Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 80.90; H, 10.62.

The product (VIII) was found to be identical by mixture melting point and infrared spectral comparison (chloroform solution) with a sample provided by Professor G. Ourisson.[21]

The ketone (VIII, 0.10 g.) from δ-apoallobetulin was heated during 1.5 hr. in a refluxing solution prepared from ethanol (25 ml.) and sodium (1 g.). After cooling, and dilution with dilute hydrochloric acid, starting material was recovered in almost quantitative yield.

Osmium tetroxide hydroxylation of δ-apoallobetulin (III). Hydroxylation of δ-apoallobetulin (0.6 g.) with osmium tetroxide (0.37 g.) in pyridine (10 ml.) solution was accomplished as described previously for the direct oxidation of α-apoallobetulin to diketone VII. The crude dark purple oil was dissolved in 1:1 petroleum ether–benzene and chromatographed on activated alumina (20 g.). Elution with chloroform, after first using a series of petroleum ether–benzene mixtures, benzene and ether respectively, gave a solid which recrystallized from methanol-water as almost colorless crystals; yield 0.2 g., m.p. 220–225°. Another recrystallization from the same solvent raised the melting point to 230–235° and yielded 0.16 g. A pure specimen was obtained from 95% ethanol as colorless plates, m.p. 242–244°, $[\alpha]_D^{22}$ +75.6°, (*c*, 1.23), λ_{max}^{KBr} 2.85 and 2.98 μ.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.29; H, 10.97.

ORONO, ME.

[CONTRIBUTION FROM THE MEDICAL RESEARCH LABORATORY, DEPARTMENT OF MEDICINE, VETERANS ADMINISTRATION HOSPITAL, INDIANAPOLIS, AND THE DEPARTMENT OF BIOCHEMISTRY, INDIANA UNIVERSITY SCHOOL OF MEDICINE]

Some Reactions of Methyl 3α-Hydroxy-12α-methoxy-9(11)-cholenate

ROBERT T. BLICKENSTAFF AND EMERSON L. FOSTER

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The tosylate (**2**) of methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate reacts with chloride ion, collidine, dimethylformamide and sodium methoxide to give the corresponding 3β-chloro (**3**), Δ⁸- (**4**), 3β-formoxy (**15**) and 3β-methoxy (**20**) derivatives. Compounds **2**, **3**, and **4** are readily chlorinated in the 12-position with hydrogen chloride; under the same conditions **15** loses the 3β-formate group. The tosylate (**9**) of methyl 12α-acetoxy-3α-hydroxy-9(11)-cholenate also was converted to the corresponding 3β-chloro (**10**), Δ⁸ (**11**), and 3β-formoxy (**12**) derivatives. Three compounds (**3**, **6**, and **7**) exhibit high activity in a seroflocculation reaction.

Bile acid derivatives containing two active groups (halogen, ester, unsaturation) in the ring system have been shown to be active seroflocculants.[1] Compounds containing three such active groups have not been examined, and methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate seemed to be a convenient starting material for the synthesis of such a series.

As outlined in Fig. 1, the key intermediate for the preparation of five of the desired derivatives was methyl 12α-methoxy-3α-tosyloxy-9(11)-cholenate (**2**). It was prepared by room temperature tosylation of **1** under standard conditions,[1c] but resisted our attempts at crystallization.[2] The reaction of the tosylate **2** with pyridinium chloride[1c] gave a crystalline 3β-chloro derivative (**3**), and dehydrotosylation[1d] of **2** gave an oily diene (**4**).[3] Chlorination of compounds **2**, **3** and **4** following the

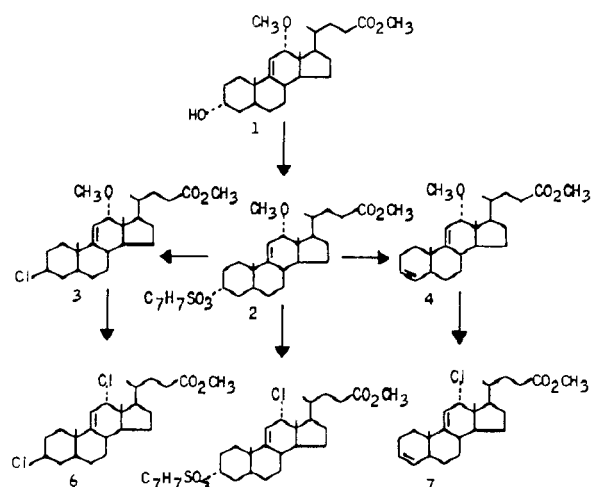

Figure 1

(1) (a) F. C. Chang and D. H. Sprunt, *J. Am. Chem. Soc.*, **76**, 3213 (1954); (b) F. C. Chang *et al.*, *J. Am. Chem. Soc.*, **79**, 2161 (1957); (c) F. C. Chang *et al.*, *J. Am. Chem. Soc.*, **79**, 2164 (1957); (d) F. C. Chang *et al.*, *J. Am. Chem. Soc.*, **79**, 2167 (1957); (e) R. T. Blickenstaff and F. C. Chang, *J. Am. Chem. Soc.*, **80**, 2726 (1958); (f) R. T. Blickenstaff and F. C. Chang, *J. Am. Chem. Soc.*, **81**, 2835 (1959); (g) R. T. Blickenstaff, *J. Am. Chem. Soc.*, **82**, 3673 (1960).

(2) The same compound had been obtained by Sarett [*J. Biol. Chem.*, **162**, 591 (1946)] in the form of an oil. It was dehydrotosylated to a diene which was not isolated, but was hydrolyzed to the acid. The acid was hydrogenated to 12α-methoxy-9(11)-cholenic acid.

(3) Issidorides, Fieser, and Fieser recently have reported [*J. Am. Chem. Soc.*, **82**, 2002(1960)] that dehydrotosylation of methyl 3α-tosyloxycholanate in refluxing lutidine produces an olefin mixture estimated to comprise 25% of the Δ²- and 75% of the Δ³-isomer. Methyl 2-cholenate is evidenced in the mixture by an infrared absorption band at 15.05 μ, which is in addition to the 14.70 μ band of pure methyl 3-cholenate. Our dehydrotosylation products **4** and **11** exhibit a weak band at 14.85 μ. An infrared curve of compound 4 determined on a Beckman IR5 contains only the single maximum below 15 μ, and an absorption minimum at 15.05 μ. We believe these products to be predominantly Δ⁸ and have so named them, but we cannot exclude the possibility of Δ² contamination.

method of Turner *et al.*[4] gave crystalline 12α-chloro products, methyl 12α-chloro-3α-tosyloxy-9(11)-cholenate (5), methyl 3β,12α-dichloro-9(11)-cholenate (6), and methyl 12α-chloro-3,9(11)-choladienate (7), respectively. Assignment of the 12-chloro configuration as α is based on the generalization of Mattox *et al.*[5] The replacement of methoxyl by chlorine is accompanied by the loss of an intense infrared peak at 9.11 to 9.14 μ (C—O—C),[6a] and the appearance of a new, weak band at 14.2–14.3 μ (C—Cl). The latter is readily distinguished from the 13.9 μ band of the 3β-Cl compounds.[6b]

Tosylation of the known methyl 12α-acetoxy-3α-hydroxy-9(11)-cholenate (Fig. 2, compd. 8)

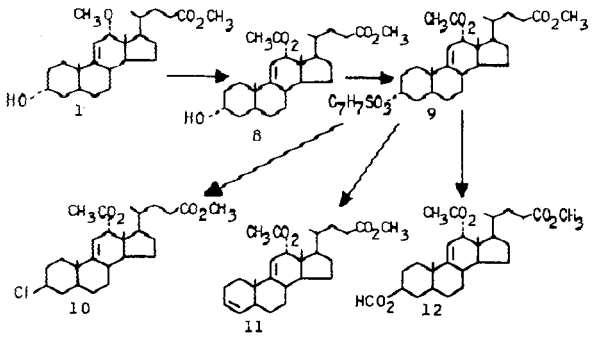

Figure 2

gave methyl 12α-acetoxy-3α-tosyloxy-9(11)-cholenate (9), which underwent substitution and elimination reactions satisfactorily to produce methyl 12α-acetoxy-3β-chloro-9(11)-cholenate (10) and methyl 12α-acetoxy-3,9(11)-choladienate (11), respectively. It also reacted with *N,N*-dimethylformamide to give methyl 12α-acetoxy-3β-formoxy-9-(11)-cholenate (12).[7] Compounds 9–12, all oils, were purified by chromatography.

The product resulting from the reaction of the tosylate 2 with dimethylformamide depends on the temperature; at 78° methyl 3β-formoxy-12α-methoxy-9(11)-cholenate (Fig. 3, compd. 15), is obtained, while at reflux the methoxyl group is replaced and methyl 3β,12α-diformoxy-9(11)-cholenate (18) is the product. Formylation is accompanied by the appearance of characteristic maxima in the infrared spectra at 5.74–5.76 μ and 8.5 μ.

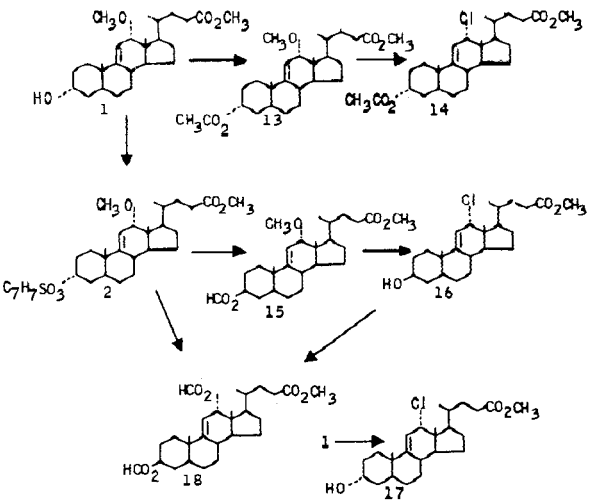

Figure 3

Chlorination [which proceeds smoothly with methyl 3α-acetoxy-12α-methoxy-9(11)-cholenate (13) to give methyl 3α-acetoxy-12α-chloro-9(11)-cholenate (14)[4]] of the monoformate 15 resulted in loss of the formate moiety, producing methyl 12α-chloro-3β-hydroxy-9(11)-cholenate (16), in spite of our efforts to maintain an anhydrous system. The structure of this product is shown by its nonidentity to the known 3α-epimer 17, by its 9.66 μ maximum in the infrared curve[8] (compared with a 9.60 μ band for 17), and by the fact that when it was esterified with formic acid and sulfuric acid at room temperature, the 12-chloro group was simultaneously displaced giving methyl 3β,12α-diformoxy-9(11)-cholenate (18).

The action of potassium and methyl iodide on methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate produced methyl 3α,12α-dimethoxy-9(11)-cholenate (Fig. 4, compd. 19). The 3-epimer, methyl

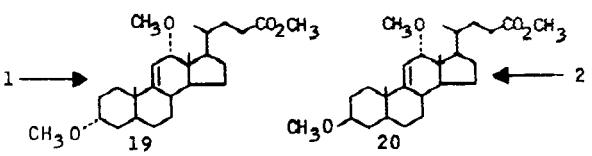

Figure 4

3β,12α-dimethoxy-9(11)-cholenate (20), was obtained from the tosylate 2 and sodium methoxide in refluxing methanol.

When screened in a serofloccculation reaction[1b] against cancer and normal sera, compounds 3, 6, and 7 were found to be very active. Compounds 4, 10, 15, and 18 possess moderate activity, compounds 5, 11, 12, 13, 14, and 20 are inactive and compounds 2, 9, and 19 are too insoluble to be tested.

(4) R. B. Turner *et al.*, *J. Biol. Chem.*, **162**, 571 (1946).

(5) V. R. Mattox *et al.*, *J. Biol. Chem.*, **173**, 283 (1948).

(6) (a) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, J. Wiley and Sons, Inc., New York, 1958, p. 115. (b) p. 331.

(7) F. C. Chang and R. T. Blickenstaff, *J. Am. Chem. Soc.*, **80**, 2906 (1958). The β-configuration for the 3-formate group in compounds 12, 15, and 18 is assigned on the basis of the analogous conversion of methyl 3α-tosyloxycholanate to methyl 3β-formoxycholanate. As compounds 12, 15, and 18 are oils, however, their stereochemical homogeneity may be questioned.

(8) A. R. H. Cole, R. N. Jones, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 5571 (1952).

EXPERIMENTAL[9]

Methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate (1). A sample, m.p. 157–159.5°[10] was recrystallized from methanol-water to give diamond-shaped plates, m.p. 161.5–163.5°, $[\alpha]_D$ +136°, (lit.[11] m.p. 162–162.5°, $[\alpha]_D$ +131°), $\lambda_{max}^{CS_2}$ 5.70, 8.55, 9.10, 9.60 μ.

Methyl 12α-methoxy-3α-tosyloxy-9(11)-cholenate (2). *p*-Toluenesulfonyl chloride (4 g.) was added to 28 ml. of anhydrous pyridine containing 4 g. of methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate (which had been dried by distilling benzene from it). After 19 hr. at room temperature the solution was diluted with ice, which deposited an oil. The supernatent was decanted and the residue was washed with water, dilute hydrochloric acid twice, and again with water, and dried in ether over sodium sulfate. The 4.7 g. of glass obtained on evaporation of the ether was sufficiently pure for subsequent reactions. The analytical sample, chromatographed on alumina (ligroin[12]-ether), resisted crystallization attempts; $[\alpha]_D$ +98°, $\lambda_{max}^{CS_2}$ 5.71, 8.40, 8.49, 9.11, 10.35, 10.75, 11.42, 11.85, 12.28 μ.

Anal. Calcd. for $C_{32}H_{46}O_6S$: C, 68.91; H, 8.13; S, 5.75. Found: C, 68.79; H, 8.30; S, 5.55.

Methyl 3β-chloro-12α-methoxy-9(11)-cholenate (3). A solution of 1.00 g. of the tosylate 2 and 1.05 g. of pyridinium chloride in 10 ml. of pyridine was warmed at 80–85° for 24 hr. Addition of water separated an oil which was washed several times with water and with dilute hydrochloric acid, and again with water, then dried in ether over sodium sulfate. The residue from evaporation of the ether was chromatographed on alumina (ligroin-ether), giving 0.50 g. of an oil which slowly crystallized on standing. Recrystallization in methanol gave 0.30 g. of product, m.p. 83–85.8°, used in the preparation of 6. The analytical sample crystallized from methanol-acetone (4:1) as platelets, m.p. 85.8–88.0°, $[\alpha]_D$ +117°, $\lambda_{max}^{CS_2}$ 5.72, 9.14, 13.95 (w) μ.

Anal. Calcd for $C_{26}H_{41}O_3Cl$: C, 71.46; H, 9.46; Cl, 8.11. Found: C, 71.82; H, 9.24; Cl, 8.44.

Methyl 12α-methoxy-3,9(11)-choladienate (4). A solution of 0.70 g. of the tosylate 2 was heated in 10 ml. of refluxing collidine for 2 hr., cooled to room temperature and diluted with water and ether. The ether layer was washed with dilute hydrochloric acid, aqueous sodium bicarbonate, and water, then dried over sodium sulfate. Evaporation of the ether left a residue which was chromatographed on alumina (ligroin-benzene) to give 0.31 g. of an oil, $[\alpha]_D$ +96°, $\lambda_{max}^{CS_2}$ 5.70, 8.54, 9.11, 14.85 μ.

Anal. Calcd. for $C_{26}H_{40}O_3$: C, 77.95; H, 10.06. Found: C, 77.72; H, 10.05.

Methyl 12α-chloro-3α-tosyloxy-9(11)-cholenate (5). Anhydrous hydrogen chloride was passed into an ice-cooled solution of 1.00 g. of 2 in 10 ml. of chloroform for 30 min. The solution was cooled for an additional 30 min., then evaporated *in vacuo*. The oily residue precipitated from benzene-ligroin as 0.67 g. of an amorphous solid, m.p. 118–119°. The analytical sample crystallized from ether-ligroin (b.p. 35–60°) in the form of platelets, m.p. 119–120.2°, $[\alpha]_D$ +108°, $\lambda_{max}^{CS_2}$ 5.70, 8.40, 8.49, 10.34, 10.55, 10.71, 14.2 (w) μ.

Anal. Calcd. for $C_{32}H_{45}O_5ClS$: C, 66.48; H, 8.02; Cl, 6.13; S, 5.55. Found: C, 66.69; H, 8.24; Cl, 6.25; S, 5.41.

Methyl 3β,12α-dichloro-9(11)-cholenate (6). Chlorination of 0.63 g. of 3 in a manner identical to the preparation of 5 gave a crude product which crystallized on standing. Recrystallization from acetone gave 0.46 g. of plates, m.p. 112–115°, $[\alpha]_D$ +140°, $\lambda_{max}^{CS_2}$ 5.70, 8.5, 11.40, 13.89, 14.3 (w) μ.

Anal. Calcd. for $C_{25}H_{38}O_2Cl_2$: C, 68.01; H; 8.68; Cl, 16.06. Found: C, 68.32; H, 8.89; Cl, 15.90.

Methyl 12α-chloro-3,9(11)-choladienate (7). Similar chlorination of 0.60 g. of 4 gave a crude material which crystallized out of benzene-ligroin (b.p. 35–60°), 0.31 g., m.p. 85–90°. Recrystallization in ligroin (b.p. 35–60°) gave laths, m.p. 89.6–91.2°, $[\alpha]_D$ +137°, $\lambda_{max}^{CS_2}$ 5.71, 8.55, 11.55 (w), 14.2 (w) μ.

Anal. Calcd. for $C_{25}H_{37}O_2Cl$: C, 74.20; H, 9.21; Cl, 8.76. Found: C, 74.27; H, 9.36; Cl, 8.70.

Methyl 12α-acetoxy-3α-hydroxy-9(11)-cholenate (8) was prepared from 1 according to Turner *et al.*;[4] m.p. 106.5–109.0°, $[\alpha]_D$ +195° (methanol), [lit. m.p. 107–107.5°, $[\alpha]_D$ +195° (methanol)], $\lambda_{max}^{CS_2}$ 5.78, 8.09, 8.60, 9.66, 9.89, 10.24, 10.90 μ.

Methyl 12α-acetoxy-3α-tosyloxy-9(11)-cholenate (9). Tosylation of 8 in a manner similar to the preparation of 2 gave a liquid tosylate. The analytical sample was chromatographed on alumina (ligroin-ether) to give an oil, $[\alpha]_D$ +145°, $\lambda_{max}^{CS_2}$ 5.71, 8.01, 8.36, 8.45, 9.80, 10.31, 10.69, 11.8, 12.24 μ.

Anal. Calcd. for $C_{34}H_{49}O_7S$: C, 67.87; H, 8.15; S, 5.33. Found: C, 67.54; H, 8.31; S, 5.75.

Methyl 12α-acetoxy-3β-chloro-9(11)-cholenate (10). The reaction of 1.0 g. of 9 with pyridinium chloride in a manner similar to the preparation of 3 gave 0.8 g. of an oil, $[\alpha]_D$ +171°, $\lambda_{max}^{CS_2}$ 5,73, 8.07, 9.85, 10.2, 13.9 (w) μ.

Anal. Calcd. for $C_{27}H_{41}O_4Cl$: C, 69.73; H, 8.89; Cl, 7.62. Found: C, 69.26; H, 8.67; Cl, 7.21.

Methyl 12α-acetoxy-3,9(11)-choladienate (11) was prepared by dehydrotosylation of 9 in collidine (as in the preparation of 4), giving an oil, $[\alpha]_D$ +103°, $\lambda_{max}^{CS_2}$ 5.73, 8.05, 9.85, 10.21, 14.9 μ.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.23; H, 9.63.

Methyl 12α-acetoxy-3β-formoxy-9(11)-cholenate (12). A solution of 0.50 g. of the tosylate 9 in 7 ml. of dimethylformamide was warmed at 75–85° for 40 hr. The addition of ice separated an oil which was extracted with benzene. The benzene solution was dried over sodium sulfate and evaporated in vacuo leaving 0.23 g. of crude product. This was chromatographed on Florisil[13] twice (ligroin-ether) to give an oil, $[\alpha]_D$ +134°, $\lambda_{max}^{CS_2}$ 5.7 (sh), 5.74, 7.91, 8.03, 8.4, 9.1, 9.8 μ.

Anal. Calcd. for $C_{28}H_{30}O_6$: C, 70.70; H, 9.11. Found: C, 70.91; H, 8.81.

Methyl 3α-acetoxy-12α-methoxy-9(11)-cholenate (13) was prepared from 1 according to Turner *et al.*,[4] was chromatographed on alumina (ligroin-ether) to give an oil which was sufficiently pure for preparation of the chloro compound 14. The oil gradually solidified; recrystallization in methanol-water gave needles, plates and rods, m.p. 79.8–81.4°, $[\alpha]_D$ +136° (lit., m.p. 102–103°, $[\alpha]_D$ +140°), $\lambda_{max}^{CS_2}$ 5 .70, 8 .01, 9.1, 9.6–9.7 μ.

Methyl 3α-acetoxy-12α-chloro-9(11)-cholenate (14), prepared from 13 according to Turner, *et al.*,[4] was obtained as large plates, m.p. 138.6–142.2°, $[\alpha]_D$ +149°, (lit. m.p. 137.5–138.5°, $[\alpha]_D$ + 154°), $\lambda_{max}^{CS_2}$ 5.72, 8.06, 9.69, 14.2–14.3 (w) μ.

Methyl 3β-formoxy-12α-methoxy-9(11)-cholenate (15). Treatment of 2.0 g. of tosylate 2 with dimethylformamide as

(9) Microanalyses by Galbraith Laboratories, Knoxville, Tenn. Melting points were taken on an electrical hot-stage and are uncorrected. Optical rotations were determined in 1% chloroform solutions at about 25°, using a Keston polarimeter attachment to a Beckman DU spectrophotometer. Infrared spectra were determined in carbon disulfide solution with an Infracord; medium and strong bands are reported (except for common C—H stretching and bending bands). In the seroflocculation testing dimethylformamide was used throughout as the organic solvent, rather than alcohol as described previously (Ref. 1b).

(10) Kindly furnished by Dr. E. P. Oliveto of the Schering Corporation.

(11) B. F. McKenzie *et al.*, *J. Biol. Chem.*, **175**, 249 (1948).

(12) The "ligroin" used in these experiments was Skellysolve B, b.p. 63–70°, except where the notation 35–60° indicates Skellysolve F (Skelly Oil Co.), purified by sulfuric acid treatment and distillation.

(13) Floridin Company.

in the preparation of **12**, gave 1.60 g. of an oil which was chromatographed on Florisil (ligroin-benzene and ether). The early fractions, 0.15 g., were identified as the 3,9(11)-diene **4** (infrared), and were followed by the desired formate, 1 45 g., an oil, $[\alpha]_D$ +116°, $\lambda_{max}^{CS_2}$ 5.70, 5.75, 8.39, 8.48, 9.11 μ.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.40; H, 9.86.

Methyl 12α-chloro-3β-hydroxy-9(11)-cholenate (**16**). Chlorination of 0.40 g. of compound 15 under conditions identical to the preparation of 5, gave a crude product whose infrared curve lacks formate bands. Crystallization of this material from ligroin-benzene gave 0.13 g. of rods, m.p. 121–127.5°, m.p. of mixture with **17** 108–120°, $[\alpha]_D$ +150°. The analytical sample was recrystallized in chloroform-ligroin (35–60°), m.p. 131–135.2°, $[\alpha]_D$ +154°, $\lambda_{max}^{CS_2}$ 5.71, 8.5–8.6, 9.66, 11.42, 14.3–14.4 (w) μ.

Anal. Calcd. for $C_{25}H_{39}O_3Cl$: C, 70.98; H, 9.29; Cl, 8.38. Found: C, 71.28; H, 9.53; Cl, 8.15.

Methyl 12α-chloro-3α-hydroxy-9(11)-cholenate (**17**), prepared from **1** according to Mattox *et al.*,[14] was difficult to obtain in a sharply-melting form. The best sample crystallized from ligroin-ether, m.p. 113–126°, $[\alpha]_D$ +143°, (lit., m.p. 119–124°, $[\alpha]_D$ +149°), $\lambda_{max}^{CS_2}$ 5.70, 8.5–8.6, 9.60, 14.2–14.3 (w) μ.

Methyl 3β,12α-diformoxy-9(11)-cholenate (**18**). A solution of 1.00 g. of **2** in 10 ml. of dimethylformamide was refluxed 48 hr., cooled, diluted with water, and extracted with benzene. The benzene layer was dried over sodium sulfate and evaporated to give 0.60 g. of crude product. Chromatography on Florisil gave 0.21 g. of an unidentified oil (eluted by ligroin) and 0.35 g. of crude product (ligroin-ether 2:1). The latter was rechromatographed on Florisil to give an oil $[\alpha]_D$ +16° (0.77%), $\lambda_{max}^{CS_2}$ 5,70, 5.76, 8.4–8.5 μ.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found: C, 70.24; H, 8.99.

The same product was obtained in an attempt to esterify methyl 12α-chloro-3β-hydroxy-9(11)-cholenate. A solution of 0.26 g. of **16** and 4 drops of concd. sulfuric acid in 20 ml. of 88% formic acid stood at room temperature 4 hr., was poured on ice, and extracted with ether. The ether layer was washed with water, dried over sodium sulfate, and evaporated leaving a chlorine-free (Beilstein) residue. Chromatography on Florisil gave 0.18 g. of an oil with an infrared curve corresponding to that of the diformate prepared from **2**.

Methyl 3α,12α-dimethoxy-9(11)-cholenate (**19**). An excess of potassium was added to a solution of 2.00 g. of **1** in 20 ml. of benzene. After the mixture had refluxed 2 hr. and cooled, methyl iodide (5 ml.) was added, and the mixture again refluxed for 3 hr. Methanol was added to the cooled mixture to destroy the excess potassium. The solution was evaporated to dryness and the residue extracted with ether. Evaporation of the ether left 1.77 g. of an oil which was chromatographed on alumina (ligroin-benzene) and crystallized from acetone, m.p. 190–195° (d), $[\alpha]_D$ +142°, $\lambda_{max}^{CS_2}$ 5.71, 7.92, 8.55, 9.10 μ.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96: H, 10.30. Found: C, 74.81: H, 9.82.

Methyl 3β,12α-dimethoxy-9(11)-cholenate (**20**). A methanolic solution of 2.20 g. of tosylate **2** and 0.40 g. of sodium methoxide was refluxed 60 hr. The methanol was evaporated and the residue extracted with ether; evaporation of the ether left 1.90 g. of crude product. Chromatography on alumina gave 0.94 g. of product **20** (eluted by ligroin-benzene) and 0.8 g. of recovered **2** (eluted by ether). The first fraction was rechromatographed on alumina to give the analytical sample, an oil, $[\alpha]_D$ +113, $\lambda_{max}^{CS_2}$ 5.70, 7.96, 8.52, 9.11, 14.3 μ.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.30. Found: C, 74.52; H, 10.28.

Acknowledgment. The authors are grateful to Mr. George Hayes for conducting the serological tests.

INDIANAPOLIS 7, IND.

(14) V. R. Mattox *et al.*, *J. Biol. Chem.*, **164**, 569 (1946).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Alkaline Degradation of Guaran and Characterization of "β"-D-Isosaccharinic Acid[1]

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Salts of "β"-D-isosaccharinic acid and 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid are obtained in equivalent amounts from oxygen-free alkaline solutions of guaran. An analysis method for mixtures of "α"- and "β"-D-isosaccharinic acids is presented. "β"-D-Isosaccharinic acid is obtained crystalline as its tetrabenzoate, and 5-*O*-α-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone and 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid have been synthesized.

That bases may be useful for the exploration of the chain structures of polysaccharides was first proposed by Corbett, Kenner, and Richards.[2] To show that the procedure is practicable, they treated the linear polysaccharide laminaran with an alkaline solution and isolated the predicted D-glucometasaccharinic acids.[3] Whistler and Corbett[4] have proposed that alkaline degradation can be used to determine branching in a polysaccharide. To test this proposition, the saccharinates from the alkaline treatment of guaran have been identified.

Guaran is a D-galacto-D-mannoglycan (33:67) consisting of a chain of β-(1 → 4)-linked D-mannopyranosyl units with single-unit side chains of D-galactopyranose residues linked α-(1 → 6) to one-half of the D-mannopyranosyl units.[5] The poly-

(1) Journal Paper No. 1690 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

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