3β,12β-Dihydroxycholanic Acid*

FREDERIC C. CHANG, NEVILLE F. WOOD, AND WILLIAM G. HOLTON

Department of Pharmacognosy, University of Tennessee, Memphis 3, Tennessee

Received November 17, 1964

A related synthesis of the three 3,12 isomers of desoxycholic acid, including the heretofore unreported 3β,12β compound, is described. 3β,12β-Dihydroxycholanic acid is not "α-lagodesoxycholic acid," and thus the identity of the latter remains undetermined. A comparison of some physical constants of the four isomeric acids and their methyl esters is presented.

Of the four possible 3,12-dihydroxy-5β-cholanic acids, only the venerable[1,2] desoxycholic (3α,12α) acid (I) is familiar and readily accessible. The 3β,12α isomer II is known[3] and has been identified recently as a metabolite found in both human and rabbit feces[4]; the 3α,12β acid III, synthesized some years ago,[5] has not been recorded since; and the 3β,12β compound IV has not been reported previously.

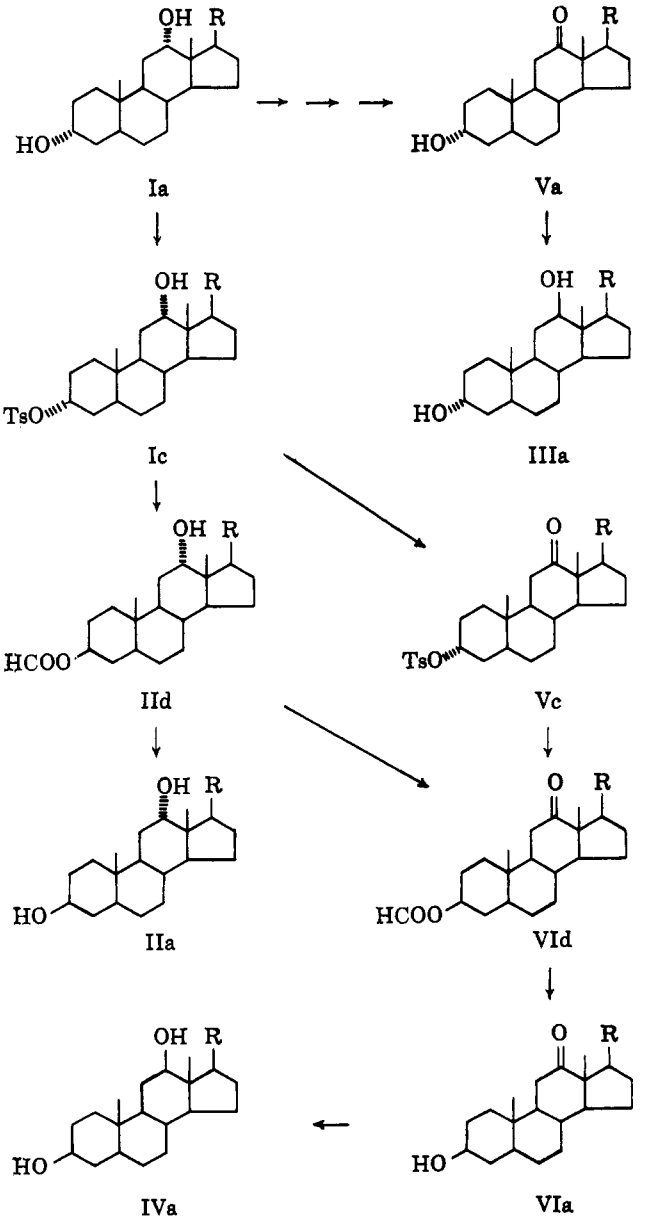
Our interest in cholanic acid derivatives and in 3,12-dihydroxy steroids[6] led us to studies of the 3,12-dihydroxycholanic acids, and the present paper is a report of a related synthesis of the three uncommon isomeric acids, including the 3β,12β one (see Scheme I).

(Methyl desoxycholate) 3-tosylate[7] (Ic) was inverted by reaction[8] with N,N-dimethylformamide (DMF) to the corresponding 3β-formate IId in 63% yield. Since Ic can be obtained in nearly quantitative yield from methyl desoxycholate, the route to 3β,12α-dihydroxycholanic acid (II) *via* Ic is superior to previously published preparations.[3,4b] The most recent process[4b] involved aluminum *t*-butoxide reduction of methyl 3-keto-12α-hydroxycholanate, an intermediate obtainable by selective Oppenheimer oxidation of methyl desoxycholate, and subsequent catalytic reduction of the ketone. The latter step yields only a slight excess of the 3β-hydroxy epimer, and the over-all yield of II from Ia by the two steps, calculated from the published figures, is 27%. The DMF route to II is thus simpler and more efficient.

A side product of the DMF inversion reaction is methyl 12α-hydroxy-3-cholenate (11%) which can be separated easily from the 3-formate by column chromatography. Unlike in other 3β-formates previously reported,[8] the formate group of IId is not cleaved cleanly to hydroxyl by passage through an alumina column, but selective hydrolysis to the methyl ester diol IIa proceeds smoothly under either acidic (methanol–HCl)[9] or basic (methanol–sodium methoxide) ester-exchange conditions. Consequently, the best route to IIa is to hydrolyze the total DMF product selectively and to separate the resulting mixture of of IIa and methyl 12α-hydroxy-3-cholenate by chromatography. A new crystalline derivative of IIa, the bistrifluoroacetate, was prepared.

SCHEME I

A RELATED SYNTHESIS OF ISOMERIC 3,12-DIHYDROXY-5β-CHOLANIC ACIDS



R = —CHCH₂CH₂COOCH₃ (CH₃)

TsO = *p*-toluenesulfonate

* To Professor Louis F. Fieser.

(1) L. F. Fieser and M. F. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 53–89.

(2) Elsevier's "Encyclopedia of Organic Chemistry," Series III, Vol. 14, Elsevier Publishing Co., Amsterdam, 1940, p. 183; Vol. 14S, 1962, p. 3223S.

(3) K. Matumoto, *J. Biochem.* (Tokyo), **36**, 173 (1944).

(4) (a) E. Heftmann, E. Weiss, H. K. Miller, and E. Mosettig, *Arch. Biochem. Biophys.*, **84**, 324 (1959); (b) H. Danielsson, P. Eneroth, K. Hellström, and J. Sjövall, *J. Biol. Chem.*, **237**, 3657 (1962).

(5) B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, **25**, 918 (1942); M. Sorkin and T. Reichstein, *ibid.*, **26**, 2097 (1943).

(6) (a) F. C. Chang, *J. Chinese Chem. Soc.* (Taiwan), **9**, 53 (1962); (b) F. C. Chang, *Tetrahedron Letters*, 2057 (1963); (c) F. C. Chang, *Clin. Chem.*, **10**, 490 (1964); (d) F. C. Chang, *J. Pharm. Sci.*, **53**, 1014 (1964).

(7) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **21**, 926 (1938); F. C. Chang, R. T. Blickenstaff, A. Feldstein, J. R. Gray, G. S. McCaleb, and D. H. Sprunt, *J. Am. Chem. Soc.*, **79**, 2164 (1957).

(8) F. C. Chang and R. T. Blickenstaff, *ibid.*, **80**, 2906 (1959).

(9) T. Reichstein and M. Sorkin, *Helv. Chim. Acta*, **25**, 797 (1942).

TABLE I

COMPARISON OF PHYSICAL CONSTANTS OF ISOMERIC 3,12-DIHYDROXY-5β-CHOLANIC ACIDS AND THEIR METHYL ESTERS

Structure of dihydroxy compd.	M.p., °C.[a]	Specific rotation, deg.[b]	R_f values[c] ABJ50:49	AB50	EG85	Characteristic infrared absorption bands, μ[d]
3α,12α						
Acid	173.8–174.4	+45.0	0.45			2.95, 5.80, 9.65
Me ester	70.0–108.5	+41.3		0.17	0.07	2.75, 2.88, 5.80, 9.66
3β,12α						
Acid	174.5–176.0	+39.0	0.50			2.80, 2.92, 5.78 (sh), 5.85, 9.70
Me ester	156.5–158.2	+34.8		0.23	0.15	2.74, 2.86, 5.77, 9.72
3α,12β						
Acid	172.5–173.6	+37.8	0.52			2.82, 2.95, 5.86, 9.65, 9.82 10.00
Me ester	Amorph.	+35.0		0.24	0.20	2.76, 2.92, 5.80, 9.70, 9.93
3β,12β						
Acid	204.3–205.6	+27.0	0.57			2.80, 3.00, 5.84, 9.73, 9.95
Me ester	115.8–116.3	+33.5		0.28	0.24	2.76, 2.90, 5.78, 9.73, 9.96

[a] See text for discussion. [b] Acid rotations were determined in dioxane, Me esters in chloroform; additional values in other solvents, see Experimental. [c] Thin layer chromatography. Acids were developed in ligroin–ethyl acetate–acetic acid, 50:49:1 (ABJ50:49); esters in ligroin–ethyl acetate 1:1 (AB50), in chloroform–acetone, 85:15 (EG85). [d] Spectra were determined on Perkin-Elmer Infracord No. 137; acids, 1% in KBr; esters, 6% in chloroform.

Not unexpectedly, the DMF reaction on 12α-mesylates failed to give inverted 12β-alcoholic products. At 78°, the 12α-mesylates were largely unaffected by DMF; at higher temperatures dehydrotosylation took place. Thus, favored routes to the 12β acids III and IV involve Raney nickel reduction (discussion in a later section) of the corresponding 3-hydroxy-12-keto derivatives Va and VIa. To Va, the steps from Ia to its 3-cathylate and oxidation of the latter to the 12-keto cathylate have been described by Fieser and Rajagapolan.[10] For selective hydrolysis of the 12-keto cathylate to Va, methanol–sodium methoxide was found effective; since the cathylate group is stable to acid, the methanolic HCl method is inapplicable.

The 3β-hydroxyl derivative VIa was prepared through the corresponding 3-formate VId, which was available by alternate routes. The first involves oxidation of IId, obtained through the DMF inversion reaction as described above. The second consists of the DMF and oxidation reactions performed in the reverse order; the tosylate Ic is oxidized to the 3-tosylate 12-ketone Vc, and the latter is subjected to the DMF inversion. Both routes are feasible, although oxidation proceeded somewhat more smoothly on the formate IId than on the tosylate Ic.

A version of the first route, starting with Ic *via* the total product of the DMF inversion reaction, proved to be most convenient for the preparation of ketone VIa. The total DMF reaction product (predominantly 3β-formate) was oxidized by potassium chromate in acetic acid; treatment with methanolic HCl of the resulting mixture, followed by crystallization from benzene–ligroin, gave VIa in 57% yield. This procedure excels in not requiring a purification step until the final crystallization of VIa is reached. However, the olefin present in the DMF reaction mixture is lost in this process.

Catalytic Reductions.—Reichstein and his colleagues,[5] in studying catalytic hydrogenations of 12-keto derivatives of cholanic acid derivatives, initially found that methyl 12-ketocholanate could be reduced with Raney nickel catalyst in methanolic solution to the corresponding 12β-hydroxy compounds in reasonable yields, but, with the latter keto compound, they encountered difficulties in separating the 12-hydroxyl epimers. Later, the Reichstein group reported that, by conducting the Raney nickel reduction in methanolic potassium hydroxide solution, 3α,12β-dihydroxy acid III was obtained in good yield and could be separated more easily from I. Their subsequent syntheses[11] of several 12β-hydroxy acids were carried out under similar alkaline conditions.

Our hydrogenations of ketones Va and VIa with Raney nickel as catalyst proceeded smoothly without added base, and each pair of methyl esters epimeric at C-12 were separated by chromatography over Florisil. The hydrogenations, however, were attended by unexpected long-range effects.[12] Ketones Va and VIa, differing only in configuration of the hydroxyl group in ring A, absorbed hydrogen at appreciably different rates, and yielded markedly different 12β:12α-hydroxyl ratios. Thus, under identical conditions, ketone Va with a 3α-hydroxyl required 8 hr. for complete hydrogenation, and the ratio of 3α,12β to 3α,12α product was approximately 1:1, whereas ketone VIa (3β-hydroxyl) was hydrogenated in 4 hr., and the ratio of 3β,12β to 3β,12α acid was 9:1. Similar experiments with 12-ketocholanic acid derivatives, either unsubstituted in ring A or with 3-formoxy or 3-ethoxycarboxy groups, indicate that rates of hydrogenation and product ratio of epimers vary considerably.[13]

Both 12β-hydroxyl acids III and IV are crystalline, but their derivatives appear to have poor crystallizing tendencies. Reichstein, *et al.*,[5] had prepared from III its methyl ester, methyl ester 3-monoacetate, methyl ester 12-monoacetate, and methyl diacetate, but were unable to obtain any of these in crystalline form, in contrast to their success in crystallizing analogous derivatives in the etiocholanic acid series.[11] Our efforts to crystallize these derivatives of III have also failed, but we have succeeded in obtaining the bistrifluoroacetate of IIIa in crystalline form.

(10) L. F. Fieser and S. Rajagopolan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).

(11) V. Wenner and T. Reichstein, *Helv. Chim. Acta*, **27**, 965 (1944); M. Sorkin and T. Reichstein, *ibid.*, **29**, 1218 (1946); S. Pataki, K. Meyer, and T. Reichstein, *ibid.*, **36**, 1295 (1953).

(12) D. H. R. Barton, F. McCapra, P. J. May, and F. Thudium, *J. Chem. Soc.*, 1297 (1960).

(13) These hydrogenation studies, now in progress, will be communicated separately.

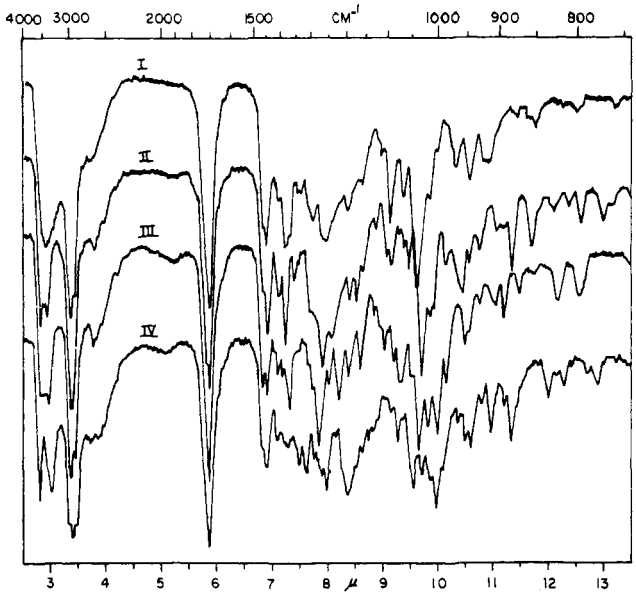


Figure 1.—Infrared spectra of the isomeric 3,12-dihydroxycholanic acids: I, 3α,12α; II, 3β,12α; III, 3α,12β; IV, 3β,12β (1% in KBr as pellets; Perkin-Elmer Infracord No. 137).

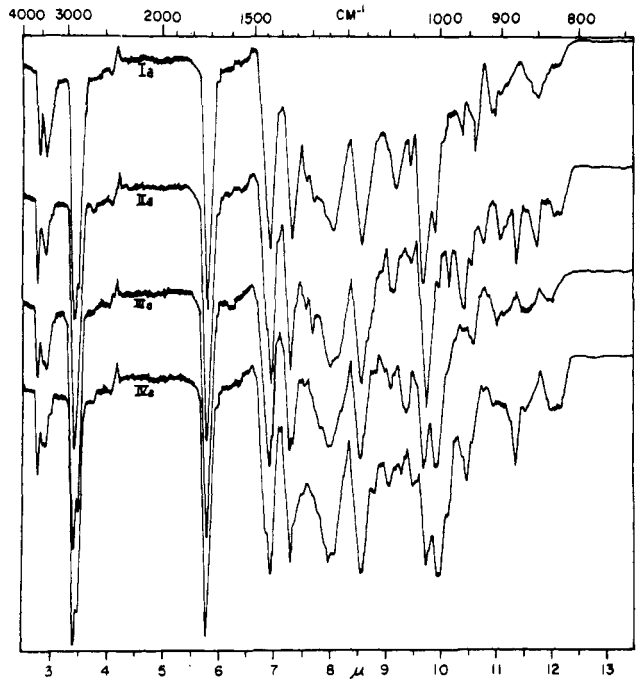


Figure 2.—Infrared spectra of the isomeric methyl 3,12-dihydroxycholanates: Ia, 3α,12α; IIa, 3β,12α; IIIa, 3α,12β; IVa, 3β,12β (6% in chloroform; Perkin-Elmer Infracord No. 137).

The methyl ester of new acid IV crystallized only after long standing, and no other derivatives of the ester have been obtained crystalline.

The physical characteristics of the isomeric acids and their methyl esters are summarized in Table I.

Melting Points and Solvation.—The notoriously erratic melting point behavior of desoxycholic acid is shared by its isomers to the extent that the melting point is unreliable as a criterion of purity. Melting points of the acids are difficult to reproduce, being dependent on the rate and duration of heating, and are extremely sensitive to trace amounts of solvent. Apparently, like desoxycholic acid, the isomers have marked tendency to form solvates with a number of solvents, perhaps as choleic acid type complexes which lose solvent with reluctance even at elevated temperatures and reduced pressures.

The melting points of the acids reported in Table I were taken on a Hershberg apparatus on analytically pure samples in Pyrex capillaries introduced into the heating bath within 2° of the melting point. The bath temperature was regulated to rise at the rate of 0.33°/min. in the melting range.

Unlike methyl desoxycholate,[14] the two crystalline isomeric methyl esters have satisfactory melting points.

Specific Rotations and Molecular Rotation Differences.—Rotations of some of the acids and methyl esters were determined in selected solvents for comparison with literature values, but, in addition, all acids were done in dioxane (for solubility reasons), and all methyl esters in chloroform, in order to obtain consistent molecular rotation differences. Molecular rotation differences for 3α–3β and 12α–12β hydroxyl groups, derived from pertinent pairs of values, agree reasonably well with published ranges of values.[15]

Column and Thin Layer Chromatography.—Synthesis of the two 12β compounds fortuitously involved column chromatographic separations between epimers that differed sufficiently in R_f values to make separation feasible. Thus, separations of 3α,12β from 3α,-12α compound and of 3β,12α from 3β,12β compound were successful. A separation of 3α,12β and 3β,12α mixture would have been difficult.

T.l.c. R_f values of the four acids and methyl esters as shown in Table I show the closeness of the R_f values of the 3α,12β and 3β,12α isomers. However, better separations were obtained by the special technique of multiple development[16] using chloroform–acetone mixtures, the best being a 9:1 mixture of these solvents.

Infrared Spectra.—Infrared spectra of the isomeric acids and their methyl esters are presented in Figures 1 and 2. The curves are sufficiently distinctive for purposes of identification of individual compounds, but of special interest for assignment of structure is a band at 10.00 μ most clearly seen in the ester curves, characteristic of the 12β-hydroxyl group in cholane derivatives.[17] In contrast, the 12α-hydroxyl group absorption is hardly distinguishable from the 3α- and 3β-hydroxyl bands, all falling near 9.7 μ.

(14) We were unable to confirm the report of R. Charonnat and B. Gauthier [*Compt. rend.*, **224**, 279 (1947)] that methyl desoxycholate has a melting point of 106° when it is prepared from acid of m.p. 190°. Our product, prepared by diazomethane reaction from highly purified acid, and carefully chromatographed, whether recrystallized from benzene, ether, or butanone (all anhydrous), had melting points ranging from 80 to 108°. The acid used had been obtained by hydrolysis of recrystallized (methyl desoxycholate) diacetate, and further purified by three recrystallizations from freshly redistilled butanone, the solvent specified by Charonnat and Gauthier. This acid, shown to be homogeneous by t.l.c., after drying at 140° (0.1 mm.) for 24 hr., melted at about 174°, depending on the rate and duration of heating. However, specific rotations of both the acid and methyl ester agreed well with Charonnat's values (see Experimental).

(15) W. Klyne, E. A. Braude, and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press Inc., New York, N. Y., 1955, p. 111.

(16) E. V. Truter, "Thin Film Chromatography," Interscience Publishers, Inc., New York, N. Y., 1963, p. 60.

(17) Unpublished comparisons in this laboratory of infrared spectra of 12α- and 12β-cholanol, and methyl 12α- and 12β-hydroxycholanate, confirm this observation.

Digitonin Precipitation.[18]—By conducting the reaction in 70% methanolic solution, the two 3β-methyl esters (3β,12α and 3β,12β) were found to precipitate with digitonin, whereas the two 3α isomers did not. Under the conditions used, the distinction between the 3α and 3β isomers is sharp.

Biological.—Koechlin and Reichstein[5] demonstrated that α-lagodesoxycholic acid, a constituent of rabbit bile,[19] is not 3α,12β-dihydroxy-5β-cholanic acid, and we can now state that neither is it the 3β,12β isomer. The identity of α-lagodesoxycholic acid is thus still unsettled.

Since 3α-hydroxy-12-ketocholanic acid, found earlier in ox bile,[20] has been shown recently by Norman and Sjövall[21] to be metabolized from desoxycholic acid by intestinal flora in the rat, the identification of 3β,12α acid in both human and rabbit feces suggests the possibility of an analogous formation of 3β-hydroxy-12-keto acid. The two keto acids, in turn, by normal biological reductive processes could give rise to small proportions of the corresponding 12β compounds, which conceivably might be present among minor components of the unidentified products of bile acid metabolism.

Experimental[22]

1. **(Methyl 3β,12α-dihydroxycholanate) 3-Formate (IId).**—Two grams of (methyl desoxycholate) 3-tosylate (Ic)[7] in 80 ml. of N,N-dimethylformamide was maintained at a constant temperature of 77.0–77.5°. The reaction,[8] monitored by thin layer chromatography (t.l.c.), showed complete disappearance of the tosylate in 60 hr. Water was added to the reaction mixture to near turbidity, and refrigeration brought out 1.28 g. of colorless crystalline product. Recrystallization was not effective for purification. However, chromatography of the mixture on a column of Florisil,[23] eluting with ligroin[24]–ether (10:1), gave 0.15 g. (11%) of methyl 12α-hydroxy-3-cholenate,[25] m.p. 111–112°; and, with ligroin–ether (5:1), 0.98 g. (63%) of (methyl 3β,12α-dihydroxycholanate) 3-formate (IId), which crystallized from ether in long transparent prisms with m.p. 160.6–161.4°; $[\alpha]_D$ +82.7°; $\lambda_{max}^{CHCl_3}$ 5.78 (C=O), 8.40, 8.52, 8.65 μ (3β-formate).[8]

Anal. Calcd. for $C_{26}H_{42}O_5$ (434.60): C, 71.85; H, 9.74. Found: C, 72.08; H, 9.95.

The final chromatographic fractions contained 0.04 g. (3%) of methyl 3β,12α-dihydroxycholanate (IIa).

From a reaction run on a larger scale in which 58.5 g. of tosylate Ic was treated with 1 l. of DMF, essentially analogous results were obtained.

2. **Methyl 3β,12α-Dihydroxycholanate[3,4] (IIa). A.**—To the formate IId (100 mg.), dissolved in 1 ml. of benzene and 3 ml. of methanol, was added at room temperature 1 ml. of 10% sodium methoxide in methanol solution. Reaction was complete in less than 1 hr. (t.l.c.). When the chilled alkaline solution was neutralized carefully with concentrated hydrochloric acid and water was added to near turbidity, dihydroxy methyl ester IIa crystallized in nearly quantitative yield. On recrystallization from methanol–water, long needles of IIa separated: m.p. 156.5–158.2°, $[\alpha]_D$ +34.8 and +39.5° (acetone); lit.[4] m.p. 157°, $[\alpha]_D$ +51.7° (acetone); $\lambda_{max}^{CHCl_3}$ 2.74, 2.86, 9.72 (OH), 5.77 μ ($COOCH_3$).

Anal. Calcd. for $C_{25}H_{42}O_4$ (406.59): C, 73.85; H, 10.41. Found: C, 74.01; H, 10.42.

B.—To the total solid mixture (4.0 g.) from the DMF reaction (part 1) dissolved in 100 ml. of methanol was added 0.5 ml. of concentrated hydrochloric acid. The reaction, monitored by t.l.c., was complete in less than 1 hr. Chromatography on Florisil permitted smooth separation of 0.52 g. (11%)[26] of methyl 12α-hydroxy-3-cholenate (eluted by ligroin–ether) and 3.28 g. (67%)[26] of IIa (eluted by ether). IIa crystallized from methanol–water was identical with the product of part A.

3. **(Methyl 3β,12α-dihydroxycholanate) Bistrifluoroacetate.**—Methyl ester IIa (100 mg.) dissolved in trifluoroacetic anhydride (0.5 ml.) had reacted completely in 15 min., as shown by t.l.c. After vacuum evaporation of excess anhydride, addition of methanol to the residual oil caused instantaneous separation of 125 mg. (85%) of crystals: m.p. 124.0–125.0°; on recrystallization from methanol–water, the melting point was unchanged; $[\alpha]_D$ +57.4°; $\lambda_{max}^{CS_2}$ 5.62 (trifluoroacetate), 5.77 ($COOCH_3$), 8.21, and 8.60 μ (trifluoroacetate).

Anal. Calcd. for $C_{29}H_{40}F_6O_6$ (598.61): C, 58.20; H, 6.74. Found: C, 57.91; H, 6.64.

4. **3β,12α-Dihydroxycholanic acid (II)** was obtained by saponification of its methyl ester IIa with 3% methanolic potassium hydroxide. After the usual processing, crystallization from ethyl acetate–ligroin afforded needles melting at 178.4–180.5° after drying at 140° (0.1 mm.): $[\alpha]_D$ +39.0° (dioxane) and +45.6° (EtOH); lit.[4b] m.p. 176°, $[\alpha]_D$ +47.3° (EtOH); λ_{max}^{KBr} 2.80, 2.92, 9.70 (OH), 5.78 (sh) and 5.85 μ (COOH).

Anal. Calcd. for $C_{24}H_{40}O_4$ (392.56): C, 73.43; H, 10.27. Found: C, 73.15; H, 10.47.

The compound, when crystallized from chloroform, gave long transparent prisms, m.p. 141.0–142.5°. When heated at 110°, the crystals gradually became opaque and finally melted at 174.5–176.0°.

Anal. Calcd. for $C_{24}H_{40}O_4 \cdot CHCl_3$ (511.95): C, 58.64; H, 8.07; Cl, 20.78. Found: C, 59.05; H, 7.83; Cl, 20.65.

5. **Methyl 3α-Hydroxy-12-ketocholanate[27] (Va). A.**—(Methyl 3α-hydroxy-12-ketocholanate) cathylate[10] in benzene, when treated at room temperature with methanolic sodium methoxide as in part 2A, was hydrolyzed quantitatively to Va. Crystallized from aqueous acetone, Va separated as long, transparent prisms, m.p. 112.0–113.6° (lit.[27] m.p. 111.5–113.5°).

B.—(Methyl 3α-hydroxy-12-ketocholanate) benzoate,[28] similarly treated with methanolic sodium methoxide, yielded Va, a product identical with that of part A, according to melting point and t.l.c.

6. **Methyl 3α,12β-Dihydroxycholanate[5] (IIIa). A.**—Methyl 3α-hydroxy-12-ketocholanate (Va, 2.5 g.) dissolved in 50 ml. of methanol was hydrogenated at a pressure of 3 atm. in a Parr apparatus in the presence of 5 g. of Raney nickel.[29] The reaction (monitored by t.l.c.) was complete in 8 hr. The product, according to t.l.c. determination,[30] was an approximately equal mixture of the 3α,12β and 3α,12α esters. This was confirmed by separation of the epimeric esters by careful column chromatography over Florisil. Methyl 3α,12β-dihydroxycholanate was eluted first (ligroin–ether, 2:1). The product, homogeneous by t.l.c., even after rechromatography resisted crystallization attempts and hardened to a glass. Physical constants were determined on this rechromatographed material [dried at 100° (0.1 mm.)]: $[\alpha]_D$ +35.0°, lit.[5] $[\alpha]_D$ +43.6° (acetone); $\lambda_{max}^{CHCl_3}$ 2.76, 2.92, 9.70, 9.93 (OH), and 5.80 μ ($COOCH_3$).

Anal. Calcd. for $C_{25}H_{42}O_4$ (406.59): C, 73.85; H, 10.41. Found: C, 73.42; H, 10.33.

(18) The reaction was carried out by mixing 70% aqueous methanolic solutions of digitonin and the methyl ester. The digitonin solution was saturated; the ester solution was slightly below saturation. Although methyl 3β,12α-dihydroxycholanate was reported[3] to form a precipitate with digitonin in alcoholic solution, details were not given, and the fairly extensive literature on digitonide formation of steroids includes only meager reference to bile acids and their congeners. We will report separately details of the reaction and other aspects of the digitonin precipitation of cholanic acid derivatives.

(19) S. Kishi, *Z. Physiol. Chem.*, **238**, 210 (1936).

(20) H. Wieland and S. Kishi, *ibid.*, **214**, 47 (1933).

(21) A. Norman and J. Sjövall, *J. Biol. Chem.*, **233**, 872 (1958).

(22) Microanalyses were by Galbraith Laboratories, Knoxville, Tenn.; Weiler and Strauss, Oxford, England; and A. Bernhardt, Mülheim, Germany. Melting points were taken on an electrical micro hot stage (except for the acids; see text). Optical rotations were determined in 2% chloroform solutions, except where stated; infrared spectra on Perkin-Elmer Infracord No. 137.

(23) Product of Floridin Co., 60–100 mesh.

(24) Ligroin in this work was Skellysolve B (Skelly Oil Co.), b.p. 63–70°, further purified by sulfuric acid treatment and distillation.

(25) F. C. Chang, A. Feldstein, J. R. Gray, G. S. McCaleb, and D. H. Sprunt, *J. Am. Chem. Soc.*, **79**, 2167 (1957).

(26) These percentages are based on tosylate Ic.

(27) B. Riegel and R. B. Moffett, *J. Am. Chem. Soc.*, **65**, 1971 (1943).

(28) B. F. McKenzie, V. R. Mattox, L. L. Engel, and E. C. Kendall, *J. Biol. Chem.*, **173**, 271 (1948).

(29) Product No. 28, Raney Catalyst Co., Chattanooga, Tenn., to whom we are indebted for a generous supply of catalyst.

(30) See ref. 16, p. 112.

B.—The epimeric mixture (3.0 g.) above, when converted to the bistrifluoroacetates as in part 3 and dissolved in methanol, yielded on refrigeration 1.64 g. (37%) of crystalline (methyl 3α,12β-dihydroxycholanate) bistrifluoroacetate (see part 7), m.p. 74°. T.l.c. shows that this crystalline product contained a trace of epimeric 3α,12α bistrifluoroacetate.

7. (Methyl 3α,12β-dihydroxycholanate) bistrifluoroacetate was prepared from IIIa as described for the 3β,12α compound (part 3). The yield was nearly quantitative; crystallized from methanol, it separated as needles which partially melted at 59–61°, resolidifying on slight cooling and remelting at 73.3–74.8°: $[\alpha]_D$ +49.4°; $\lambda_{max}^{CS_2}$ 5.63 (trifluoroacetate), 5.75 (C=O), 8.22, 8.60 μ (trifluoroacetate).

Anal. Calcd. for $C_{29}H_{40}F_6O_6$ (598.61): C, 58.20; H, 6.74. Found: C, 57.91; H, 6.64.

8. 3α,12β-Dihydroxycholanic acid[5] (III) obtained quantitatively by alkaline hydrolysis of the chromatographically homogeneous fractions of ester IIIa, as for acid II, crystallized from acetone in transparent elongated prisms with hexagonal ends: m.p. 172.5–173.6°, $[\alpha]_D$ +37.8° (dioxane); lit.[5] m.p. 178–180°, $[\alpha]_D$ +38.4 (dioxane); λ_{max}^{KBr} 2.82, 2.95, 9.65, 9.82, 10.00 (OH), and 5.86 μ (COOH), identical (mixture melting point and infrared spectra) with a sample prepared by Sorkin and Reichstein.[5,31]

Anal. Calcd. for $C_{24}H_{40}O_4$ (392.56): C, 73.43; H, 10.27. Found: C, 73.39; H, 10.35.

9. (Methyl 3α-hydroxy-12-ketocholanate) Tosylate (Vc). A.—Vc, prepared by tosylation[7] in pyridine solution of methyl 3α-hydroxy-12-ketocholanate (Va), when recrystallized from benzene–ligroin separated in rosettes of needles: m.p. 159.6–160.2°; $[\alpha]_D$ +74.5°; $\lambda_{max}^{CHCl_3}$ 5.78 (ester C=O), 5.88 (ketone), 8.43 (C–O–C), 8.53, and 10.77 μ (tosylate).

Anal. Calcd. for $C_{32}H_{46}O_6S$ (558.69): C, 68.79; H, 8.30. Found: C, 68.66; H, 8.42.

B.—The tosylate Ic (22.4 g.) was oxidized by the potassium chromate–acetic acid procedure[10] to give 15.0 g. (67%) of product, m.p. 154–155°. Recrystallized from benzene–ligroin, Vc melted at 159–160° and was identical with the product of part 9A, according to infrared and t.l.c.

10. (Methyl 3β-hydroxy-12-ketocholanate) Formate (VId). A.—Methyl 3β-hydroxy-12-ketocholanate[32] (VIa), dissolved in formic acid (reagent grade), had undergone formylation completely (t.l.c.) within 50 min. The formate VId crystallized on addition of water and, when recrystallized from ligroin, separated as rosettes of needles: m.p. 132.5–134.0°; $[\alpha]_D$ +82.7°; $\lambda_{max}^{CS_2}$ 5.70 (formate C=O), 5.75 ($COOCH_3$), and 5.83 μ (C-12 C=O).

Anal. Calcd. for $C_{26}H_{40}O_5$ (418.60): C, 72.19; H, 9.32. Found: C, 72.39; H, 9.42.

B.—The formate IId was quantitatively oxidized by potassium chromate–acetic acid to VId. The ketone crystallized from benzene–ligroin as long, thin needles, whose melting point and infrared spectrum were identical with those of the product VId in part A.

C.—The tosylate Vc underwent the DMF inversion reaction, carried out essentially as described for compound Ic, affording analogous products. Chromatography on Florisil gave methyl 12-keto-3-cholenate[33] (12%), eluted with ligroin–ether (5:1), crystallized from methanol: m.p. 119.0–119.5°, $[\alpha]_D$ +90.0°, $\lambda_{max}^{CHCl_3}$ 5.76 (ester C=O) and 5.86 μ (C-12 C=O).

Anal. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.69; H, 10.25. Found: C, 77.67; H, 9.91.

The second product from chromatography was (methyl 3β-hydroxy-12-ketocholanate) formate (VId, 58% yield; eluted with ligroin–ether, 2:1), identical with the product of part 10A, and the third was methyl 3β-hydroxy-12-ketocholanate (VIa, 4% yield; eluted with ether).

11. Methyl 3β-Hydroxy-12-ketocholanate (VIa). A.—The formate VId (100 mg.) was dissolved by warming in 2 ml. of a methanolic hydrochloric acid solution (100 ml. of CH_3OH:0.25 ml. of concentrated HCl) and allowed to react at room temperature. The reaction was complete in 1 hr. (t.l.c.) and, on addition of water, a solid crystallized which melted at 126.5–127.0° (lit. m.p. 115–117°,[32a] 120–126°[32b]).

B.—The total DMF reaction product (10.5 g.) from 17.7 g. of tosylate Ic, as described in part 1, consisting of methyl 12α-hydroxy-3-cholenate, (methyl 3β,12α-dihydroxycholanate) 3-formate (IId), and methyl 3β,12α-dihydroxycholanate (IIa), was dissolved in 200 ml. of acetic acid. A solution of potassium chromate (8.0 g. in 15 ml. of water) was added slowly and the whole was stirred overnight. Crystallization occurred on addition of water.

The filtered and water-washed product, after air drying (9.8 g.), was dissolved in 55 ml. of methanol, cooled to room temperature, and treated with 0.25 ml. of concentrated hydrochloric acid. After stirring for 1 hr. and addition of 150 ml. of water, the precipitate that formed was collected and thoroughly washed with water. T.l.c. showed that this product (9.5 g.) was largely methyl 3β-hydroxy-12-ketocholanate (VIa). Crystallization from benzene–ligroin (1:2) gave in two crops a total of 7.9 g. (57%) of needles, m.p. 125.5–127.5°. This product was identical (t.l.c. and infrared spectrum) with VIa prepared in part 11A.

12. Methyl 3β,12β-Dihydroxycholanate (IVa).—Raney nickel reduction of methyl 3β-hydroxy-12-ketocholanate (VIa), performed identically as with the corresponding 3α epimer (part 6A), was complete (t.l.c.) in 4 hr., and the ratio of 12β to 12α products was 9:1 as determined by t.l.c.[30] The product mixture responded nicely to chromatographic separation on Florisil. Early fractions eluted with ligroin–ether (2:1) yielded 62% of homogeneous (t.l.c.) 3β,12β ester IVa, and later fractions gave a mixture of IVa and 3β,12α compound.

The homogeneous 3β,12β methyl ester IVa resisted numerous attempts at crystallization from solvents, but, after storage as an oil for a period of over 12 months, the ester was found to crystallize from anhydrous ether in huge prisms: m.p. 115.8–116.3°; $[\alpha]_D$ +33.5 and +28.7° (dioxane); $\lambda_{max}^{CHCl_3}$ 2.76, 2.90, 9.73, 9.96 (OH), and 5.78 μ ($COOCH_3$).

Anal. Calcd. for $C_{25}H_{42}O_4$ (406.59): C, 73.85; H, 10.41. Found: C, 74.08; H, 10.75.

13. (Methyl 3β,12β-dihydroxycholanate) bistrifluoroacetate, prepared as for the 3β,12α isomer (part 3), failed to crystallize although the product was homogeneous by t.l.c.; its infrared spectrum showed characteristic trifluoroacetate bands at 5.63, 8.22, and 8.60 μ.

14. 3β,12β-Dihydroxycholanic Acid (IV).—The 3β,12β ester IVa was hydrolyzed (as in part 4) quantitatively to the acid, which crystallized from ethyl acetate–ligroin (1:2) as fine needles, m.p. 174.0–176.0°. After drying at 140° (0.1 mm.), the melting point was 204.3–205.6°; $[\alpha]_D$ +27.0° (dioxane); λ_{max}^{KBr} 2.80, 3.00, 9.73, 9.95 (OH), and 5.84 μ (COOH).

Anal. Calcd. for $C_{24}H_{40}O_4$ (392.56): C, 73.43; H, 10.27. Found: C, 73.34; H, 10.20.

Solvates.—Acid IV crystallized nicely from benzene as well-shaped needles, which after being dried at 100° (0.1 mm.) melted at temperatures near 180° (melt unclear), depending on the rate and duration of heating.

Anal. Calcd. for $C_{24}H_{40}O_4 \cdot 0.2C_6H_6$ (408.18): C, 74.15; H, 10.17. Found: C, 74.36; H, 10.00.

When crystallized from chloroform, IV crystallized as short rods, solvated with chloroform. After drying at 122° (0.1 mm.), the melting point was 173.5–177.5 (melt unclear).

Anal. Calcd. for $C_{24}H_{40}O_4 \cdot 0.2CHCl_3$ (416.44): C, 69.79; H, 9.73. Found: C, 70.02; H, 9.80.

When crystallized from ethyl acetate, IV crystallized as dense prisms, m.p. about 170°. When heated at 140° (0.1 mm.) for several hours, the crystals became opaque and discolored, and melted at 203–204°.

15. Methyl 3α,12α-Dihydroxycholanate (Ia). A. By Reaction with Diazomethane.—Two grams of desoxycholic acid (I) of high purity, prepared as described below, was esterified with diazomethane and the product was chromatographed over a Florisil column. Practically all ether-eluted fractions were homogeneous by t.l.c. The ethereal eluates, when concentrated, crystallized slowly to give dense crystals which, after drying at 56° (0.1 mm.) for 12 hr., melted at 75.5–95.5°: $[\alpha]_D$ +41.3 and +45.0 (dioxane), +47.8° (butanone); lit.[14] m.p. 105–106°, $[\alpha]_D$ +48° (butanone). Products crystallized from benzene or butanone even after long periods of drying had wide melting-point ranges. A sample from benzene melted at 70–108°.

B. By Reaction by the Acetyl Chloride Method.—When Ia was prepared by esterification of desoxycholic acid (same batch as used in part 15A) and recrystallized with ether–petroleum

(31) We wish to thank Professor Reichstein for a comparison sample.

(32) (a) K. Kyogoku, *Z. Physiol. Chem.*, **246**, 99 (1937); (b) J. Press, P. Grandjean, and T. Reichstein, *Helv. Chim. Acta*, **26**, 598 (1943).

(33) Gives a positive tetranitromethane test and is catalytically (PtO_2) hydrogenated to methyl 12-ketocholanate, m.p. 108–109°.[5a]

ether,[34] the nicely crystalline prisms had a melting point range of 78–104°. Thin layer chromatographic examination of the product showed the presence of unchanged desoxycholic acid.[35]

16. **3α,12α-Dihydroxycholanic (Desoxycholic) Acid (I).**—(Methyl desoxycholate) diacetate,[9] m.p. 118.0–119.5°, was hydrolyzed to the acid as in part 4. The acid was dried and successively recrystallized three times from freshly distilled butanone; the melting point, after drying at 140° (0.1 mm.) for 24 hr., was 173.8–174.4°. The melting point of the acid before the third crystallization was essentially the same (see text): $[\alpha]_D$ +49.8 (dioxane), +40.3 ($CHCl_3$ and trace of CH_3OH), +54.1° (EtOH); lit.[2] $[\alpha]_D$ +49 (butanone), +48 (dioxane), +53° (alcohol)]; λ_{max}^{KBr} 2.95, 9.65 (OH), and 5.80 μ (COOH).

Anal. Calcd. for $C_{24}H_{40}O_4$ (392.56): C, 73.43; H, 10.27. Found: C, 73.35; H, 10.12.

(34) B. Riegel, R. B. Moffett, and A. V. McIntosh, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 237.

(35) Examination by t.l.c. of methyl cholanates prepared by the methanol–hydrochloric acid method[28] show that in all instances traces of acid remain, even after recrystallization.

Acknowledgment.—This investigation was supported in part by Public Health Service Research Grant CA-05011 from the National Cancer Institute. Terry Richardson and Jo Thedford contributed valuable technical assistance.

Substitution at Unactivated Carbon. The Synthesis of 18- and 19-Substituted Derivatives of 11β-Hydroxyprogesterone*,[1,2]

ROBERT H. HESSE AND MAURICE M. PECHET

Research Institute for Medicine and Chemistry, Cambridge 42, Massachusetts

Received February 24, 1965

A series of optically active 18- and 19-substituted 11-oxygenated progesterones has been prepared using as a key step the Barton reaction. Included in this series are 21-desoxyaldosterone, 11β,18-epoxyprogesterone, 11β,18-dihydroxyprogesterone 18-acetate, 11β-hydroxyprogesteron-18-oic 11,18-lactone, 11β,19-dihydroxyprogesterone, 11β-hydroxy-19-oxoprogesterone 11,19-cyclohemiacetal, 11β-hydroxyprogesteron-19-oic 11,19-lactone, and 11β,19-epoxyprogesterone. The values for the molecular rotations of these steroids are dependent on the nature of the oxygen function at C-18 or -19 and are characteristic for each series. The presence of an 11,19-oxide bridge retards the proton-catalyzed migration of the $\Delta^{5,6}$ double bond into conjugation with the 3-ketone. In the presence of mild acid, 11β,18-dihydroxyprogesterone 3,20-bisketal is converted into 11β,18-epoxyprogesterone. A mechanism is proposed for this surprising transformation.

The interesting chemical and biological properties of 18- and 19-substituted steroids have focused considerable attention on the problem of chemical reactions at "unactivated" carbon atoms. Recent efforts toward this end have resulted in a number of methods for introducing substituents at "unactivated" carbon.[3–10] One method of considerable scope and utility is the Barton reaction.[3–5]

As one aspect of our studies of the Barton reaction, we have undertaken the preparation of a series of 11,18-substituted progesterones as potential biosynthetic precursors of *d*-aldosterone. The corresponding 11,19-oxygenated isomers were also prepared, since it was felt that a series of isomeric 18- and 19-substituted compounds would be useful for the study of physical properties as well as chemical reactivity and biological activity.

Nitrosolation of 11β-hydroxyprogesterone 3,20-bisethylene ketal (**1**)[11] gave a crystalline nitrite **2** which on irradiation underwent rearrangement to yield the isomeric 18- and 19-oximes **3** and **4**. Oxime **3** was shown to be the 18-substituted isomer by acid-catalyzed conversion into the nitrone **5**.[12] The other oxime was shown to be the 19-substituted isomer by transformation to the known 11β-hydroxy-19-norprogesterone (**6**).[13] (See Scheme I.)

Treatment of the 18-oxime **3** with acetic acid and aqueous sodium nitrite[14] gave an oily product which showed considerable carbonyl absorption at 1710 $cm.^{-1}$ in the infrared. This suggested a cleavage of the 20-ketal.[15] The 18-oxime bisketal **3** was therefore treated with dry sodium nitrite in glacial acetic acid to give a small yield of the desired 11→18-hemiacetal bisketal **8**. The major product of this reaction was a gummy solid with a strong ester band at 1740 $cm.^{-1}$. Since this material, on treatment with sodium hydroxide in methanol, was converted into the 11→18-hemiacetal bisketal **8**, it was formulated as the hemiacetal acetate **7**.[16,17]

* To Professor Louis F. Fieser.

(1) Communication No. 33 from the Research Institute for Medicine and Chemistry. For No. 32, see N. Basu and D. H. R. Barton, *Tetrahedron Letters*, 3151 (1964).

(2) A preliminary account of this work was presented at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 1962, Abstracts p. 22C.

(3) D. H. R. Barton, J. M. Beaton, L. E. Geller, and M. M. Pechet, *J. Am. Chem. Soc.*, **82**, 2640 (1960).

(4) M. Akhtar, D. H. R. Barton, J. M. Beaton, and A. G. Hortmann, *ibid.*, **85**, 1512 (1963).

(5) M. Akhtar, "Advances in Photochemistry," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1964, p. 263.

(6) P. Buchschacher, M. Cereghetti, H. Wehrli, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **42**, 2122 (1959).

(7) G. Cainellio, M. L. Mihailovic, P. Arigoni, and O. Jeger, *ibid.*, **42**, 1124 (1959).

(8) M. Akhtar and D. H. R. Barton, *J. Am. Chem. Soc.*, **83**, 2213 (1961).

(9) Ch. Meystre, K. Heusler, J. Kalvoda, P. Weiland, G. Anner, and A. Wettstein, *Experientia*, **17**, 475 (1961).

(10) E. J. Corey and W. R. Hertler, *J. Am. Chem. Soc.*, **80**, 2903 (1958).

(11) B. J. Magerlin and R. H. Levin, *ibid.*, **75**, 3654 (1953).

(12) The same nitrone had previously been obtained by J. M. Beaton in this institute by treatment of the nitrone from aldosterone acetate oxime with zinc and acetic acid.

(13) A. Bowers, C. Casas-Campillo, and C. Djerassi, *Tetrahedron*, **2**, 165 (1958).

(14) S. G. Brooks, R. M. Evans, G. F. H. Green, J. S. Hunt, A. G. Long, B. Mooney, and L. J. Wyman, *J. Chem. Soc.*, 4614 (1958).

(15) The 20-ketal in this series is quite labile and is cleaved by prolonged contact with acid-washed alumina or by 60% aqueous acetic acid at room temperature.

(16) There is evidence[17] that the 11β-hydroxyl participates in this reaction to give an 11β,18-oxonium ion intermediate. This intermediate may be trapped by a variety of internal or external nucleophiles; see, for instance, ref. 4.

(17) Participation of the 11β-hydroxyl is indicated by the rapidity of the reaction (<2 min. at 5°) and by the observation of A. G. Hortmann of this institute that 11-keto 18-oximes, on treatment with nitrous acid, give nitrimines rather than aldehydes.