anhydride in pyridine solution at 27° during 18 hr. gave the corresponding **triacetate**, identified by the n.m.r. spectrum: 0.67 (C-18 methyl), 0.82, 0.92 (C-21, C-26, C-27 methyls), 2.08, 2.12, 2.14 (acetate methyls) p.p.m.

5α-Chlorocholestane-3β,6β,19-triol 3-Acetate (XVIII).—A solution of 0.20 g. of XV in 50 ml. of methanol was treated with a solution of 0.10 g. of sodium borohydride in 0.5 ml. of water and kept for 1 hr. at 27°. It was poured into ice–water, kept for 1 hr., and extracted with ether. Evaporation of the washed and dried ether extract gave colorless crystals: m.p. 125–127°; λ_{max}^{KBr} 3.05 μ; $[\alpha]^{28}D$ +11° (*c* 0.86, $CHCl_3$).

Anal. Calcd. for $C_{29}H_{49}ClO_4$: C, 70.06; H, 10.17. Found: C, 69.87; H, 9.67.

3β,5β-Dihydroxy-6-oxocholestane-19-nitrile 3-Benzoate (XIX).—A suspension of 3.0 g. of XIII in 40 ml. of 5% potassium hydroxide in absolute ethanol was stirred for 1 hr., diluted with 15 ml. of absolute ethanol, and stirred for an additional 4 hr. The resulting solution was diluted with 200 ml. of ether, washed three times with brine, dried over sodium sulfate, and evaporated under reduced pressure. The residue was dissolved in 20 ml. of pyridine and allowed to react with 3 ml. of benzoyl chloride at 70° during 3 hr. The solution was poured into 60 ml. of 5% sodium bicarbonate solution, and the resulting precipitate was filtered after 0.5 hr. and washed with 5% sodium bicarbonate solution and water. Recrystallization from acetonitrile gave 1.3 g. (40%) of shiny plates: m.p. 230–232°; $[\alpha]^{25}D$ +18° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 3.0, 5.7, 5.8 μ.

Anal. Calcd. for $C_{34}H_{47}NO_4$: C, 76.51; H, 8.88; N, 2.62. Found: C, 76.42; H, 9.01; N, 2.41.

3β,5β-Dihydroxy-6-oxocholestane-19-nitrile 3-Acetate (XX).—This compound was obtained from XIII by the same method described for XIX except that acetic anhydride was substituted for the benzoyl chloride. There was obtained a yield of 12% of colorless fine needles: m.p. 175–176° after chromatography on alumina and recrystallization from acetone–petroleum ether; $[\alpha]^{25}D$ +12° (*c* 0.55, chloroform); λ_{max}^{KBr} 2.9, 4.5, 5.7, 5.8, 8.0 μ.

Better yields were obtained when the acetylation was carried out with acetic anhydride in acetic acid in the presence of *p*-toluenesulfonic acid.[19]

Anal. Calcd. for $C_{29}H_{45}NO_4$: C, 73.85; H, 9.62. Found: C, 73.68; H, 9.40.

3β,5β,6β-Trihydroxycholestane-19-nitrile 6-Acetate 3-Benzoate (XXI).—To a solution of 0.90 g. of XIX in a mixture of 35 ml. of dioxane and 25 ml. of methanol there was added, dropwise, a solution of 0.30 g. of sodium borohydride in 1 ml. of water. The mixture was kept at 27° for 1 hr., cooled in ice, and diluted with 200 ml. of water and extracted with ether. The united ether extracts were washed with water, dried ($NaSO_4$), filtered, and evaporated under reduced pressure to afford 0.90 g. of crude reduction product, m.p. 219–221°. The triol monobenzoate was kept in pyridine and acetic anhydride for 18 hr., and the solution was diluted with water. The product was filtered and recrystallized from methanol to furnish colorless needles: m.p. 220–222°; $[\alpha]^{25}D$ −43° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 3.1, 5.7, 5.8 μ.

Anal. Calcd. for $C_{36}H_{51}NO_5$: C, 74.83; H, 8.90. Found: C, 74.77; H, 8.70.

3β,5β,6β-Trihydroxycholestan-19-oic Acid 6,19-Lactone 3-Benzoate (XXII).—A solution of 0.20 g. of XXI in 30 ml. of methanol was treated with a stream of hydrogen chloride for 5

min., kept for 1 hr. at 27°, and evaporated. The crystalline residue (imino ether hydrochloride) was dissolved in 20 ml. of methanol, treated with 0.10 g. of sodium borohydride in 1 ml. of water, and kept for 1 hr. at 27°. The mixture was diluted with water, and the resulting precipitate was filtered and recrystallized from methanol to give a nearly quantitative yield of colorless crystals: m.p. 248–250°; $[\alpha]^{28}D$ +53° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 3.0, 5.7, 5.8 μ.

Anal. Calcd. for $C_{34}H_{48}O_5$: C, 76.08; H, 9.01. Found: C, 75.98; H, 9.02.

3β,5β,6β-Trihydroxycholestan-19-oic Acid 6,19-Lactone (XXIII).—A solution of 0.10 g. of the crude imino ether hydrochloride described in the preparation of XXIII was prepared in 5% methanolic potassium hydroxide solution and kept for 2 hr. at 27°. It was diluted with water and the resulting precipitate was collected and recrystallized from ethyl acetate to give colorless plates: m.p. 263–264°; λ_{max}^{KBr} 3.00, 3.1–3.2, 5.7 μ; $[\alpha]^{28}D$ +23° (*c* 0.30, $CHCl_3$).

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25. Found: C, 75.12; H, 10.22.

The Constitution of a Galactomannan from the Seed of *Gleditsia amorphoides*[1]

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A galactomannan composed of 28.6% D-galactose and 71.4% D-mannose was isolated in 30% yield from the seeds of *Gleditsia amorphoides*. Periodate oxidation showed that all the mannose and galactose units were attacked since hydrolysis of the reduced oxopolysaccharide gave only glycerol (1 mole) and erythritol (2.7 moles). The periodate consumption was 4.8 moles for every four hexose residues and at the same time 1 mole of formic acid was produced. Hydrolysis of the methylated polysaccharide yielded 2,3,4,6-tetra-O-methyl-D-galactose (1 mole), 2,3,6-tri-O-methyl-D-mannose (2.1 moles), and 2,3-di-O-methyl-D-mannose (1.1 moles).

Gleditsia amorphoides is a leguminous tree that grows in the northern part of the Argentine Republic where it is known as "espina corona". Its fruit has been studied by Riqué and Pardo[2a] who showed that its composition was similar to that of locust bean fruit.[2a] As the industrial applications of the galactomannan contained in its seed are continually increasing in this country it was of interest to study whether its structure is similar to those of the already known galactomannans.

The polysaccharide was extracted from the dry and coarsely ground seed with hot water. Addition of ethanol to the aqueous extract precipitated the polysaccharide (yield 30%) as a fibrous material. This crude product was purified by fractional precipitation from its aqueous solution by increasing the concentration of ethanol stepwise; nearly 90% of the initial weight precipitated at an ethanol concentration between 22 and 26% (wt. of ethanol/wt. of solution), no significant precipitation was obtained at lower or higher concentration (upper limit 50%). After two reprecipitations the polysaccharide showed a nitrogen content of less than 0.4% and a rotatory power of $[\alpha]^{25}D$ +22.4° (water).

Evidence of its homogeneity was provided by (a) a single, sharp peak in the sedimentation pattern obtained with the ultracentrifuge, (b) its precipitation from aqueous solution over a narrow concentration range of ethanol, and (c) the fact that the product obtained after purification through its acetate[2b] displays the same physical properties as the product purified by precipitation with ethanol.

The infrared spectrum of the polysaccharide showed absorption bands at 817 and 874 $cm.^{-1}$ thus indicating the presence of α-linked D-galactopyranose units and β-linked D-mannopyranose units, respectively.[3]

Acid hydrolysis of the purified galactomannan which had a D.P. (degree of polymerization) of 116 determined chemically[4] was shown to give rise to D-galactose (28.6%) and D-mannose (71.4%) in a molar ratio of 1:2.7. Variable results were obtained in periodate oxidation studies. With 0.01 *N* periodate, 4 moles of periodate were consumed for every four hexose units and hydrolysis of the corresponding reduced oxopolysaccharide gave rise to 1 mole of mannose for every four hexose units as well as glycerol and erythritol and trace amounts of galactose. However, when the oxidation was carried out with 0.1 *N* periodate, 4.8 moles of the oxidant were consumed for every four hexose residues and hydrolysis of the corresponding polyalcohol in this case gave glycerol (1 mole) and erythritol (2.7 moles)[4,5] with only trace amounts of galactose and mannose.

This resistance of some mannose residues to the periodate oxidation has been observed in several cases (guar[6,7] and fenugreek,[8] galactomannans of Lucerne and Clover seeds[9]) and has been attributed[9] to a steric effect resulting from the highly ramified structure of the galactomannan in which mannose units form the branching points. Present knowledge, however, indicates that this phenomenon is most likely due to cyclic acetal formation.

The fact that only traces of mannose and galactose survived the periodate treatment indicate that no significant amounts of (1 → 3) linkage are present. The large proportion of erythritol released upon acid hydrolysis of the polyalcohol serves as evidence that the main polymeric linkage was of the (1 → 4) type and the ratio of this erythritol to the glycerol indicated a branching point, on the average, every three units in the backbone. The molar proportion of periodate consumed and of formic acid produced (1 mole/every four hexose units) corroborate these findings.

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