[Chem. Pharm. Bull. 26(9)2788—2792(1978)]

UDC 547.922.04 : 547.931.04

Syntheses of 22- and 23-Hydroxylated Bile Alcohols[1,2]

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(Received March 17, 1978)

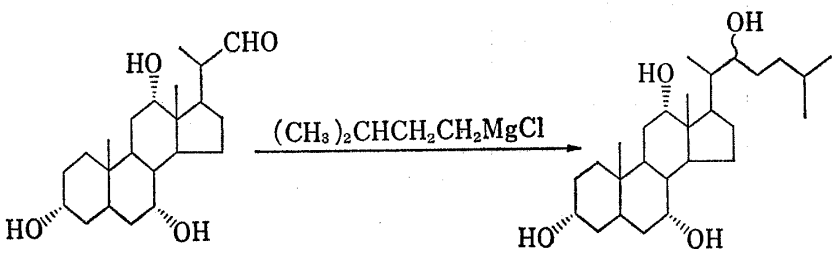
The syntheses of four new bile alcohols, 5β-cholestane-3α,7α,12α,22α-tetrol, 5β-cholestane-3α,7α,12α,22β-tetrol, 5β-cholestane-3α,7α,12α,23α-tetrol, and 5β-cholestane-3α,7α,-12α,23β-tetrol, were described. The 22-hydroxy compounds were prepared from bisnorcholyl aldehyde by the Grignard reaction with 3-methylbutylmagnesium chloride. The 23-hydroxy compounds were prepared from norcholic acid by the condensation with diisobutylcadmium and the subsequent lithium aluminum hydride reduction of the resulting 3α,7α,12α-trihydroxy-5β-cholestan-23-one. One of the synthetic tetrols, 5β-cholestane-3α,7α,12α,23β-tetrol was identical with a bile alcohol isolated from a patient with cerebrotendinous xanthomatosis.

Keywords——steroid; new bile alcohols; synthesis; disorder of cholesterol metabolism; structural establishment

The presence of bile alcohols in bile and feces of patients with cerebrotendinous xanthomatosis is a topic of current interest.[4]

During the course of our studies on the bile alcohols found in the patients with cerebrotendinous xanthomatosis, it was necessary to prepare C_{27} bile alcohol derivatives, hydroxylated at the 22- or 23-position. This paper describes the synthesis of the following bile alcohols: 5β-cholestane-3α, 7α, 12α, 22α-tetrol (IIa); 5β-cholestane-3α, 7α, 12α, 22β-tetrol (IIb); 5β-cholestane-3α, 7α, 12α, 23α-terol (VIIa); 5β-cholestane-3α, 7α, 12α, 23β-tetrol (VIIb).

The synthetic route to the 22-hydroxy compounds (IIa and IIb) is shown in Chart 1. As starting material we used the known bisnorcholyl aldehyde (I) prepared according to a published procedure[5] from cholic acid. Treatment of the C_{22} steroid aldehyde (I) with an



I

IIa (22α)
IIb (22β)

Chart 1

1) This paper is Part X of a series entitled "Comparative biochemical studies of bile acids and bile alcohols." Part IX, M. Murata, T. Kuramoto, and T. Hoshita, *Steroids*, **31**, 319 (1978).
2) The following IUPAC names apply to the steroids discussed in this manuscript: bisnorcholyl aldehyde, 3α,7α,12α-trihydroxy-23,24-dinor-5β-cholan-22-al; norcholic acid, 3α,7α,12α-trihydroxy-24-nor-5β-cholan-23-oic acid; cholic acid, 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid.
3) Location: *Kasumi, 1-2-3, Hiroshima.*
4) *a*) T. Setoguchi, G. Salen, G.S. Tint, and E.H. Mosbach, *J. Clin. Invest.*, **53**, 1393 (1974); *b*) S. Shefer, B. Dayal, G.S. Tint, G. Salen, and E.H. Mosbach, *J. Lipid Res.*, **16**, 280 (1975); *c*) T. Hoshita, M. Yasuhara, K. Kihira, and T. Kuramoto, *Steroids*, **27**, 657 (1976); *d*) M. Yasuhara, T. Kuramoto, T. Hoshita, E. Itoga, and S. Kito, *Steroids*, **31**, 333 (1978).
5) K. Kihira, T. Kuramoto, and T. Hoshita, *Steroids*, **27**, 383 (1976).

excess of 3-methylbutylmagnesium chloride in ether yielded a mixture of two 22-epimeric 5β-cholestane-3α,7α,12α,22-tetrols (IIa and IIb). Silica gel column chromatography of the mixture has led to the isolation of the more polar tetrol (IIa, the major product), mp 203—204°, $[\alpha]_D$ +20°, and the less polar tetrol (IIb, the minor product), mp 127—128.5°, $[\alpha]_D$ +34°.

Spectral analysis permitted positive identification of the synthetic 22-hydroxy compounds. The molecular weights determined for IIa as well as IIb were consistent with that of the expected formula $C_{27}H_{48}O_4$. Mass spectra (MS) of the trimethylsilyl (TMS) ethers of IIa and IIb did not differ significantly and each displayed a molecular ion at *m/e* 724 and two series of peaks, one at *m/e* 634, 544, 454, and 364, and a second at *m/e* 653, 563, 473, 383, and 293. The former series results from the consecutive loss of one, two, three, and four molecules of trimethylsilanol (TMS–OH). The latter series results from scission of the bond between C-22 and C-23 followed by the successive losses of TMS–OH. The base peak at *m/e* 173 is a side chain fragment resulting from scission of the bond between C-20 and C-22. The ion found at *m/e* 253 represents loss of the side chain and three nuclear TMS ether groups. Infrared (IR) spectra of IIa and IIb were practically identical to each other, and each exhibited a strong absorption band due to hydroxyl groups and a series of bands in the fingerprint region, characteristic of the cholic acid-type nucleus.[6] Nuclear magnetic resonance (NMR) spectrum of IIb exhibited five methyl resonances, two singlets at δ 0.87 and 1.02, and three doublets at δ 0.86, 0.89 and 1.46, which are assignable to the C-18, C-19, C-26, C-27 and C-21 methyl groups, respectively. The spectrum also showed four carbinyl hydrogen signals at δ 3.70, 3.94, 4.08, and 4.28, which are assignable to the C-3βH, C-22H, C-7βH, and C-12βH, respectively. NMR spectrum of IIa was similar to that of IIb, except for the chemical shift differences in the resonances of C-18 and C-27 methyls.

Tentative assignments of configuration at C-22 were made on the basis of optical rotation differences. It has already been pointed out by Barton, *et al.*[7] that the 22α-hydroxylated steroids are more levorotatory than their analogs of the 22β configuration. Hence, the more polar epimer (IIa), being the more levorotatory of the two epimers, may be assigned the 22α configuration, 5β-cholestane-3α,7α,12α,22α-tetrol (22*S*); the less polar epimer (IIb), the 22β configuration, 5β-cholestane-3α,7α,12α,22β-tetrol (22*R*).

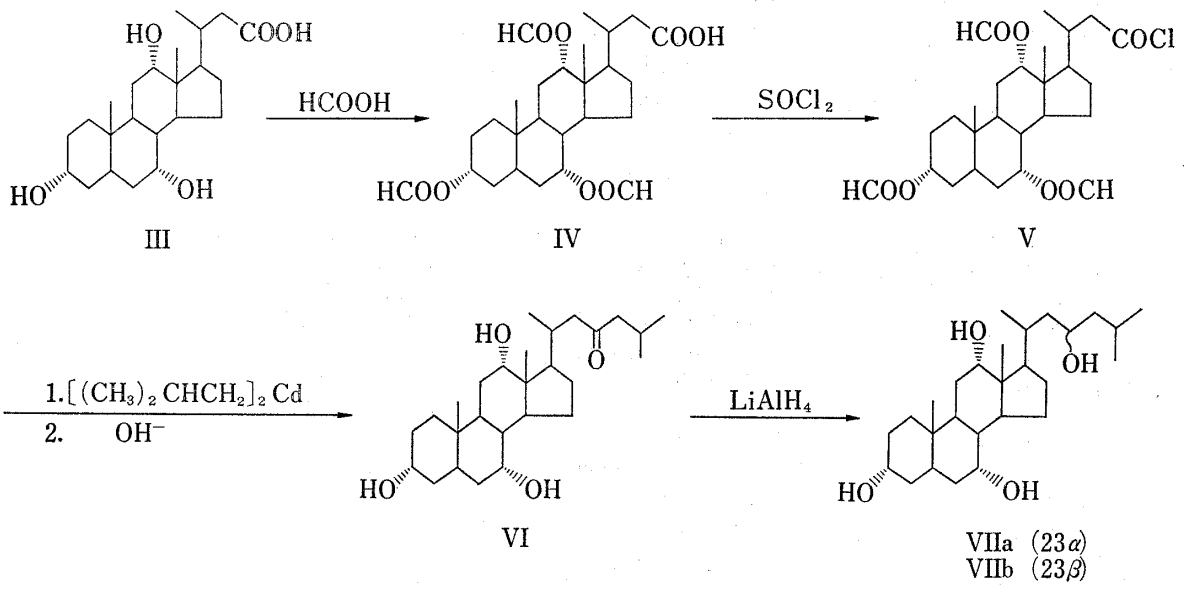


Chart 2

6) G.A.D. Haslewood, "Bile Salts," Methuen and Co., London, 1967, p. 34.
7) D.H.R. Barton, J.P. Poyser, and P.G. Sammes, *J. Chem. Soc.*, **1972**, 53.

The synthetic route to 5β-cholestane-3α,7α,12α,23-tetrols (VIIa and VIIb) is shown in Chart 2. Starting norcholic acid (III) was prepared from cholic acid according to a procedure described previously.[8] Formylation of norcholic acid (III) followed by treatment with thionyl chloride gave the acid chloride (V). The acid chloride (V) was not isolated but was freed of any residual thionyl chloride by repeated evaporation with benzene. Treatment of the acid chloride (V) with an excess of diisobutylcadmium followed by alkaline hydrolysis yielded 3α,7α,12α-trihydroxy-5β-cholestan-23-one (VI), mp 173—174°. IR spectrum of VI showed the presence of a carbonyl group (1710 cm^{-1}). The 23-ketone (VI) was reduced with lithium aluminum hydride and the resulting mixture of two 23-epimeric 5β-cholestane-3α,7α,12α,23-tetrols (VIIa and VIIb) was separated by silica gel column chromatography.

The structures of VIIa (eluted first from the column), mp 189—191°, $[\alpha]_D$ +41°, and VIIb (eluted second), mp 232—233°, $[\alpha]_D$ +52° were confirmed by spectral analysis. MS of the TMS ethers of VIIa and VIIb were essentially identical to each other. The spectra were characterized by a molecular ion at *m/e* 724, by a series of fragments at *m/e* 634, 544, 454, and 364, resulting the successive loss of one, two, three, and four molecules of TMS–OH, by ions at *m/e* 159 (the base peak), and 667, resulting from scissions of the bond between C-22 and C-23, and of the bond between C-23 and C-24, respectively, and by an ion at *m/e* 253, representing loss of the side chain plus three nuclear TMS ether groups. IR spectra of the epimers did not differ significantly and each exhibited a strong hydroxyl band and a series of bands due to the cholic acid-type nucleus.[6] NMR spectrum of VIIa showed the presence of five methyl groups and four carbinyl protons. NMR spectrum of VIIb was identical with that of VIIa, except for the chemical shift differences in the resonance of C-26 and C-27 methyls.

The more polar 23-hydroxy compound (VIIb) was more dextrorotatory than the less polar 23-epimer (VIIa). It has been reported[7,9] that in the optical rotation differences among several pairs of 23-epimeric steroid alcohols, the 23β-epimers consistently possessed more highly positive rotation than their 23α-counterparts. Hence, VIIb may be assigned the 23β configuration, 5β-cholestane-3α,7α,12α,23β-tetrol (23*R*); VIIa, the 23α configuration, 5β-cholestane-3α,7α,12α,23α-tetrol (23*S*).

Recently Yasuhara, *et al.*[4d] have isolated three minor bile alcohols from feces of a patient with cerebrotendinous xanthomatosis. By direct comparison with the specimen synthesized in the present work, one of the minor bile alcohols was shown to be 5β-cholestane-3α,7α,12α,23β-tetrol. The biosynthetic bile alcohol and the synthetic reference compound had identical mass and IR spectra and identical *Rf* and relative retention time (t_R) values when examined by thin-layer and gas-liquid chromatography.

Experimental

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were taken in ethanol solution with a JASCO DIP-180 polarimeter at 25°. Molecular weights were determined from the molecular ions using high resolution mass spectra, which were recorded with a JEOL JMS-01SG mass spectrometer, with an accelerating potential of 10 kV, an ionization potential of 70 eV and a source temperature of 230°. Thin-layer chromatography (TLC) was carried out on silica gel G (Merck) using a 10% solution of phosphomolybdic acid in ethanol as the detection reagent. The following solvent systems were employed: OEA, isooctane–ethyl acetate–acetic acid, 30:8:1; EA, ethyl acetate–acetone, 7:3, CE, chloroform–ethanol, 4:1. Gas–liquid chromatography (GLC) was run on a Shimadzu GC-6A gas chromatograph using glass column (2 m×4 mm) packed with 1.5% OV-1, 3% OV-17, 3% QF-1 or 3% Poly I-110 on Gas-Chrom Q (80—100 mesh). The samples were injected into the gas chromatograph as their TMS ethers, which were prepared with hexamethyldisilazane and trimethylchlorosilane in pyridine at room temperature. All retention times are reported relative to TMS ether of methyl cholate (relative t_R=1.00). IR spectra were taken on a JASCO IRA-1 spectrometer as KBr discs. NMR spectra were obtained at 100 MHz on a JEOL JNM-PS-100 spectrometer using pyridine-d_5 as solvent. Chemical shifts are given in the

8) T. Shimizu and T. Kazuno, *Z. Physiol. Chem.*, **244**, 167 (1936).
9) J.E. van Lier and L.L. Smith, *J. Pharm. Sci.*, **59**, 719 (1970).

δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet, d, doublet, m, multiplet). MS of TMS ethers were obtained on a Shimadzu-LKB 9000 gas chromatograph–mass spectrometer. The following operating conditions were employed: column, 2% OV-1 (2 m×3 mm); column temperature, 270°; ion source temperature, 310°; electron energy, 70 eV; trap current, 60 μA; accelerating voltage, 3500 V.

5β-Cholestane-3α,7α,12α,22α-tetrol (IIa) and 5β-Cholestane-3α,7α,12α,22β-tetrol (IIb)——To a solution of 1.6 g of bisnorcholyl aldehyde (I) in 40 ml of dry benzene was added a solution of 3-methylbutylmagnesium (prepared from 1.3 g of magnesium and 11 ml of 3-methylbutyl chloride) in 100 ml of dry ether. The reaction mixture was refluxed for 2 hr. After cooling to 0° in ice, 500 g of crushed ice and 20 ml of 2 N H_2SO_4 were added in order to decompose the Grignard products. The reaction mixture was extracted three times with 100 ml portions of ethyl acetate. The combined ethyl acetate extracts were washed with water until the washings were neutral, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting residue (1.7 g) was chromatographed on a column of silica gel (Merck, 50 g). The column was eluted with ethyl acetate and then with ethyl acetate–acetone mixtures.

Elution with ethyl acetate and crystallization from ethyl acetate gave crystals (80 mg) of 5β-cholestane-3α,7α,12α,22β-tetrol (IIb): mp 127—128.5°; $[\alpha]_D$ +34° (c=2.2, MeOH); M^+, 436.35504 (Calcd. for $C_{27}H_{48}O_4$: 436.35526); TLC (Rf): 0.70 (EA), 0.94 (CE); GLC (relative t_R): 1.09 (OV-1), 0.72 (OV-17), 0.54 (QF-1), 0.55 (poly I-110); IR ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1080, 1040, 1020, 980, 950, 915 (cholic acid-type nucleus); NMR: 0.87 (s, 3H, 18-CH_3), 0.86 (d, J=6 Hz, 3H, 26-CH_3), 0.89 (d, J=6 Hz, 3H, 27-CH_3), 1.02 (s, 3H, 19-CH_3), 1.46 (d, J=6 Hz, 3H, 21-CH_3), 3.70 (m, 1H, C-3βH), 3.94 (m, 1H, C-22αH), 4.08 (m, 1H, C-7βH), 4.28 (m, 1H, C-12βH); MS of the TMS ether (m/e): 724, 653, 634, 563, 544, 473, 454, 383, 364, 293, 253, 173.

Elution with a 7: 3 mixture of ethyl acetate–acetone and crystallization from ethyl acetate gave crystals (365 mg) of 5β-cholestane-3α,7α,12α,22α-tetrol (IIa): mp 203—204°; $[\alpha]_D$ +20° (c=1.9, MeOH); M^+, 436.35662 (Calcd. for $C_{27}H_{48}O_4$: 436.35526); TLC (Rf): 0.12 (EA), 0.55 (CE); GLC (relative t_R): 1.28 (OV-1), 0.86 (OV-17), 0.66 (QF-1), 0.69 (Poly I-110); IR ν_{max}^{KBr} (cm^{-1}) 3360 (OH), 1080, 1045, 1020, 983, 953, 918 (cholic acid-type nucleus); NMR: 0.89 (d, J=6 Hz, 6H, 26/27-CH_3), 0.92 (s, 3H, 18-CH_3), 1.04 (s, 3H, 19-CH_3), 1.46 (d, J=6 Hz, 3H, 21-CH_3), 3.66 (m, 1H, C-3βH), 3.93 (m, 1H, C-22βH), 4.06 (m, 1H, C-7βH), 4.30 (m, 1H, C-12βH); MS of the TMS ether (m/e): 724, 653, 634, 563, 544, 473, 454, 383, 364, 293, 253, 173.

3α,7α,12α-Trihydroxy-5β-cholestan-23-one (VI)——A solution of 4 g of norcholic acid (III) in 30 ml of formic acid was heated at 55° for 5 hr. The solution was then diluted with 600 ml of water. The precipitated formate (IV) was filtered, washed with water until free from formic acid, and dried in a desiccator over NaOH. The formate (IV) gave a single spot when examined by TLC using the system OEA, and showed in its IR spectrum no hydroxyl absorption.

To the 4.5 g of IV was added 12 ml of thionyl chloride. The reaction was allowed to proceed at room temperature for 2 hr. The excess of thionyl chloride was completely removed at room temperature *in vacuo* followed by repeated evaporation with benzene, and the resulting acid chloride (V) was used without purification.

To a solution of diisobutylcadmium (prepared from 3.5 g of magnesium, 19.5 ml of isobutyl bromide, and 25 g of cadmium chloride) in 120 ml of dry ether was added a solution of the acid chloride (V) in 50 ml of dry benzene. The reaction mixture was refluxed for 5 hr. After addition of 500 g of crushed ice and 20 ml of 2 N H_2SO_4, the layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layer and the ethyl acetate extract were combined, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting residue was dissolved in 50 ml of 2 N methanolic KOH, and the solution was refluxed for 2 hr. After dilution with 500 ml of water, the precipitated products were extracted with three 100 ml portions of ethyl acetate. Evaporation of the solvent from the washed and dried extracts left a residue (4.1 g), which was recrystallized from ether to yield 2.1 g of 3α,7α,12α-trihydroxy-5β-cholestan-23-one (VI): mp 173—174°; M^+, 434.33774 (Calcd. for $C_{27}H_{46}O_4$: 434.33961); TLC (Rf): 0.57 (EA); GLC (relative t_R): 1.36 (QF-1); IR ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1710 (C=O), 1080, 1040, 980, 950, 915 (cholic acid-type nucleus).

5β-Cholestane-3α,7α,12α,23α-tetrol (VIIa) and 5β-Cholestane-3α,7α,12α,23β-tetrol (VIIb)——To a solution of 1.2 g of the 23-ketone (VI) in 40 ml of tetrahydrofuran was added 1 g of lithium aluminum hydride at 0°. The reaction mixture was refluxed for 5 hr. After cooling to 0°, crushed ice (500 g) and 2 N H_2SO_4 (20 ml) were added to decompose the excess of litium aluminum hydride. The precipitated products were extracted with four 100 ml portions of ethyl acetate. The combined ethyl acetate extracts were washed with water, 5% Na_2CO_3 solution, and water successively, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting residue (1.1 g) was chromatographed on a column of silica gel (Merck, 30 g) in the same manner used for the separation of the 22-hydroxy compounds.

Elution with ethyl acetate from the silica gel column and crystallization from acetone gave crystals (223 mg) of 5β-cholestane-3α,7α,12α,23α-tetrol (VIIa): mp 189—191°; $[\alpha]_D$ +41° (c=1.8, MeOH); M^+, 436.35244 (Calcd. for $C_{27}H_{48}O_4$: 436.35526); TLC (Rf): 0.60 (EA), 0.90 (CE); GLC (relative t_R): 1.22 (OV-1), 0.82 (OV-17), 0.63 (QF-1), 0.65 (Poly I-110); IR ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1080, 1040, 980, 950, 920 (cholic acid-type nucleus); NMR: 0.88 (s, 3H, 18-CH_3), 0.95 (d, J=6 Hz, 3H, 26-CH_3), 0.98 (d, J=6 Hz, 3H, 27-CH_3), 1.00 (s, 3H, 19-CH_3), 1.38 (d, J=6 Hz, 3H, 21-CH_3), 3.70 (m, 1H, C-3βH), 4.10 (m, 2H, C-7βH and C-23βH), 4.32 (m, 1H, C-12βH); MS of the TMS ether (m/e): 724, 667, 634, 544, 454, 364, 253, 159.

Elution with a 39:1 mixture of ethyl acetate–acetone and crystallization from ethyl acetate gave crystals (111 mg) of 5β-cholestane-3α,7α,12α,23β-tetrol (VIIb): mp 232—233°; $[\alpha]_D$ +52° (c=1.9, MeOH); M^+, 436.35561 (Calcd. for $C_{27}H_{48}O_4$: 436.35526); TLC (Rf): 0.23 (EA), 0.65 (CE); GLC (relative t_R): 1.22 (OV-1), 0.83 (OV-17), 0.65 (QF-1), 0.68 (Poly I-110); IR ν_{max}^{KBr} (cm^{-1}): 3340 (OH), 1080, 1030, 980, 950, 918 (cholic acid-type nucleus); NMR: 0.89 (s, 3H, 18-CH_3), 1.01 (s, 3H, 19-CH_3), 1.00 (d, J=6 Hz, 3H, 26-CH_3), 1.03 (d, J=6 Hz, 3H, 27-CH_3), 1.39 (d, J=6 Hz, 3H, 21-CH_3), 3.70 (m, 1H, C-3βH), 4.10 (m, 2H, C-7βH and C-23αH), 4.32 (m, 1H, C-12βH); MS of the TMS ether (m/e): 724, 667, 634, 544, 454, 364, 253, 159.

Acknowledgement The authors are indebted to Prof. A. Hayashi and Dr. F. Matsuura of the Department of Chemistry, Faculty of Science and Technology, Kinki University, for their help in the measurement of combined gas chromatography–mass spectrometry.

This work was supported in part by a grant (code No. 76-125) from the Naito Foundation.