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SYNTHESES OF DEUTERIUM LABELED BILE ALCOHOLS

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SUMMARY

The following major bile alcohols, identified in urine of healthy humans and in bile and urine of patients with cerebrotendinous xanthomatosis, were synthesized as internal standards for mass spectral analyses: [26,27-D_6]5β-cholestane-3α,7α,12α,25-tetrol, [26,27-D_6](23S)-5β-cholestane-3α,7α,12α,23,25-pentol, [27-D_3]5β-cholestane-3α,7α,12α,25,26-pentol, and [2,4-D_4]27-nor-5β-cholestane-3α,7α,12α,24,25-pentol.

Key Words: bile alcohols, 5β-cholestane-3α,7α,12α,25-tetrol, 5β-cholestane-3α,7α,12α,25,26-pentol, 5β-cholestane-3α,7α,12α,23,25-pentol, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, deuterium-labeled.

INTRODUCTION

Bile alcohols have been identified in bile, feces, and urine of patients with cerebrotendinous xanthomatosis (CTX)(1-5) and also in bile, serum and urine of healthy humans (6-11). The major biliary bile alcohol of the CTX patients is 5β-cholestane-3α,7α,12α,25-tetrol (1) and the major urinary bile alcohol is (23S)-5β-cholestane-3α,7α,12α,23,25-pentol (4). In healthy humans the major bile alcohol in urine and serum is 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (6,11) and as a minor constituent 5β-cholestane-3α,7α,12α,25,26-pentol (9) has been identified.

This paper deals with the chemical syntheses of deuterium labeled 5β-cholestane-3α,7α,12α,25-tetrol, (23S)-5β-cholestane-3α,7α,12α,23,25-pentols, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol and 5β-cholestane-3α,7α,12,25,26-pentol as internal standards for gas chromatography-mass spectrometry by the routes shown in the Scheme. The use of the deuterium labeled bile alcohols ensures the accurate qualification and quantification of the bile alcohols in clinical investigations of healthy humans and CTX patients.

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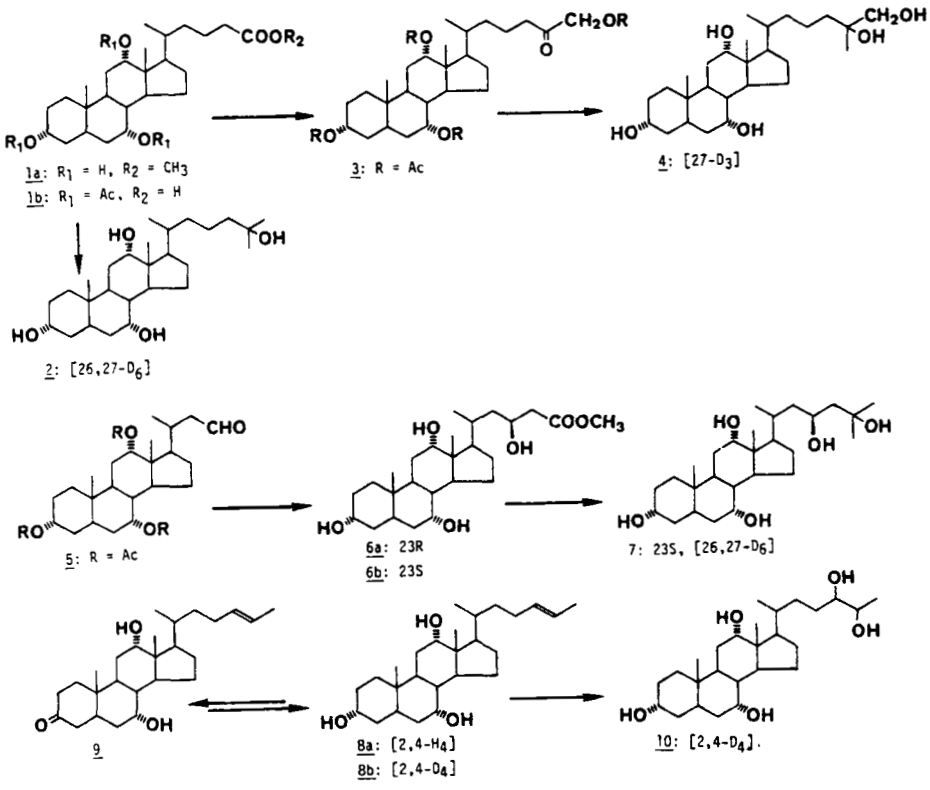

DISCUSSION

On the syntheses of the deuterium labeled bile alcohols as internal standards for mass spectrometry, the most important point is where the deuteriums are labeled.

5β-Cholestane-3α,7α,12α,25-tetrol and (23S)-5β-cholestane-3α,7α,12α,23,25-pentol exhibited the fragment ion at m/z 131 as the base ion, and other ions observed were quite weak (less than 5% compare to the base ion) in their mass spectra analyzed as trimethylsilyl (TMS) ethers (1,12). The base ion at m/z 131 consisted of the $(CH_3)_2COTMS$ moiety due to the scission of the bond between C24 and C25. Therefore, deuteriums were introduced at C26 and C27. Methyl 3α,7α,12α-trihydroxy-25-homo-5β-cholan-25-oate (1) was treated with CD_3MgI to yield [26,27-D_6]5β-cholestane-3α,7α,12α,25-tetrol (2). By the similar route, (23S)-5β-cholestane-3α,7α,12α,23,25-pentol was labeled with deuteriums at C26 and C27. Methyl (23S)-3α,7α,12α,23-tetrahydroxy-25-homo-5β-cholan-25-oate (6b) was prepared from 3α,7α,12α-triacetoxy-24-nor-5β-cholan-23-al (5) by Reformatsky

reaction with ethyl α-bromoacetate. The ester (6b) was then reacted with CD_3MgI to yield [26,27-D_6](23S)-5β-cholestane-3α,7α,12α,23,25-pentol (7). In the mass spectra of the deuterium labeled bile alcohols (2 and 7) as TMS ethers the base ions were observed at m/z 137.

5β-Cholestane-3α,7α,12α,25,26-pentol was labeled at C27, because the mass spectrum of this bile alcohol as TMS ether shows a series of fragments at m/z 349, 439, 529, and 619 due to the scission of the bond between C25 and C26 losing CH_2OTMS (103 mass units)(13). 3α,7α,12α-Triacetoxy-25-homo-5β-cholan-25-oic acid (1b) was converted into 3α,7α,12α,26-tetraacetoxy-27-nor-5β-cholestan-25-one (3) by subsequent treatment with oxalyl chloride, ethereal diazomethane, and then acetic acid. Reaction of the 25-keto bile alcohol (3) with CD_3MgI afforded [27-D_3]5β-cholestane-3α,7α,12α,25,26-pentol (4). The mass spectrum of the labeled bile alcohol (4) as TMS ether showed the series of fragments at m/z 352, 442, 532, and 622 which were larger by 3 mass units than those of the corresponding unlabeled bile alcohol.

The mass spectrum of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol as TMS ether shows a series of fragments at m/z 321, 411, and 501 due to the scission of the bond between C24 and C25 (9). Therefore the bile alcohol was labeled at C2 and C4. 27-Nor-5β-cholest-24-ene-3α,7α,12α-triol (8a) was converted to 7α,12α-dihydroxy-27-nor-5β-cholest-24-en-3-one (9) by treatment with Ag_2CO_3-celite. The 3-keto bile alcohol (9) was refluxed repeatedly with CD_3OD in the presence of K_2CO_3 and then reduced by $NaBH_4$ to [2,4-D_4]5β-cholest-24-ene-3α,7α,12α-triol (8b). The labeled bile alcohol (8b) was then hydroxylated at C24 and C25 with OsO_4 to yield [2,4-D_4]27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (10). The mass spectrum of the labeled bile alcohol (10) as TMS ether showed a series of fragments at m/z 325, 415, and 506 which were larger by 4 mass units than those of the corresponding unlabeled bile alcohol.

EXPERIMENTAL

Melting points are uncorrected. The nmr (proton) spectra were obtained in deuterio-methanol or deuterio-pyridine solution using tetramethylsilane as an internal standard and were recorded on a Hitachi R-40 spectrometer (90 MHz). Mass spectra were recorded on a Shimadzu QP-1000 gas chromatography-mass

spectrometer as TMS ethers. The IR spectra were recorded on a Shimadzu IR-408 spectrophotometer as KBr-pellets.

Usual work-up refers to dilution with 5 volumes of water or 1N HCl solution, extraction with organic solvent, washing with water to neutrality, drying over Na_2SO_4, and evaporation of the solvent.

[26,27-D_6]5β-Cholestane-3α,7α,12α,25-tetrol (2).

Ten ml of a solution of methyl 3α,7α,12α-trihydroxy-25-home-5β-cholan-25-oate (1a, 714 mg) in anhydrous ether was added to a solution containing CD_3MgI prepared by adding 3.4 ml of CD_3I to 1 g of Mg and in 20 ml of anhydrous ether. Usual work-up (1N HCl and ethyl acetate) gave a residue of crude product (730 mg). Repeated crystallization of the product from ethyl acetate gave colorless crystals of [26,27-D_6]5β-cholestane-3α,7α,12α,25-tetrol (2, 305 mg); mp, 188-189°C (Lit. (14) 188-189°C); IR, 3400; nmr (methanol), 0.70 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.99 (3H, d, J = 6 Hz, 21-Me), 3.30 (1H, m, 3β-H), 3.72 (1H, m, 7β-H), 3.87 (1H, m, 12β-H).

3α,7α,12α,26-Tetraacetoxy-27-nor-5β-cholestan-25-one (3).

3α,7α,12α-Triacetoxy-25-homo-cholan-25-oic acid (1b, 1.1 g) was added to a solution of oxalyl chloride (0.6 ml) in anhydrous benzene (20 ml). The reaction mixture was refluxed for 2 hr and evaporated to dryness. The resulting acid chloride was dissolved in 10 ml of anhydrous benzene and added to a solution of excess diazomethane in ether. After standing at room temperature for 2 hr, the solvent was evaporated to dryness and refluxed with 20 ml of acetic acid for 1hr. After usual work-up (water and ether), the product was purified by column chromatography of silica gel with benzene-ethyl acetate (7:3) to give 1.0 g of 3α,7α,12α,26-tetraacetoxy-27-nor-5β-cholestan-25-one (3) as a colorless syrup; IR, 1720; nmr (methanol), 0.76 (3H, s, 18-Me), 0.82 (3H, d, J = 6 Hz, 21-Me), 0.94 (3H, s, 19-Me), 1.96 (3H, s, CH_3-CO-), 2.00 (3H, s, CH_3-CO-), 2.07 (6H, s, CH_3-CO- x 2), 4.60 (2H, s, 26-H_2), 4.47 (1H, m, 3β-H), 4.81 (1H, m, 7β-H), 4.99 (1H, m, 12β-H).

[27-D_3]5β-Cholestane-3α,7α,12α,25,26-pentol (4).

Ten ml of a solution of 3 (900 mg) in anhydrous ether was added to a solution containing CD_3MgI prepared from 1.5 g of Mg and 5 ml of CD_3I in 20 ml

of anhydrous ether. Usual work-up (1N HCl and ethyl acetate) gave a residue (723 mg). The residue was hydrolyzed with 5% methanolic KOH at 60°C for 2hr. After usual work-up (water and ethyl acetate), the product was purified by column chromatography of silica gel with ethyl acetate-acetone (2:8) to give a residue (405 mg). Repeated crystallization from benzene gave crystals of [27-D_3]5β-cholestane-3α,7α,12α,25,26-pentol (4, 310 mg): mp, 188-190°C (Lit. (15) 178°C); IR, 3400; nmr (methanol), 0.70 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.00 (3H, d, J = 6 Hz, 21-Me), 3.20 (1H, m, 3β-H), 3.29 (2H, s, 26-H_2), 3.73 (1H, m, 7β-H), 3.89 (1H, m, 12β-H).

Methyl (23R and 23S)-3α,7α,12α,23-tetrahydroxy-25-homo-5β-cholestan-25-oates (6a and 6b).

3α,7α,12α-Triacetoxy-24-nor-5β-cholan-23-al (5, 1.1 g) prepared from norcholic acid as described previously (16) was dissolved in anhydrous benzene and added to a solution containing 6 ml of ethyl α-bromoacetate, 10 g of Zn, and a few mg of Cu and I in anhydrous benzene (100 ml). The reaction mixture was refluxed for 2 hr. After usual work-up (1N HCl and with ether), the resulting residue was hydrolyzed with 5% methanolic KOH by refluxing for 1 hr. After usual work-up (1N HCl and ethyl acetate), the resulting residue was then methylated with ethereal diazomethane and purified by column chromatography of silica gel (100 g) eluting with ethyl acetate graded by acetone. The eluants were monitored on TLC. Faster eluted fractions containing methyl (23R)-3α,7α,12α,23-tetrahydroxy-25-homo-5β-cholan-25-ate (6a) was combined and the solvent was evaporated. Repeated crystallization from ethyl acetate gave crystals of 6a (252 mg); mp, 188-189°C (219-220°C as free form); IR, 1749, 3400; nmr (as free form, pyridine), 0.84 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 1.33 (3H, d, J = 6 Hz, 21-Me), 3.64 (1H, m, 3β-H), 4.01 (1H, m, 7β-H), 4.21 (1H, m, 12β-H), 4.61 (1H, m, 23-H).

Fractions containing methyl (23S)-3α,7α,12α,23-tetrahydroxy-25-homo-5β-cholan-25-oate (6b) were combined and the solvent was evaporated. Repeated crystallization from ethyl acetate gave crystals of 6b (218 mg); mp, 170-171°C (238.5-240°C as free form); IR, 1740, 3400; nmr (as free form, pyridine), 0.83 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 1.34 (3H, d, J = 6 Hz, 21-Me), 3.64 (1H, m,

3β-H), 4.00 (1H, m, 7β-H), 4.19 (1H, m, 12β-H), 4.61 (1H, m, 23-H).

[26,27-D_6](23S)-5β-Cholestane-3α,7α,12α,23,25-pentol (7).

A solution of 6b (100 mg) in anhydrous tetrahydrofuran was added to a solution of CD_3MgI prepared from 1 g of Mg and 2.5 ml of CD_3I in anhydrous ether (20 ml). The reaction mixture was refluxed for 2 hr. After usual work-up (1N HCl and ethyl acetate), repeated crystallization from ethyl acetate gave crystals of [26,27-D_6](23S)-5β-cholestane-3α,7α,12α,23,25-pentol (7, 12 mg), mp, 208-209°C (Lit.(12) 209-210°C); IR, 3400; nmr (methanol), 0.70 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.01 (3H, s, J = 6 Hz, 21-Me), 3.00-4.10 (4H, m, 3β-, 7β-, 12β-, and 23-H).

7α,12α-Dihydroxy-27-nor-5β-cholest-24-en-3-one (9).

Twenty ml of benzene solution of 27-nor-5β-cholest-24-ene-3α,7α,12α-triol (8a, 500 mg) prepared as described previously (9) was refluxed with 5 g of Ag_2CO_3/celite for 12 hr. The reaction mixture was filtered and the solvent was evaporated. Repeated crystallization from methanol gave crystals of 7α,12α-dihydroxy-27-nor-5β-cholestan-24-en-3-one (9, 250 mg); mp, 194-197°C; IR, 1640, 3400; nmr (pyridine), 0.80 (3H, s, 18-Me), 0.97 (3H, s, 19-Me), 1.19 (3H, d, J = 6 Hz, 21-Me), 1.66 (3H, d, J = 6 Hz, 26-Me), 3.99 (1H, m, 7β-H), 4.16 (1H, m, 12β-H), 5.37 (2H, m, 24- and 25-H).

[2,4-D_4]27-nor-5β-cholestan-24-en-3α,7α,12α-triol (8b).

A solution of 9 (250 mg) in 10 ml of CH_3OD was added with 0.5 ml of 40% K_2CO_3 solution dissolved in D_2O. The reaction mixture was refluxed for 12 hr and the solvent was evaporated to dryness. The resulting residue was dissolved in 10 ml of CH_3OD and refluxed for 12 hr and the solvent was evaporated. The resulting residue was again dissolved in 10 ml of CH_3OD and refluxed for 12 hr. To this reaction mixture 50 mg of $NaBH_4$ was added and the mixture was stirred at room temperature for 2 hr. The reaction mixture was diluted with 5 volumes of water and resulting precipitates were combined by filtration and washed with water to neutrality. The precipitates were crystallized from methanol to give [2,4-D_4]27-nor-5β-cholest-24-ene-3α,7α,12α-triol (8b, 212 mg).

[2,4-D_4]27-Nor-5β-cholestane-3α,7α,12α,24,25-pentol (10).

Deuterium labeled 27-nor-5β-cholest-24-ene-3α,7α,12α-triol (8b, 200 mg) was

acetylated by heating at 100°C for 3 hr with 12 ml of acetic anhydride and 2 ml of pyridine. The reaction product was evaporated to dryness and dissolved in 20 ml of anhydrous ether and 0.4 ml of pyridine. To this solution 300 mg of OsO_4 dissolved in 5 ml of anhydrous ether was added. The reaction mixture was kept at room temperature for 48 hr. After removal of the solvent, resulting residue was refluxed with 50% ethanol solution containing $NaHSO_3$ for 2 hr. Resulting black residue was filtered off and washed with 50 ml of ethanol. The filtrate was added with 2 g of KOH and hydrolyzed by heating at 60°C for 2 hr. Usual work-up (water and ethyl acetate) gave a residue of [2,4-D_4]27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (10, 224 mg). Repeated crystallization from ethyl acetate gave crystals of 10 (93 mg): mp, 174-175°C; IR, 3400; nmr (pyridine), 0.80 (3H, s, 18-Me), 0.97 (3H, s, 19-Me), 1.20 (3H, d, J = 6 Hz, 21-Me), 1.43 (3H, d, J = 6 Hz, 26-Me), 3.60 (1H, s, 3β-H), 3.97 (1H, m, 7β-H), 4.13 (1H, m, 12β-H), 3.70-4.05 (2H, m, 24- and 25-H).

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