Bile Acids and Steroids. XX[1]. *Studies on the Hog Bile Acids (Part 4)*[2]. *Synthesis of 3β, 6β-Dihydroxy-5β-cholanoic Acid and 3β, 6α-Dihydroxy-5α-cholanoic Acid*

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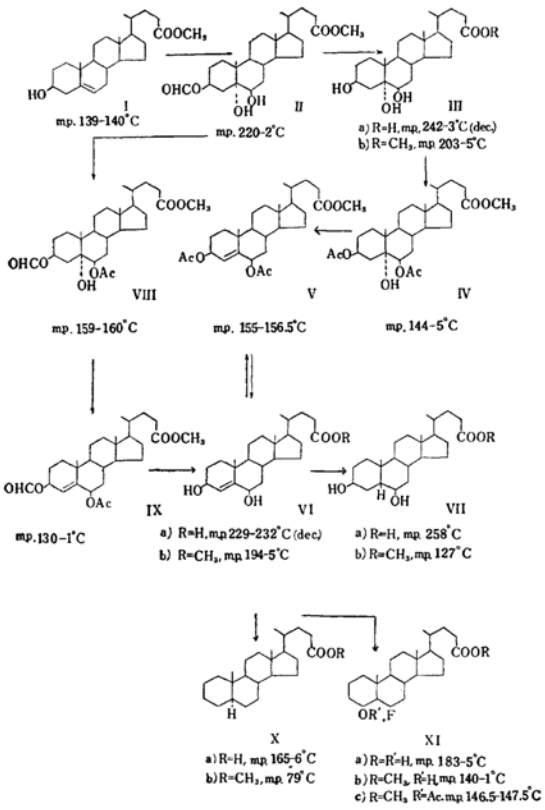
(Received September 30, 1960)

3β, 6β-Dihydroxy-5β-cholanoic Acid. — Although 3β, 6β-dihydroxy-5β-cholanoic acid has been synthesized earlier[3-5], the methods then used were unprofitable for the preparation because the yield was low and the treatment difficult. The most convenient method for this purpose was as follows (chart 1). The starting

Chart I



material, methyl 3β-hydroxy-Δ^5-cholenoate (I) was prepared from methyl α-hyodeoxycholate in high yield according to Bharucha's method[6]. A trans-hydroxylation of the double bond was done according to the method that was performed in the cholesterol series by Fieser[7] and the trans triol III (acid, m. p. 242~243°C with decomp.; methyl ester, m. p. 203~205°C) was obtained in a comparatively good yield. As seen in Table I, it is clear that the configuration of the hydroxyl group at position 6 in the triol III thus obtained belongs to the β-series by comparison with the molecular rotations[8] of the analogous derivatives in the cholesterol series. By acetylation with acetic anhydride, the triol IIIb afforded a diacetate IV with m. p. 144~145°C which was followed by dehydration according to the Darzens' method[9] to afford an unsaturated derivative V with m. p. 155~156.5°C in a quantitative yield. The unsaturated diacetate V was saponified with alkali to yield an unsaturated diol VIa, m. p. 229~232°C (decomp.), in high yield. This acid VIa was esterified with an ethereal diazomethane to furnish its methyl ester VIb, m. p. 194~195°C, in 89% yield, which was sparingly soluble in ether. This methyl ester VIb was also obtained from the monoformate IX by the following route: When the 6-acetate VIII, m. p. 159~160°C, obtained from the monoformate II was dehydrated according to Darzens' method[9] as in the previous case, it afforded an unsaturated derivative IX, m.p. 130~131°C, which was saponified to give the identical diol VI with that obtained from the diacetate V. Since all of the unsaturated derivatives, V, VI and IX described above were positive to the Rosenheim test[10], it shows that these compounds have acetyl, formyl or hydroxyl group at the allyl position[11]. The ultraviolet (λ_{max} 205.5 mμ) and the infrared absorption spectrum (ν_{max} 1658 cm^{-1}) show the presence of a double bond at position 4-5. The unsaturated diol VIb obtained on the above

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2) Part 3 of this series: K. Takeda, T. Kubota and J. Kawanami, *J. Biochem.* (*Japan*), **44**, 51 (1957).
3) M. Tsukamoto, ibid., **32**, 451 (1940).
4) M. Tsukamoto, ibid., **32**, 467 (1940).
5) J. S. Moffatt, *J. Chem. Soc.*, **1947**, 812.
6) K. R. Bharucha, G. C. Buckley, C. K. Cross, L. J. Rubin and P. Ziegler, *Can. J. Chem.*, **34**, 982 (1956).
7) L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).
8) L. F. Fieser and M. Fieser, "Steroids", Reinhold Publish. Co., New York (1959), p. 204.
9) G. Darzens, *Compt. rend.*, **152**, 1601 (1911).
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11) R. Schoenheim and E. A. Evans, Jr., *J. Biol. Chem.* **114**, 567 (1936).

TABLE I. MOLECULAR ROTATIONS OF 6-EPIMERS

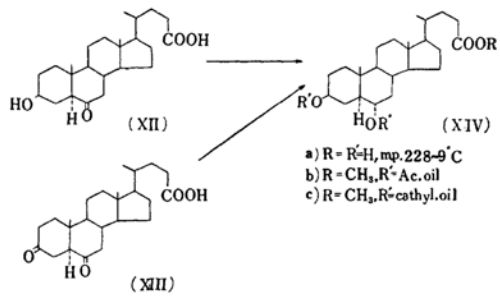
Substituent	Skeletal	6β	6α*	$\Delta^{\alpha-\beta}$
3β,6-Diol	5α-Cholestane	+ 53	+154	+101
	5α-Cholanoic acid	0 (Py.)	+135	+135
3β,5α,6-Triol	Cholestane	+ 4 (Di.)	+ 88 (Di.)	+ 84
	Cholanoic acid	− 41 (Py.)	+ 94 (Py.)	+135
	Methyl cholanoate	− 34 (Py.)	+346 (Me.)	+370
3β,5α,6-Triol 3,6-diacetate	Cholestane	−232	+ 28	+260
	Methyl cholanoate	−243	+ 41 (Di.)	+283
Δ^4-3β,6-Diol	Cholestane	− 53	+117	+170
	Cholanoic acid	0 (Py.)	+109 (Py.)	+109
Δ^4-3β,6-Diacetate	Cholestane	− 58	+ 89	+147
	Methyl cholanoate	− 78	+ 78	+156

* Molecular rotations of the 6α-derivatives, in bile acid are unpublished data by the author. Unless otherwise noted, the solvent is chloroform. Di., Py. and Me. are dioxane, pyridine and methanol, respectively.

saponification was again acetylated back to the parent unsaturated diacetate V. Hence, it was defined that allyl rearrangement or epimerization did not take place in the course of the saponification. In absolute ethanol the unsaturated diol VIb was hydrogenated with the Adams' catalyst to absorb hydrogen very rapidly (1 hr.) in comparison with the case in cholestene diol (28 hr.)[12] yielding the anticipated methyl 3β,6β-dihydroxy-5β-cholanoate VIIb, m. p. 127°C, in a quantitative yield. This compound shows the absence of the double bond in both the ultraviolet and the infrared absorption spectrum and it was also negative to the Rosenheim test. The methyl ester VIIb was saponified with methanolic potassium hydroxide to give 3β,6β-dihydroxy-5β-cholanoic acid VIIa, m. p. 258°C, in 87% yield. While catalytic hydrogenation was carried out in 95% ethanol solution, there occurred simultaneous hydrogenolysis as in the case of the diacetate[13] and the following reduction products were separated after acetylation: 45.5% of methyl 5α-cholanoate, Xb m. p. 79°C, 27% of methyl acetoxy cholenoate, XIc m. p. 146.5~147.5°C and an oil which was saponified to afford 5% of 3β,6β-dihydroxy-5β-cholanoic acid, VIIa m. p. 255°C. In the above methyl acetoxycholenoate XIc, the positions of both the hydroxyl group and the double bond could not be determined on account of the scarcity of the substance.

3β, 6α-Dihydroxy-5α-cholanoate. — Reduction of the steroid six-membered ketone by metal and alcohol generally produces almost exclusively equatorial alcohols. Then the reduction of 3β-hydroxy-6-oxo-5α-cholanoic acid (XII) with sodium and *n*-butanol was carried out and it

Chart 2



gave an acid with m. p. 228~229°C. This reduction product afforded a diacetate XIVb and a dicathylate XIVc by treatment in the usual method[14]. Since the attempted partial saponification of the diacetate XIVb was unsuccessful, it was assumed that the newly formed hydroxyl group is equatorial. Also, as seen in Table I, comparison of molecular rotations shows that this product belongs to the 6α-series. With the above observation, it could be deduced that this reduction product is 3β, 6α-dihydroxy-5α-cholanoic acid. The identical compound was also obtained from 3,6-dioxo-5α-cholanoic acid (XIII) by the same reduction.

Experimental[15]

3β, 6β-Dihydroxy-5β-cholanoic Acid.—*Methyl 3β, 5α, 6β-trihydroxy-cholanoate 3-Formate (II).* — This preparation corresponded to the treatment on the

12) V. Prelog and E. Tagmann, *Helv. Chim. Acta*, **27**, 1880 (1944).

13) O. Mancera, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **16**, 192 (1951); S. Nishimura and K. Mori, This Bulletin, **32**, 103 (1959).

14) L. F. Fieser et al., *J. Am. Chem. Soc.*, **74**, 3309 (1952).

15) All melting points are uncorrected. Infrared spectra were recorded in Nujol mulls with a Koken DS 301 double beam infrared spectrophotometer, unless otherwise noted, and the ultraviolet spectra were taken in 95% ethanol solution using a Hitachi EPS 2 spectrophotometer. Optical rotations were determined in a 1-dm. tube for chloroform solutions, unless otherwise specified. The alumina used for chromatography in this experiment was Merck's reagent grade standardized according to Brockmann and the chromatography was usually performed according to the method described by T. Reichstein. (T. Reichstein and W. Schopee, *Discussions Faraday Soc.*, No. 7, (1949)) The extracts were dried over anhydrous sodium sulfate before evaporation unless stated otherwise.

study of cholesterol series[7]. Methyl 3β-hydroxy-Δ^5-cholenoate (I), m. p. 139~140°C, (5.871 g.) (prepared from methyl α-hyodeoxycholate ditosylate according to Bharucha's method[6]) was added to 88% of formic acid (59 ml.) and warmed for 5 min. on a boiling water bath, and so the crystals precipitated were dissolved to separate as oil. To this reaction mixture cooled to room temperature, 30% of aqueous hydrogen peroxide (6 ml.) was added. The white deposit initially precipitated was dissolved by occasionally shaking for 30 min. After standing overnight at room temperature, the reaction mixture was diluted with water to separate an oily precipitate. The extract with ether-chloroform mixture (4 : 1) was washed successively with water, dilute hydrochloric acid and again water. The extract was evaporated to afford an oily product (9.448 g.). Trituration of the oily residue with methanol (45 ml.) coupled with 5% methanolic hydrochloric acid (5 ml.) caused the crystallization of II as cubes. After standing overnight at room temperature, the crystals obtained were collected and washed thoroughly with methanol to give methyl 3β,5α,6β-trihydroxycholanoate 3-formate (II), 1.657 g. (24.4%), cubes, m. p. 220~222°C. The melting point was not elevated by further recrystallization from methanol. $[\alpha]_D^{19} = -17.7 \pm 2°$ (*c* 0.962). infrared spectrum: ν_{max} 3939, 3454 (OH); 1722, 1710 (C=O); 1183, 1169 cm^{-1} (C-O).

Found: C, 69.43; H, 9.46. Calcd. for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40%.

Methyl 3β,5α,6β-Trihydroxycholanoate (IIIb).—i) From the above filtrate—The filtrate was concentrated under diminished pressure to cause the crystallization. After cooling well the crystals were collected and washed with cold methanol to give crystals with m. p. 193~202°C (1.947 g.: 30.5%) as a first crop and those with m. p. 168~172°C as a second crop (1.086 g.: 17%) were obtained on concentration of the mother liquor. These are combined and recrystallized from methanol to methyl 3β,5α,6β-trihydroxycholanoate (IIIb), cubes, m. p. 203~205°C. $[\alpha]_D^{20} = -7.6 \pm 2°$ (*c*=0.996, pyridine). Infrared spectrum: ν_{max} 3568, 3488 (OH); 1740 (C=O); 1173 cm^{-1} (C-O).

Found: C, 70.95; H, 9.86. Calcd. for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02%.

ii) From the formate II—The formate II (591 mg.) was dissolved in methanol (3 ml.) coupled with ten drops of 10% methanolic hydrochloric acid and allowed to stand overnight at room temperature (22~25°C). After removal of the solvent under reduced pressure, the crystals obtained were collected and washed with methanol to afford methyl 3β,5α,6β-trihydroxycholanoate (IIIb), 497 mg. (90%), m. p. 202~205°C. The sample showed no depression on admixture with the triol (IIIb) obtained above and the infrared spectrum of the sample was also identical with that of IIIb.

3β,5α,6β-Trihydroxycholanoic Acid (IIIa).—After a solution of the formate II (591 mg.) in 0.5 N methanolic potassium hydroxide (25 ml.) was allowed to stand overnight at room temperature, the solvent was removed under reduced pressure, water was added and it was acidified with dilute hydrochloric acid under cooling. After being kept for 1 hr. at room temperature, the precipitate was collected by filtration, washed thoroughly with water and dried in vacuo overnight at 100°C. The crude acid obtained in 96% yield (167 mg.) was recrystallized from acetone-methanol to yield 3β,5α,6β-trihydroxycholanoic acid (IIIa), 64 mg., m. p. 242~243°C (decomp.). $[\alpha]_D^{21} = -10.3 \pm 2°$ (*c* 0.987, pyridine). Infrared spectrum: ν_{max} 3575, 3433, about 3000 (OH); 1710, 1692, 1674 cm^{-1} (C=O).

Found: C, 70.67; H, 9.89. Calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87%.

Methyl 3β,5α,6β-Trihydroxycholanoate 3,6-Diacetate (IV).—In warming with acetic anhydride (4 ml.) on a boiling water bath for 30 min., the triol methyl ester III (415 mg.) was dissolved. After further warming for 2 hr., the mixture was cooled, water was added carefully and it was occasionally shaken to crystallize it. After being kept for 30 min. at room temperature, the precipitate was collected and washed successively with water and methanol to furnish 3,6-diacetate, 491 mg. (99%), m. p. 142~143°C. This was further recrystallized from methanol to methyl 3β,5α,6β-trihydroxycholanoate 3,6-diacetate (IV), 296 mg. (59.5%), long prisms, m. p. 144~145°C. $[\alpha]_D^{24} = -48.1 \pm 2°$ (*c* 0.998). Infrared spectrum: ν_{max} 3540 (OH); 1741, 1724, 1710 (C=O); 1270, 1250, 1164, 1030 cm^{-1} (C-O).

Found: C, 68.76; H, 9.11. Calcd. for $C_{29}H_{46}O_7$: C, 68.74; H, 9.15%.

Methyl 3β,6β-Diacetoxy-Δ^4-cholenoate (V).—To a solution of the diacetate IV (1.215 g.) in pyridine (7 ml.), thionyl chloride (0.28 ml.) was added dropwise under cooling and shaking to give needles immediately. After shaking for ten min. at 0°C, the mixture was carefully diluted with 100 ml. of water and allowed to stand for 1 hr. at room temperature. The crystals thus obtained were collected and washed thoroughly with water and a little methanol to afford methyl 3β,6β-diacetoxy-Δ^4-cholenoate (V), 1.112 g. (95%), prismatic needles, m. p. 155~156.5°C. The Rosenheim test gave blue coloration. $[\alpha]_D^{18} = -16.3 \pm 2°$ (*c* 1.011). Infrared spectrum: ν_{max} 1738, 1726, (C=O); 1658 (C=C); 1242, 1200, 1164, 1034, 1023 cm^{-1} (C-O).

Found: C, 71.55; H, 9.08. Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08%.

3β,6β-Dihydroxy-Δ^4-cholenoic Acid (VIa).—A solution of the above ester V (3.365 g.) in 0.5 N methanolic potassium hydroxide (670 ml.) was heated under reflux for 5 hr. on a boiling water bath. After removal of the solvent under reduced pressure, the residue was diluted with water and acidified with dilute hydrochloric acid under cooling. After standing, the precipitate was collected by filtration, washed well with water and dried in vacuo overnight at 100°C to furnish a crude acid (2.662 g.: 99%). For analysis, 59 mg. of the crude acid was recrystallized from methanol to 3β,6β-dihydroxyl-Δ^4-cholenoic acid (VIa), 32 mg., scales, m. p. 229~232°C (decomp.). The Rosenheim test gave violet-blue coloration. $[\alpha]_D^{19} = 0 \pm 2°$ (*c* 0.913, pyridine). I. R. spectrum: ν_{max} 3436 (broad), 3256, about 3000 (OH); 1706 (C=O); 1658 cm^{-1} (C=C).

Found: C, 73.61; H, 9.36. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81%.

3β,6β-Dihydroxy-Δ⁴-cholenoic Acid (VIa) from Methyl 3β-Formoxy-6β-acetoxy-Δ⁴-cholenoate (IX).—Methyl 3β-formoxy-6β-acetoxy-Δ⁴-cholenoate (289 mg.) which will be described in the next section, was saponified under reflux with 0.5 N methanolic potassium hydroxide (75 ml.) for 2 hr. Isolation in the usual way gave a crude acid (187 mg.) in 79% yield. The product was recrystallized from chloroform-methanol to 3β,6β-dihydroxy-Δ⁴-cholenoic acid (VIa), 153 mg., cubes, m. p. 228~230°C (decomp.). The resenheim test gave violet-blue coloration. Its infrared spectrum was satisfactorily identical with that of the acid from diacetate V.

Found: C, 74.03; H, 9.77. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81%.

Methyl 3β,6β-Dihydroxy-Δ⁴-cholenoate (VIb).—Being esterified with ethereal diazomethane the above crude acid (2.603 g.) was violently bubbled to react and afforded a precipitate. After collecting and washing with ether, the precipitate was recrystallized from acetone to methyl 3β,6β-dihydroxy-Δ⁴-cholenoate (VIb), 2.477 g. (89%), cubes, m. p. 194~195°C. The Rosenheim test gave blue coloration and the Liebermann test violet to blue and to green. $[\alpha]_D^{22}=+22\pm2°$ (*c* 0.948). Ultraviolet spectrum: λ_{max} 205.5 mμ (ε=5800). Infrared spectrum: ν_{max} 3292 (OH); 3029 (shoulder) (C=C); 1742 (C=O); 1659 cm^{-1} (C=C).

Found: C, 74.22; H, 9.95. Calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97%.

Methyl 3β,6β-Diacetoxy-Δ⁴-cholenoate (V) from the above Ester (VIb).—The above ester VIb (85 mg.) was warmed with pyridine (1 ml.) and acetic anhydride (1 ml.) for 2 hr. on a boiling water bath. After cooling, water was carefully addded to the mixture to furnish needles which after filtering and washing, were recrystallized from methanol to methyl 3β,6β-diacetoxy-Δ⁴-cholenoate (V), 65 mg. (82.6%), fine needles, m. p. 154~155°C. The compound was identified by a mixed melting point and the infrared spectrum with the specimen described above.

Methyl 3β-Formoxy-5α,6β-dihydroxycholanoate 6-Acetate (VIII).—On warming with acetic anhydride (6 ml.) on a boiling water bath for 2 hr., the 3-formyl derivative II (580 mg.) described above had dissolved completely. After warming for a further 2 hr., the mixture was cooled and water was added carefully to give crystals which after filtering and washing with water and a little methanol, were recrystallized from methanol to methyl 3β-formoxy-5α,6β-dihydroxycholanoate 6-acetate (VIII), 500 mg. (80%), fine needles, m. p. 159~160°C. $[\alpha]_D^{18}=-46\pm2°$ (*c* 1.006). Infrared spectrum: ν_{max} 3495 (OH); 1733, 1721, 1711 (C=O); 1282, 1185, 1040 cm^{-1} (C-O).

Found: C, 68.09; H, 9.01. Calcd. for $C_{28}H_{44}O_7$: C, 68.26; H, 9.00%.

Methyl 3β-Formoxy-6β-acetoxy-Δ⁴-cholenoate (IX).—To a solution of the above 5α-hydroxy derivative VIII (431 mg.) in pyridine (2 ml.), thionyl chloride (0.1 ml.) was added dropwise under cooling and shaking to give needles immediately. After shaking for ten min. at 0°C, 50 ml. of water was added carefully to the mixture and it allowed to stand for 1 hr. at room temperature. The crystals obtained after filtering and washing thoroughly with water and a little methanol, were further recrystallized from methanol to methyl 3β-formoxy-6β-acetoxy-Δ⁴-cholenoate (IX), 334 mg. (80.7%), needles, m. p. 130~131°C, sinter at 123~124°C. $[\alpha]_D^{18}=-5\pm2°$ (*c* 0.911). Infrared spectrum: ν_{max} 1738, 1725 (C=O); 1659 (C=C); 1248, 1183, 1161, 1038 cm^{-1} (C-O).

Found: C, 70.98; H, 8.96. Calcd. for $C_{28}H_{42}O_6$; C, 70.85; H, 8.92%.

Methyl 3β,6β-Dihydroxy-5β-cholanoate (VIIb).—A suspension of Adams catalyst (73 mg.) in absolute ethanol (20 ml.) was prereduced with hydrogen at 20°C to absorb 18 ml. of hydrogen for 20 min. To the above mixture, the methyl 3β,6β-dyhydroxy-Δ⁴-cholenoate (VIb) (1.134 g.) which was dissolved in absolute ethanol (150 ml.) by warming and successively being cooled to room temperature, was added and shaken under hydrogen for 1 hr. to uptake 63.5 ml. of hydrogen. (calcd. for 1 mol. equiv. 67.5 ml. at 20°C). The filtrate from platinum moll was evaporated under reduced pressure to give a sirup which was recrystallized from methanol to methyl 3β,6β-dihydroxy-5β-cholanoate (VIIb), 1.096 g. (96.3%), needles, m. p. 127°C, softening at 78~80°C. The Rosenheim test was negative and the Liebermann test gave cherry-red to bordeaux coloration. $[\alpha]_D^{21}=+17\pm2°$ (*c* 1.062). No appreciable absorption in the ultraviolet spectrum. Infrared spectrum: ν_{max} 3602, 3377 (OH); 1740 (C=O); 1174, 1023 cm^{-1} (C-O).

Found: C, 73.89; H, 10.41. Calcd. for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41%.

3β,6β-Dihydroxy-5β-cholanoic Acid (VIIa).—After a solution of the saturated ester VIIb (150 mg.) in 0.2 N methanolic potassium hydroxide (15 ml.) was heated under reflux for 2 hr., the mixture was evaporated, diluted with water and acidified with dilute hydrochloric acid to Congo red to give a crude acid. The precipitate after filtering and washing thoroughly with water, was recrystallized from a large volume of acetone to 3β,6β-dihydroxy-5β-cholanoic acid (VIIa), 126 mg. (87%), scales, m. p. 258°C. The Liebermann test gave pink coloration. $[\alpha]_D^{20}=+21\pm2°$ (*c* 1.004, methanol). Infrared spectrum: ν_{max} 3497, about 3000 (OH); 1710 cm^{-1} (C=C).

Found: C, 73.53; H, 10.33. Calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27%.

On the Hydrogenation of Methyl 3β,6β-Dihydroxy-Δ⁴-cholenoate (VIb) in 95% of Ethanol.—A suspension of methyl 3β,6β-dihydroxy-Δ⁴-cholenoate (VIb) (460 mg.) in 95% of ethanol (40 ml.) was dissolved by warming and successively cooled to room temperature and then shaken with prereduced Adams catalyst (35 mg.) under hydrogen atmosphere for 24 hr. at 26°C to absorb 30.9 ml. of hydrogen (calcd. for 1 mol. equiv., 28 ml.). The filtrate from the catalyst was evaporated under diminished pressure to give a residue which was recrystallized from methanol to methyl 5α-cholanoate (Xb), 130 mg. scales, m. p. 78~79°C. The filtrate from the above crystals was evaporated to dryness to afford an oil (326 mg.) which was acetylated with pyridine (3 ml.) and acetic anhydride (3 ml.) by being kept overnight at room temperature. Working up in the usual way gave an oil (341 mg.) which was followed by chromatography on alumina. The petroleum ether eluate gave methyl 5α-cholanoate, 63 mg., scales,

m. p. 78~79°C. The fractions eluted with petroleum ether and petroleum ether-benzene (9 : 1 and 8 : 2) gave fine needles from methanol, 129 mg. (27%), m. p. 146.5~147.5°C. The last fractions eluted with petroleum ether-benzene (1 : 1), benzene and benzene-chloroform (9 : 1) gave an oil (39 mg.).

Methyl 5α-Cholanoate (Xb).—Methyl 5α-cholanoate was both scales (130 mg.) obtained by the recrystallization from methanol and scales (63 mg.) from the first elution on the chromatography described above. The total yield was 45.4%. The Liebermann test was negative. A mixed melting point with methyl 5β-cholanoate showed definite depression, melting at 60~61°C. The infrared spectrum of this compound was different from that of the normal series. $[\alpha]_D^{18}=+21\pm2°$ (*c* 1.018). Infrared spectrum: ν_{max} 1736 (C=O); 1197, 1167 cm^{-1} (C-O).

Found: C, 80.31; H, 11.32. Calcd. for $C_{25}H_{42}O_2$: C, 80.15; H, 11.30%.

5α-Cholanoic Acid (Xa).—After a solution of the above ester Xb (103 mg.) in 0.5 N methanolic potassium hydroxide (2 ml.) was heated under reflux for 2 hr., the mixture was diluted with water, acidified with dilute hydrochloric acid and extracted with ether. The ethereal layer was washed with water and evaporated to give crystals which were recrystallized from methanol to 5α-cholanoic acid, 82 mg. (83%), scales, m. p. 165~166°C. $[\alpha]_D^{21}=+21\pm2°$ (*c* 1.013). Infrared spectrum: ν_{max} about 3000 (broad) (OH); 1710 cm^{-1} (C=O).

Found: C, 80.03; H, 11.27. Calcd. for $C_{24}H_{40}O_2$: C, 79.94; H, 11.18%.

The Second Elution on the Chromatography.—This compound was assumed to be methyl ?-acetoxy-$\Delta^?$-cholenoate from both the elementary analysis and the infrared spectrum. The Liebermann test gave from red to violet and to brown coloration successively. $[\alpha]_D^{22}=+14\pm2°$ (*c* 1.009). Infrared spectrum: ν_{max} 1738 (C=O); 1694 (C=C); 1254, 1167, 1036 cm^{-1} (C-O).

Found: C, 75.50; H, 10.19. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83%.

Acid from the above Ester XIa.—After a solution of the above ester acetate XIc (51 mg.) in 0.2 N methanolic potassium hydroxide was heated under reflux for 2 hr., the mixture was cooled, diluted with water and acidified with dilute hydrochloric acid to afford a precipitate which was collected, washed will with water and dried in vacuo at 100°C to yield a crude acid. The crude acid was recrystallized from methanol to furnish the pure acid, 33 mg. (74.5%), fine needles, m. p. 183~185°C. The Liebermann test gave from red to bordeaux coloration. Infrared spectrum: ν_{max} 3397, about 3000 (broad) (OH); 1693 cm^{-1} (C=O).

Found: C, 73.50; H, 10.52. Calcd. for $C_{24}H_{38}O_3\cdot CH_3OH$: C, 73.85; H, 10.41%.

Ester from the above Acid XIb.—Being esterified with ethereal diazomethane in the usual manner, the above acid (21 mg.) gave fine needles from methanol, 7 mg., m. p. 140~141°C. The Rosenheim test was negative. No appreciable absorption in the ultraviolet spectrum. Infrared spectrum: ν_{max} 3541 (OH); 3013 (shoulder) (C=C); 1712 (C=O); 1619 cm^{-1} (C=C).

3β,6β-Dihydroxy-5β-cholanoic Acid (VIIa) from the Third Elution on the Chromatography.—Since attempts to crystallize the oil failed, the oil (39 mg.) wasaaponified with 0.2 N methanolic potassium hydroxide in the usual way to give 3β,6β-dihydroxy-5β-cholanoic acid (VIIa), 23 mg. (5%), scales, (from acetone-methanol), m. p. 250~255°C. This compound was identified by a mixed melting point and the infrared spectrum with 3β,6β-dihydroxy-5β-cholanoic acid described above. I. R. spectrum: ν_{max} 3480, about 3000 (broad) (OH); 1710 cm^{-1} (C=O).

Found: C, 73.53; H, 10.25. Calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27%.

3β, 6α-Dihydroxy-5α-cholanoic Acid. — *Reduction of 3β-Hydroxy-6-oxo-5α-cholanoic Acid.*—To a boiling solution of 3β-hydroxy-6-oxo-5α-cholanoic acid (397 mg.) in absolute *n*-buthanol (8 ml.), sodium (0.8 g.) was added dropwise. After completion of addition, the mixture was further heated for 3 hr. After the addition of water, removal of the solvent and cooling, the alkaline solution was acidified with dilute hydrochloric acid to yield a crude acid (268 mg.) which was recrystallized from ethyl acetate to crystals with m. p. 228~229°C. $[\alpha]_D^{29}=+35\pm2°$ (*c* 1.104, methanol).

Found: C, 73.03; H, 10.26. Calcd. for $C_{24}H_{30}O_4$: C, 73.43; H, 10.27%.

Its dicathylate—After esterification with ethereal diazomethane, the cathylation acording to Fieser's method[14] gave dicathylate XIV as an oil which would not crystallize. No hydroxyl band in the infrared spectrum. Its diacetate XIVb—After esterification with etheral diazomethane and acetylation with acetic anhydride and pyridine overnight at room temperature, the acid gave a diacetate XIVb as an oil which would not crystallize. Infrared spectrum shows no hydroxyl band. This diacetate was saponified with methanolic potassium carbonate overnight at room temperature to afford 3β, 6α-dihydroxy-5α-cholanoic acid with m. p. 228~229°C.

Reduction of 3,6-dioxo-5α-cholanoic acid with sodium and absolute *n*-buthanol as in the previous case gave identical acid in the mixed melting point and the infrared spectrum.

Found: C, 73.50; H, 10.29%.

Summary

(1) 3β, 6β-Dihydroxy-5β-cholanoic acid was synthesized from methyl 3β-hydroxy-Δ^5-cholenoate in high yield.

(2) 3β, 6α-Dihydroxy-5α-cholanoic acid was also synthesized from 3β-hydroxy-6-oxo-5α-cholanoic acid or 3, 6-dioxo-5α-cholanoic acid.

The author is particularly indebted to Dr. K. Takeda, Director of this Laboratory, for his discussions and encouragement during this work. Thanks are also due to Mr. M. Takasuka for infrared spectral measurements, to Messrs T. Ieki, K. Miyahara, K. Daikatsu, T. Takaoka and Miss U. Kasugai for elementary analyses and to Mr. T. Iwata for measurements of optical rotation.

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