New Bile Alcohols II: Synthesis and Mass Spectra of C_{26} Bile Alcohols[1]

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ABSTRACT AND SUMMARY

The synthesis of (23α-) and (23β-)24-nor-5β-cholestane-3α,7α,23,25-tetrols (VII-a and VII-b) and 24-nor-5β-cholestane-3α,7α,25,26-tetrol (VIII) is described. Dehydration of 24-nor-5β-cholestane-3α,7α,25-triol (II) (synthesized from methyl chenodeoxycholate via a Grignard reaction) with acetic anhydride and acetic acid yielded two unsaturated compounds. These were oxidized with osmium tetroxide to produce a mixture of 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) and 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI). After separating these diols (V and VI) by column chromatography, each compound was hydrolyzed with KOH to give 24-nor-5β-cholestane-3α,7α,23ξ,25-tetrol (VII) and 24-nor-5β-cholestane-3α,7α,25,26-tetrol (VIII), respectively. Two isomers of the tetrol (VII) were separated using alumina column chromatography (VII-a and VII-b).

INTRODUCTION

C_{27}-bile alcohols have served as intermediates in studies of biosynthesis of the primary bile acids, cholic acid, and chenodeoxycholic acid (1-3). It was assumed that the major pathway for the degradation of the sterol side chain involved 26-hydroxylation as an initial step. Recently, an alternate pathway of cholic acid biosynthesis involving 25-hydroxylation was shown in man (4) and in perfused rabbit liver (5). It will be important to isolate intermediates of the 26- and 25-hydroxylation pathway in order to ascertain the exact sequence of reactions from bile alcohol to bile acid. The C_{26}-analogs of certain C_{27}-bile alcohols may be useful as inhibitors of the normal biosynthetic pathway involving side-chain oxidation and 12α-hydroxylation. Isolation of C_{27}-bile alcohol and bile acid intermediates may then be possible. Our laboratory has reported the synthesis of C_{26}-analogs of bile alcohols of the cholic acid series (6). The present paper describes the synthesis of the following C_{26}-bile alcohols of the chenodeoxycholic acid series: 24-nor-5β-cholestane-3α,7α,23α,25-tetrol; 24-nor-5β-cholestane-3α,7α,23β,25-tetrol; and 24-nor-5β-cholestane-3α,7α,25,26-tetrol, and their characterization by physical-chemical methods.

EXPERIMENTAL PROCEDURES

All solvents were reagent grade or spectrophotometric grade and were used without further purification. Melting points were determined with a Thermolyne melting point apparatus (Thermolyne Corp., Dubuque, IA) and are uncorrected. Infrared spectra were taken on a Perkin-Elmer No. 421 grating spectrometer (Perkin-Elmer Corp., Norwalk, CT) as KBr discs. Optical rotations were recorded with a Cary 60 spectropolarimeter (Varian Instrument Division, Palo Alto, CA) using methanol as solvent.

Thin layer chromatograph (TLC) was carried out on Silica Gel G plates (Analtech Inc., Newark, DE) using a phosphomolybdic acid spray (Brinkmann Inst., Inc., Westbury, NY) as a detection reagent. The following solvent systems were employed: system BE-1, benzene-ethyl acetate (1:1, v/v); system EA-1, ethyl acetate-acetone (7:3, v/v). Column chromatography was carried out using neutral alumina, 100-200 mesh (Calbiochem, Monsey, NY) or silica gel, 40-140 mesh (J.T. Baker, Inc., Phillipsburg, NJ), with an adsorbent/compound ratio of 100:1.

Gas liquid chromatography was carried out as previously described (7). Retention times were related to 5α-cholestane (absolute time of elution = 1.2 min on 2% OV-210).

Gas chromatography-mass spectrometry was performed on a Hewlett-Packard No. 5981A E.I. mass spectrometer; conditions for the analyses were previously described (7).

24-Nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) and 24-Nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI)

The procedure used was similar to that of Hoshita (8) with modifications. A solution containing 1 g of 24-nor-5β-cholestane-3α,7α,25-triol (synthesized from chenodeoxycholic acid and methyl magnesium iodide) (II) (Fig. 1) in 60 ml of acetic acid and 20 ml of acetic anhydride was refluxed for 20 hr. The reaction mixture was evaporated and the residue was dried at 85 C, and dissolved in a mixture of 40 ml of dry ether and 1 ml of dry pyridine. One gram of osmium tetroxide was added, and the mixture was allowed to remain at room temperature for 72 hr. The solution was evaporated and the black residue was refluxed for 4 hr with 150 ml of 50% ethanol-water, containing 14 g of $NaHSO_3$. Ethanol was added and the precipitate was filtered off.

The crude sample (mixture of the diol diacetates V and VI) was separated by column chromatography on silica gel using benzene-ethyl acetate (1:1, v/v). The 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) emerged in fractions 61-100 (20 ml per tube). The isomers of the C_{26} diol, 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V), could not be separated by TLC using silica gel plates. The other diol, 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI) was eluted in fractions 150-200.

Crystallization of 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) from acetone-water (1:1, v/v) gave 510 mg (40%) of white needles with the following properties: mp 200-201 C, single spot on TLC, R_f = 0.32 (solvent system BE-1). Gas liquid chromatographic analysis (as the TMSi derivative): relative retention time = 16.6. IR (KBr disc), 3450 (OH), 2950, 2880, 1728, 1245, 1136, 1064, and 1021 cm^{-1}.

The mass spectrum of this diol (V) exhibited major fragments at m/e 446, M-60; 428, M-(60+18); 386, M-(2x60); 368, M-(2x60+18); 353, M-(2x60+18+15); 255, M-(side chain+2x60) (base peak); 253, M-(side chain+2x60+2H). The mass spectral fragments of the trimethylsilyl ether of this diol (V) are m/e 650, M^+; 592, M-(43+15); 532, M-(43+15+60); 519, M-(131); 515, M-(2x60+15); 459, M-(131+60); 399, M-(131+2x60); 375, M-(side chain); 315, M-(60+side chain); 255, M-(2x60+side chain); 131, $[(CH_3)_3C{=}O^+Si(CH_3)_3]$ (base peak). Analysis calculated

[1]The following IUPAC names apply to the steroids discussed in this manuscript: cholic acid = 3α,7α, 12α-trihydroxy-5β-cholan-24-oic acid; chenodeoxycholic acid = 3α, 7α-dihydroxy-5β-cholan-24-oic acid.

for $C_{30}H_{50}O_6$: C, 71.11; H, 9.95. Found: C, 70.57; H, 9.63.

Recrystallization of 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI) from acetone-water (2:1, v/v) gave fine crystals 210 mg (16%) with the following properties: mp 127-128 C, single spot on TLC, R_f = 0.15 (solvent system BE-1). Gas liquid chromatographic analysis (as the TMSi ether derivative); relative retention time = 16.0. IR (KBr disc), 3460 (OH), 2945, 2875, 1728, 1372, 1245, 1136, and 1062 cm^{-1}. The mass spectral fragments of this diol (VI) are m/e 650, M^+; 547, M-(103); 487, M-(103+60); 472, M-(103+60+15); 427, M-(103+2x60); 397, M-(103+60+90); 355, M-(103+90+60+42) (base peak); 337, M-(103+2x60+90); 255, M-(2x60+side chain); 253, M-(2x60+side chain+2H); 219.

24-Nor-5β-cholestane-3α,7α 25,26-tetrol

One hundred mg of 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI) was refluxed for 2 hr with 10% methanolic potassium hydroxide. The hydrolysate was poured into water and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4, and evaporated. The residue was crystallized from ethyl acetate-hexane (3:7, v/v) and recrystallized from acetone-water (1:2, v/v) yielding white needles with the following properties: mp 182.5-183 C, single spot on TLC, R_f = 0.22 (solvent system EA-1). Gas liquid chromagographic analysis (as the TMSi derivative); relative retention time = 3.22. IR (KBr disc), 3400, 2930, 2865, 1456, 1380, 1073, 975 cm^{-1}.

The mass spectrum of 24-nor-5β-cholestane-3α,7α,25,26-tetrol (VIII) showed major peaks at m/e 404, M-18; 386, M-(2x18); 368, M-(3x18); 353, M-(3x18+15); 335, M-(4x18+15); 273, M-(side chain+18); 255 (base peak), M-(side chain+2x18). The mass spectral fragments of the TMSi derivative of the tetrol (VIII) are m/e 710, M^+; 607, M-(103); 517, M-(103+90); 427, M-(103+2x90); 350, M-(4x90); 337, M-(103+3x90); 255, M-(side chain+2x90); 253, M-(side chain+2x90+2H); 243, 219, 173 (base peak).

(23α) and (23β)24-Nor-5β-cholestane-3α,7α,23,25-tetrols

To 300 mg of 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) was added 40 ml of 10% methanolic KOH. The reaction mixture was refluxed for 2 hr and then poured into water. The solution was extracted with ethyl acetate; the extract was washed with water and dried over Na_2SO_4.

The white amorphous residue was separated into two C-23 isomers using alumina column chromatography by eluting with increasing amounts of methanol in acetone-ethyl acetate (1:1, v/v). Fractions eluted with 2% methanol contained 24-nor-5β-cholestane-3α,7α,23β,25-tetrol (VII-b). Crystallization of tetrol (VII-b) from methanol-water (1:2, v/v) gave white needles (90 mg), mp 189.5-190.5 C; single spot on TLC, R_f = 0.42 (solvent system EA-1). IR (KBr disc), 3420 (OH), 2930, 1460, 1370, 1070, 993, 972, 893, 850 cm^{-1}. Optical rotation $[\alpha]_D^{25} = 30°$. Gas liquid chromatographic analysis (as TMSi derivative): relative retention time = 3.25. The mass spectral fragments of the TMSi derivative of 24-nor-5β-cholestane-3α,7α,23β,25-tetrol (VII-b) are m/e 579, M-(131); 530, M-(2x90); 489, M-(131+90); 440, M-(3x90); 399, M-(131+2x90); 384, M-(131+2x90+15); 309, M-(131+3x90); 253, M-(side chain+2x90+2H); 243, 131 (base peak). The mass spectrum of underivatized (VII-b) obtained by direct insertion exhibited major peaks at m/e 386, M-(2x18); 371, M-(2x18+15); 368, M-(3x18); 353, M-(3x18+15); 345, M-(18+59); 273, M-(side chain+18); 255 (base peak), M-(side chain+2x18). Analysis calculated for $C_{26}H_{46}O_4 \cdot 1/2H_2O$: C, 72.35; H, 10.98. Found: C, 72.30; H, 11.13.

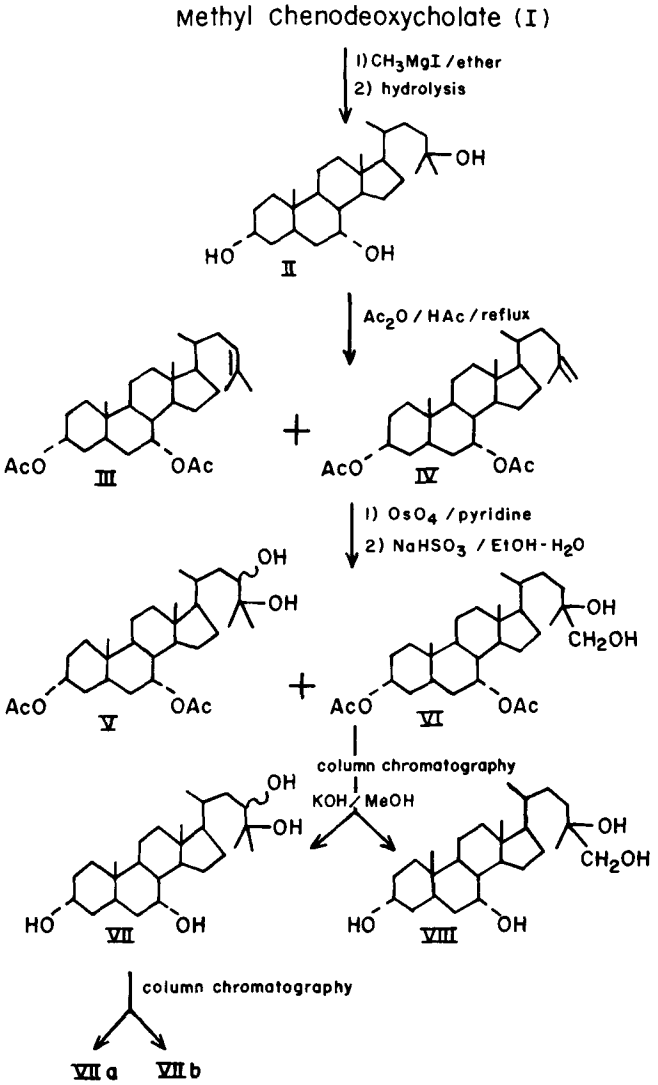

FIG. 1. Synthesis of 24-nor-5β-cholestane-3α,7α,23α,25-tetrol; 24-nor-5β-cholestane-3α,7α,23β,25-tetrol; and 24-nor-5β-cholestane-3α,7α,25,26-tetrol from methyl chenodeoxycholate. II, 24-nor-5β-cholestane-3α,7α,25-triol; III, 24-nor-5β-cholest-23-ene-3α,7α-diacetate; IV, 24-nor-5β-cholest-25-ene-3α,7α-diacetate; V, 24-nor-5β-cholestane-3α,7α-diacetoxy-23,25-diol; VI, 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol; VII, 24-nor-5β-cholestane-3α,7α,23ξ,25-tetrol; VII-a, 24-nor-5β-cholestane-3α,7α,23α,25-tetrol; VII-b, 24-nor-5β-cholestane-3α,7α,23β,25-tetrol; VIII, 24-nor-5β-cholestane-3α,7α,25,26-tetrol.

Further elution with 5% methanol in acetone-ethyl acetate yielded 24-nor-5β-cholestane-3α,7α,23α,25-tetrol (VII-a). Crystallization of 24-nor-5β-cholestane-3α,7α,23α,25-tetrol (VII-a) from acetone-water (2:1, v/v) gave white crystalline flakes (30 mg) with the following properties: mp 182.5-183 C, single spot on TLC, R_f = 0.35 (solvent system EA-1). IR (KBr disc), 3400, 2930, 1458, 1370, 1070, 992, 970, 891, 848 cm^{-1}. Optical rotation $[\alpha]_D^{25} = -9°$. Gas liquid chromatographic analysis (as TMSi derivative): relative retention time=3.26. The mass spectral fragments of the TMSi derivative of this tetrol are m/e 579, 489, 440, 399, 384, 309, 253, 243, and 131 (fragments similar to VII-b).

The mass spectrum of the underivatized tetrol (VII-a) obtained by direct insertion was similar to the spectrum of VII-b and had major peaks at m/e 386, M-(2x18); 371, M-(2x18+15); 368, M-(3x18); 353, M-(3x18+15); 345, M-(18+59); 273, M-(side chain+18); 255 (base peak), M-(side chain+2x18). Analysis calculated for $C_{26}H_{46}O_4$: C, 73.89; H, 10.97. Found: C, 73.80; H, 10.96.

TABLE I

Molecular Rotation of Tetrol Epimers VII-a and VII-b

Steroid	$[\alpha]_D^{25}$	M_D	ΔM_D
24-Nor-5β-cholestane-3α,7α,25-triol	+10°	+40	---
VII-b	+30°	+127	+87
VII-a	-9°	-38	-78

RESULTS AND DISCUSSION

This paper describes the synthesis and mass spectra of the following C_{26} steroid alcohols: 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol: 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol; (23α) and (23β)24-nor-5β-cholestane-3α,7α,23,25-tetrols; and 24-nor-5β-cholestane-3α,7α,25,26-tetrol. These compounds are analogs of certain C_{27}-bile alcohols.

24-Nor-5β-cholestane-3α,7α,25-triol (II, Fig. 1) was synthesized from methyl chenodeoxycholate (I) by a Grignard reaction (9). When the C_{26}-triol (II) (Fig. 1) was refluxed for 20 hr with glacial acetic acid-acetic anhydride (3:2, v/v), a mixture of 24-nor-5β-cholest-23-ene-3α,7α-diacetate (III) and 24-nor-5β-cholest-25-ene-3α,7α-diacetate (IV) was obtained. These compounds were not separated at this point but were oxidized with osmium tetroxide for the preparation of 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) and 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI). The two diacetoxy compounds, V and VI, were easily separated by column chromatography on silica gel.

The mass spectrum of the TMSi derivative of 24-nor-5β-cholestane-3α,7α-diacetoxy-23ξ,25-diol (V) (eluted from the column before diol VI) showed fragments at m/e 592, M-(43+15); 532, M-(43+15+60); 459, M-(131+60); 399, M-(131+2x60). The loss of 60 amu was attributed to the loss of acetic acid, while 43 represented loss of a $CH_3C{=}O$ group. The base peak at m/e 131 was due to the scission of the C-23,25 bond containing a TMSiOH group (90 amu) on carbon 25. The mass spectrum of this compound (V) was also obtained by direct insertion without derivatization. It showed fragment ions at m/e 446, 428, 386, and 368. The loss of 18 amu and 60 amu was due to the losses of water and acetic acid, respectively.

The mass spectrum of the TMSi derivative of 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI), the second compound to be eluted from the column, showed a series of fragments at m/e 547, 487, 427, 397, and 337. The loss of 103 was attributed to splitting between the C-25,26 bond of a diol containing a TMSiOH group on terminal carbon 26, similar to that observed for the TMS: derivative of 5β-cholestane-3α,7α,12α,15,16-pentol (7). The base peak at m/e 355 may arise from loss of C_3H_6 from the side chain of the fragment with m/e 397. A fragment m/e 42 was seen in the spectrum. The peak seen at m/e 255 was characteristic of a dihydroxy bile acid (10).

Further characterization of the diols (V and VI) was accomplished by infrared spectroscopy. The bands present at 3460 cm^{-1} and between 1200 and 1000 cm^{-1} were characteristic of the alcohol functional group (11). The band at 1245 cm^{-1} was characteristic of the acetate moiety (12). Bands at 2950 and 1373 cm^{-1} were characteristic of the CH_3-C=O group (13).

24-Nor-5β-cholestane-3α,7α,25,26-tetrol (VIII) was formed by hydrolyzing 24-nor-5β-cholestane-3α,7α-diacetoxy-25,26-diol (VI) in a methanolic solution of 10% KOH. The mass spectrum of the TMSi derivative of the tetrol (VIII) showed a series of fragments at m/e 607, 517, 427, and 337. The peak at m/e 607 was attributed to the cleavage between carbon atoms 25 and 26 of a tetrol containing a TMSiOH group on C-26. The remainder of the peaks were attributed to successive loss of the TMSiOH groups from the molecule (7). The peak at m/e 173 may arise from fragmentation of the side chain (m/e 275) by loss of the $CH_2\overset{+}{O}$-$SiMe_3$ moiety (m/e 103) with transfer of a proton from the steroid nucleus. This may also lead to the appearance of the peak at m/e 253. The infrared spectrum revealed bands at 3400, 2930 and 1073, 1456 and 1380 cm^{-1}. These were characteristic of the alcohol and methyl groups, respectively.

Treatment of 24-nor-5β-cholestane-3α,7α-diacetoxy-23,25-diol (V) with 10% methanolic KOH solution afforded a 1:1 mixture of the 23-epimeric 24-nor-5β-cholestane-3α,7α,23ξ,25-tetrols (VII-a and VII-b). Separation of these epimers was achieved using alumina column chromatography. The infrared spectrum and the mass spectrum of the tetrol VII-a were very similar in character to that of the tetrol VII-b. This suggested the former was the C-23 epimer of the latter (14).

The mass spectrum of the TMSi derivative of 24-nor-5β-cholestane-3α7α,23α,25-tetrol (VII-a) showed a base peak at m/e 131, which is based on the scission of the C-23,25 bond, containing a TMSiOH (90 amu) group on C-25 (15). An important series of peaks was present at m/e 579, 489, 399, and 309. These arose from the loss of the base peak, together with successive losses of TMSiOH from the parent molecule. The peak at m/e 243 is characteristic of a 3,7-bis (TMSi) structure (16).

Optical rotation measurements afforded an interesting comparison of the various bile alcohols. The molecular rotation of 24-nor-5β-cholestane-3α,7α,23ξ,25-tetrol (VII) can be considered to be made up of the molecular rotation of 24-nor-5β-cholestane-3α,7α,25-triol (II) and the molecular rotation due to the additional asymmetric center of C-23. The molecular rotations of the two epimers were quite different as seen in Table I. 24-Nor-5β-cholestane-3α,7α,23β,25-tetrol (VII-b) had a molecular rotation +127, an increase of +87, caused by the presence of the C-23-hydroxyl group in triol II. This was similar to the molecular rotation difference (+60) found between C_{27} 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,23β,25-pentol (14,17,18). The C_{27} compounds, however, cannot be used in assigning the configuration of the C_{26}-analogs since the proximity of the C_3-terminal isopropyl group is different. Consequently, on the basis of mobility of the C_{26}-analogs on TLC, we tentatively assigned the more polar compound (VII-a) as the α epimer and the less polar compound as the β-epimer.

The C_{26} compounds synthesized here will be used not only as potential enzyme inhibitors but also as model substances in studies of certain steps in the side-chain oxidation and of 12α-hydroxylation of bile acid precursors in: (a) perfused rabbit liver, and (b) in vitro microsomal and mitochondrial incubation studies.

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REFERENCES

1. Staple, E., and J.L. Rabinowitz, Biochim. Biophys. Acta 59:735 (1962).
2. Cronholm, T., and G. Johansson, Eur. J. Biochem. 16:373 (1970).

3. Danielsson, H., in "The Bile Acids: Chemistry, Physiology, and Metabolism," Edited by P.P. Nair and D. Kritchevsky, Plenum Press, New York, 1973, p. 1.
4. Shefer, S., F.W. Cheng, B. Dayal, S. Hauser, G.S. Tint, G. Salen, and E.H. Mosbach, J. Clin. Invest. 57:897 (1976).
5. Cohen, B.I., T. Kuramoto, M.A. Rothschild, and E.H. Mosbach, J. Biol. Chem. 251:2709 (1976).
6. Dayal, B., S. Shefer, G.S. Tint, G. Salen, and E.H. Mosbach, J. Lipid Res. 17:478 (1976).
7. Kuramoto, T., B.I. Cohen, and E.H. Mosbach, Anal. Biochem. 71:481 (1976).
8. Hoshita, T., J. Biochem. 52:176 (1962).
9. Ishihara, J., Ibid. 27:265 (1938).
10. Elliott, W.H., in "Biochemical Applications of Mass Spectrometry," Edited by G.R. Waller, John Wiley and Sons, New York, 1971 p. 291.
11. Bellamy, L.J., in "The Infra-Red Spectra of Complex Molecules," John Wiley and Sons, New York, 1958, p. 110.
12. Bellamy, L.J., Ibid. p. 189.
13. Bellamy, L.J., Ibid. p. 21.
14. Hoshita, T., M. Yasuhara, K. Kihira, and T. Kuramoto, Steroids 27:647 (1976).
15. Cohen, B.I., G.S. Tint, T. Kuramoto, and E.H. Mosbach, Ibid. 25:365 (1975).
16. Sjövall, J., P. Eneroth, and R. Ryhage, in "The Bile Acids: Chemistry, Physiology, and Metabolism," Edited by P.P. Nair and D. Kritchevsky, Plenum Press, New York, 1971, p. 225.
17. Entwistle, N., and A.D. Pratt, Tetrahedron 25:1499 (1969).
18. Shefer, S., B. Dayal, G.S. Tint, G. Salen, and E.H. Mosbach, J. Lipid Res. 16:280 (1975).

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