C_{26}-Analogs of naturally occurring C_{27} bile alcohols

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Abstract C_{26} Bile alcohols of the 24-nor-5β-cholestane series were prepared starting from methyl cholate. A Grignard reaction of methyl magnesium iodide with methyl cholate yielded 24-nor-5β-cholestane-3α,7α,12α,25-tetrol which was dehydrated to form a mixture of 24-nor-5β-cholest-23-ene-3α,7α,12α-triol and the corresponding Δ^{25} compound. Oxidation of the former with OsO_4 yielded 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol, while catalytic hydrogenation of a mixture of the Δ^{23} and Δ^{25} triols resulted in the formation of 24-nor-5β-cholestane-3α,7α,-12α-triol. The structures of these new compounds were confirmed by infrared and nuclear magnetic resonance spectrometry and by gas–liquid chromatography–mass spectrometry.

Bile alcohols are polyhydroxy C_{27} sterols that serve as intermediates in the biosynthesis of cholic acid and chenodeoxycholic acid from cholesterol (1, 2). It is currently assumed that the major pathway of side chain degradation of cholesterol involves 26-hydroxylated bile alcohols (3, 4), but a possible alternate route of cholic acid synthesis involving 25-hydroxylation of the side chain was recently proposed by Shefer, et al. (5). Since the naturally occurring C_{27} bile alcohols are relatively difficult to synthesize, it was thought that their more easily accessible C_{26} analogs might be useful as model compounds or inhibitors for enzymatic studies. The present paper describes the syntheses of 24-nor-5β-cholestane-3α,7α,12α-triol, 24-nor-5β-cholestane-3α,7α,12α,25-tetrol, and 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (**Fig. 1**) and their characterization by physical-chemical methods.

EXPERIMENTAL

Physical Measurements

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, Iowa), model MP-126000, and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer (Norwalk, Conn.) model 421 grating spectrophotometer as KBr discs. Absorption frequencies are quoted in reciprocal centimeters.

NMR spectra, in Hertz, were obtained in deuterated chloroform ($CDCl_3$) and dimethylsulfoxide (CD_3SOCD_3) solution using a JEOL (Medford, Mass.) PS-100 spectrometer equipped with Fourier transform capability.

GLC. The bile alcohols, as the TMSi derivatives, were analyzed on a 180 cm × 4 mm column packed with 3% QF-1 on 80/100 mesh Gas Chrom Q; column temp. 230°C (Hewlett-Packard model 7610 gas chromatograph) (Hewlett-Packard, Palo Alto, Cal.) (**Table 1**).

Mass spectra of the bile alcohols were obained with a Varian MAT-111 gas chromatograph–mass spectrometer (Varian Associates, Palo Alto, Ca.) at an ion source pressure of $2–3 \times 10^6$ mm and an electron energy of 70 eV, as described previously (6).

TLC. The bile alcohols were separated on silica gel G plates (Brinkmann Instruments, Westbury, N.J., 0.25 mm thickness), with the solvent system, chloroform–acetone–methanol 35:25:7.5 (v/v). The spots were made visible either with iodine, or with phosphomolybdic acid (3.5% in isopropanol) plus concentrated sulfuric acid (Table 1).

24-Nor-5β-cholestane-3α,7α,12α,25-tetrol (II, Fig. 2)

Compound (II) was prepared from methyl cholate (I) by a Grignard reaction. Methyl cholate (5.7 g) dissolved in dry benzene (160 ml) was added to 90

Abbreviations: CTX, cerebrotendinous xanthomatosis; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; NMR, nuclear-magnetic resonance; PMR, proton-magnetic resonance; IR, infrared.

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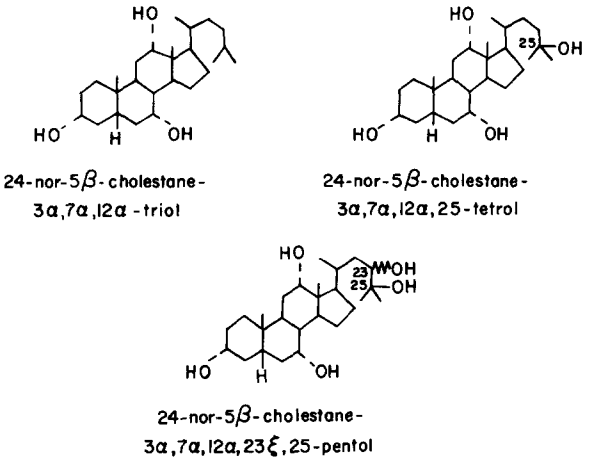

Fig. 1. Structures of 24-nor-bile alcohols.

ml of 2 M methyl magnesium iodide dissolved in dry ethyl ether (Alfa Inorganics, Inc., Ventron Corp., Beverly, Mass.). This reaction was carried out according to the procedure of Dayal et al. (7) and Pearlman (8). The crude nor-tetrol (4.8 g) was recrystallized from ethyl acetate/methanol and from methanol (yield: 83.3%). The crystals had a mp of 119–121°C. After repeated crystallization from acetone the mp was 124–126°C (literature reports values of 126–130°C (9); 177–177.5°C (10); 184–185°C (9)). IR (KBr disc) 3400 cm^{-1} (OH) (II, Fig. 3); NMR ($CDCl_3$) (Fig. 4; Table 2): δ 0.65 (s, 3H, 18-CH_3), 0.85 (s, 3H, 19-CH_3), 0.95 (d, J = 6 Hz, 3H, 21-CH_3), 1.16 (s, 6H, 26-CH_3 + 27-CH_3); mass spectrum (TMSi derivative): base peak at *m/e* 131 resulting from the scission of the C-23, C-25 bond that is α to the terminal trimethylsiloxyl group (6). A peak was detected at *m/e* 695 (M − 15) and two prominent series of peaks were observed, one at *m/e* 620 (M − 90), 530 (M − 2 × 90), 440 (M − 3 × 90) and a second at *m/e* 564 (M − 146), 474 [M − (90 + 146)], 384 [M − (2 × 90 + 146)] and 294 [M − (3 × 90 + 146)].

24-Nor-5β-cholest-23-ene-3α,7α,12α-triol (III, Fig. 2) and 24-nor-5β-cholest-25-ene-3α,7α, 12α-triol (IV)

These triols (III and IV) were synthesized using a procedure similar to that of Dayal et al. (7). Nor-tetrol (II) (3.06 g), 100 ml of acetic acid, and 70 ml of acetic anhydride were refluxed for 24 hr. The cooled solution was concentrated in vacuo and the residue was treated with 200 ml of ice-cold water and the white precipitate was collected.

Two grams of the triacetoxy mixture were hydrolyzed in a 60°C water bath with 100 ml of 6% methanolic KOH for 3 hr. The hydrolyzate was

TABLE 1. GLC and TLC of C_{26} and C_{27} bile alcohols

Compound(s)	R_f[a]	RRT[b]
V 24-Nor-5β-cholestane-3α,7α,12α-triol	0.67	1.17
III 24-Nor-5β-cholest-23-ene-3α,7α,12α-triol and IV 24-Nor-5β-cholest-25-ene-3α,7α,12α-triol	0.67	1.31
II 24-Nor-5β-cholestane-3α,7α,12α,25-tetrol	0.36	1.95
VI 24-Nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol	0.33	2.61
5β-Cholestane-3α,7α,12α-triol	0.70	1.50
5β-Cholestane-3α,7α,12α,25-tetrol	0.45	2.52
5β-Cholest-24-ene-3α,7α,12α-triol and 5β-Cholest-25-ene-3α,7α,12α-triol	0.70	1.60
5β-Cholestane-3α,7α,12α,24ξ,25-pentol	0.23	3.40

[a] Solvent system: chloroform–acetone–methanol 70:50:15 (v/v/v). Silica gel G plates, 0.25 mm thickness (Brinkmann).

[b] RRT is retention time of the TMSi derivative of the compound relative to 5α-cholestane on 3% QF-1 (1.95 min.) at a column temperature of 230°C.

poured into a beaker with crushed ice, stirred, and the white precipitate was collected. A mixture of Δ^{23} and Δ^{25} triol (III and IV) (230 mg) was separated on a 2 × 40 cm column, containing 40 g of 25% $AgNO_3$/silicic acid (11). The products were eluted with increasing amounts of ethyl acetate in benzene. Pure Δ^{23} triol was eluted with 70% ethyl acetate in benzene and Δ^{25} triol with 80% ethyl acetate in benzene. Column separations were monitored by TLC on silica gel G plates (Analtech, Newark, Del.) impregnated with $AgNO_3$ [0.25 mm layer thickness; solvent system: benzene–ethyl acetate 75:25 (v/v)]. Spots were made visible by spraying the plates with water. R_f values: Δ^{23} compound, R_f 0.5; Δ^{25} compound, R_f 0.4.

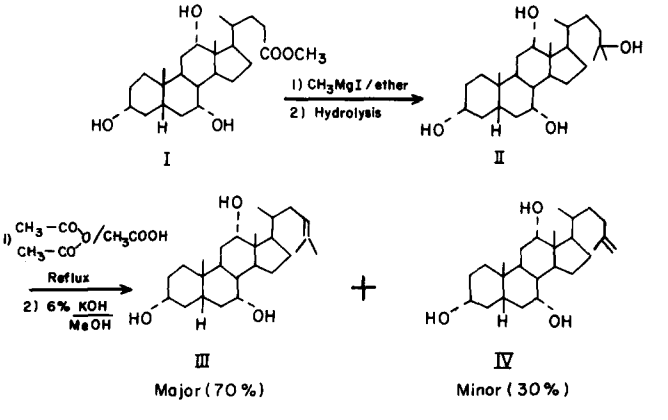

Fig. 2. Synthesis of 24-nor-5β-cholestane-3α,7α,12α,25-tetrol, 24-nor-5β-cholest-23-ene-3α,7α,12α-triol and 24-nor-5β-cholest-25-ene-3α,7α,12α-triol. I, Methyl cholate; II, 24-nor-5β-cholestane-3α,7α,12α,25-tetrol; III, 24-nor-5β-cholest-23-ene-3α,7α,-12α-triol; IV, 24-nor-5β-cholest-25-ene-3α,7α,12α-triol.

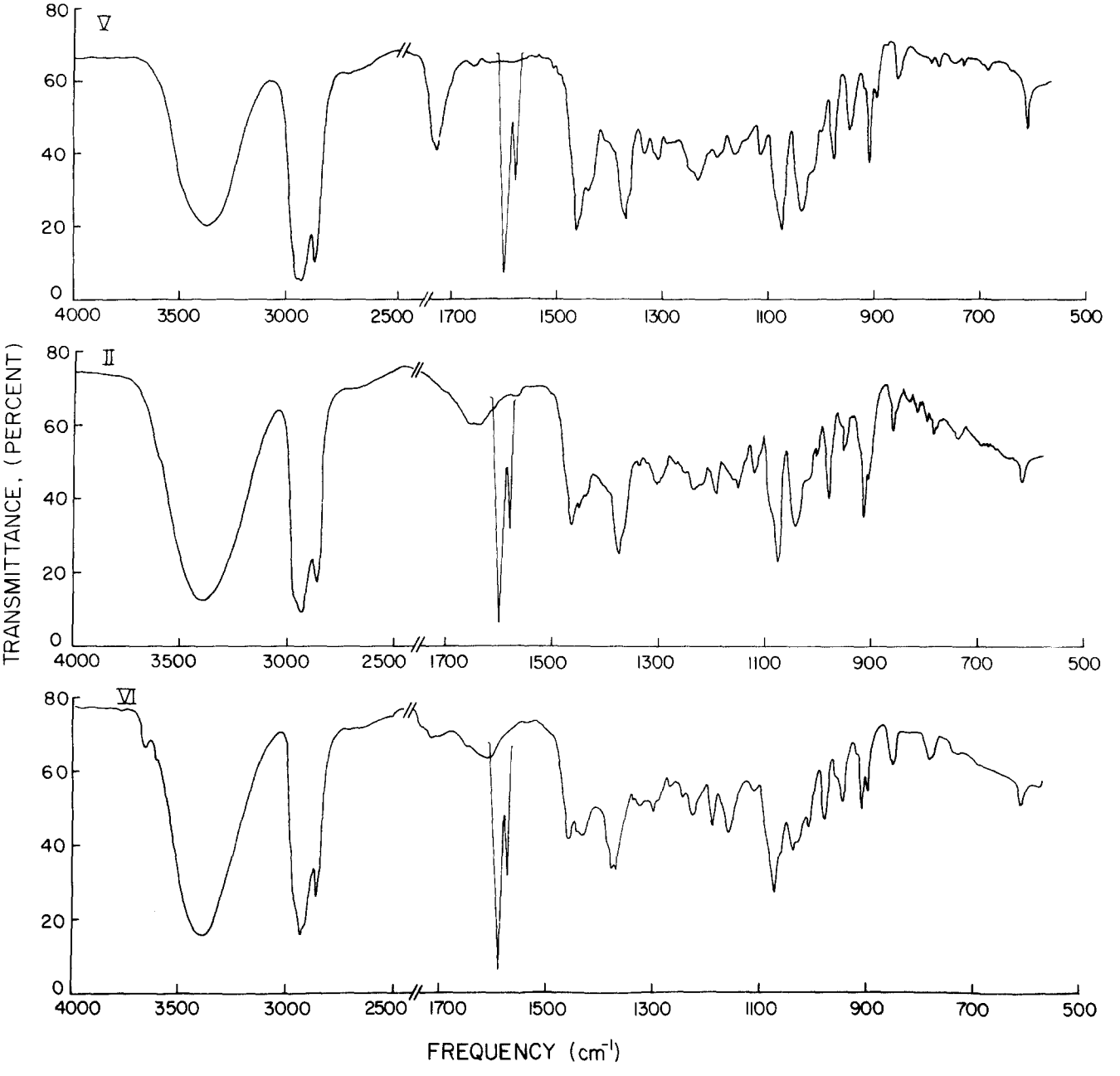

Fig. 3. Infrared spectra of 24-nor-5β-cholestane-3α,7α,12α-triol (V); 24-nor-5β-cholestane-3α,7α,12α,25-tetrol (II); and 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (VI).

Δ^{23} *Triol* was crystallized from acetone (mp 190–191°C; yield: 47%); IR (KBr disc) 3375 cm^{-1} (OH), 1365–1395 cm^{-1} (C = C). NMR ($CDCl_3$) δ 0.68 (s, 3H, 18-CH_3), 0.88 (s, 3H, 19-CH_3), 0.94 (d, J = 6 Hz, 21-CH_3), 1.60 (s, 3H, 26-CH_3), 1.68 (s, 3H, 27-CH_3). The mass spectrum of the TMSi derivative (Fig. 5) exhibited a strong peak at *m/e* 253 and a base peak at 96. Other major fragments were at *m/e* 530, 440, and 350; at *m/e* 461, 371, and 281; at *m/e* 564, 474, 384, and 294; and at *m/e* 481, 391, 301, and 211. There were also very intense peaks at *m/e* 254 and 344.

Δ^{25} *Triol* (IV, Fig. 2), crystallized from acetone, gave a mp of 166–169°C (yield: 31.3%); IR (KBr disc) 3375 cm^{-1} (OH) and a characteristic peak at 883 cm^{-1} of the end methylene group. Mass spectrum of the TMSi derivative (Fig. 6) exhibited important fragments at *m/e* 530 (M − 90), 440 (M − 2 × 90), and 350 (M − 3 × 90) and a prominent series of peaks at *m/e* 461, 371, and 281 arising from scission between C-20 and C-22. A significant molecular ion peak at *m/e* 620 and a fragmentation series at *m/e* 481, 391, 301, and 211 were apparently due to the loss of the side chain plus the entire D ring (12, 13).

24-Nor-5β-cholest-23-ene-3α,7α,12α-triacetate

This compound was prepared according to the procedure of Ikan, Markus, and Goldschmidt (14). One gram of 24-nor-5β-cholestane-3α,7α,12α,25-tetrol (Fig. 1) was refluxed for 20 hr with 50 ml acetic acid and 5 ml of acetic anhydride. The cooled solution was concentrated in vacuo and the residue was treated with ice-cold water. The oily product was extracted with benzene and evaporated, and the residue was used for the preparation of 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (Fig. 7).

24-Nor-5β-cholestane-3α,7α,12α-triol (V, Fig. 7)

A mixture of unsaturated sterols (200 mg) obtained as described in Section B above dissolved in 30 ml of ethyl acetate was hydrogenated for 12 hr at 25°C with a platinum-on-carbon catalyst (35 mg). The catalyst was filtered off and the solvent was evaporated. Crystallization of the resulting compound from ethyl acetate gave white crystalline material (130 mg; yield: 65%), mp 182–184°C; IR (KBr disc) 3370 cm^{-1} (OH) and three distinct peaks in the 1360–1380 cm^{-1} region (V, Fig. 3). NMR ($CDCl_3$) (Fig. 4; Table 2): δ 0.64 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 0.96 (d, J = 6 Hz, 3H, 21-CH_3), 0.84 (d, J = 6 Hz, 3H, 26-CH_3), 0.92 (d, J = 6 Hz, 3H, 27-CH_3). Mass spectrum (TMSi derivative): molecular ion at *m/e* 622; other major series of lines arising from the successive losses of trimethylsilanol from the molecular ion are at *m/e* 532 (M − 90), 442 (M − 2 × 90), 352 (M − 3 × 90) and for the eight carbon side chain analog at *m/e* 546, 456, and 366. Other prominent peaks were seen at *m/e* 461, 371, and 281, and *m/e* 343 and 433.

24-Nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (VI, Fig. 7)

24 - Nor - 5β - cholest - 23 - ene - 3α,7α,12α - triacetate (400 mg) was carefully dried and dissolved in 30 ml of anhydrous ethyl ether and 2 ml of anhydrous pyridine. Osmium tetroxide (0.5 g) dissolved in 6 ml of anhydrous ethyl ether was added, the solution was stoppered and left for 60 hr. The reaction mixture was worked up according to Dayal et al. (7). The crude pentol (272 mg) was purified by column chromatography on neutral alumina V with increasing amounts of methanol in ethyl acetate (15). The fractions were monitored by TLC on silica gel G plates of 0.25 mm thickness (Brinkmann) solvent chloroform–acetone–methanol 70:50:15 (v/v/v). The fractions eluted with 7–12% methanol in ethyl acetate contained 207 mg of 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol. Crystallization from ethyl acetate gave a white crystalline material, mp 172–175°C (yield: 52%); IR (KBr disc) 3390 cm^{-1} (OH)

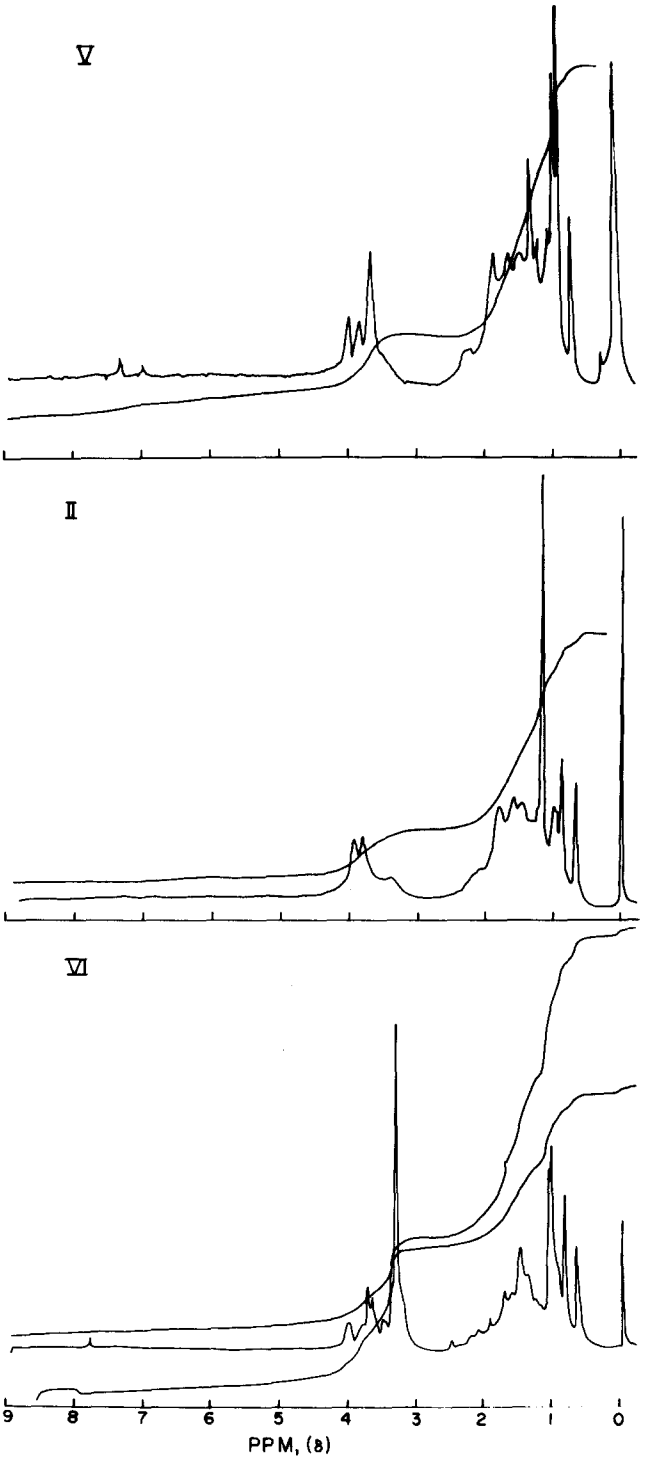

Fig. 4. 100 MHz PMR spectra (in $CDCl_3$) of 24-nor-5β-cholestane-3α,7α,12α,25-tetrol (II); 24-nor-5β-cholestane-3α,7α,-12α-triol (V); and 100 MHz PMR spectrum [in $CDCl_3$ + DMSO (d_6)] of 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (VI). The strong singlet signal in the region of δ 3.2 is due to the solvent DMSO used to obtain the spectrum of (VI).

(VI, Fig. 3); NMR [$CDCl_3$ + DMSO (d_6)] (Fig. 4; Table 2): δ 0.68 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 0.99 (d, J = 6 Hz, 3H, 21-CH_3), 1.04 (s, 3H, 26-CH_3), 1.08 (s, 3H, 27-CH_3). Mass spectrum of TMSi

TABLE 2. PMR Spectra of C_{26} bile alcohols

Compound	C-18	C-19	C-21	C-26	C-27
	Hz	*Hz*	*Hz*	*Hz*	*Hz*
24-Nor-5β-cholestane-3α,7α,12α-triol[a]	64	84	96	84	92
24-Nor-5β-cholestane-3α,7α,12α,25-tetrol[a]	65	85	95	116	116
24-Nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol[b]	68	84	99	104	108

[a] Solvent $CDCl_3$.
[b] Solvent [$CDCl_3$ + DMSO(d_6)].

derivative (Fig. 8): a prominent peak at *m/e* 131 arises from rupture of the C-23, C-25 bond (6). There were series of lines at *m/e* 667 (M − 131), 577 (M − 131 − 90), 487 (M − 131 − 2 × 90), 397 (M − 131 − 3 × 90) and 307 (M − 131 − 4 × 90). The other prominent series arises from the successive loss of trimethysilanol or trimethylsiloxyl (TMSi = 89 amu) groups and can be seen at *m/e* 618 (M − 2 × 90), 529 (M − 2 × 90 − 89), 439 (M − 3 × 90 − 89), and 349 (M − 4 × 90 − 89).

RESULTS AND DISCUSSION

This paper describes the synthesis of the following C_{26}-steroid alcohols: 24-nor-5β-cholestane-3α,7α,-12α-triol; 24-nor-5β-cholestane-3α,7α,12α,25-tetrol and 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (Fig. 1). These compounds are analogs of certain C_{27}-bile alcohols that are potential intermediates on the pathway leading from cholesterol to cholic acid. The nor-compounds will be used as model substances in studies of certain steps in the side chain oxidation of cholesterol.

24-Nor-5β-cholestane-3α,7α,12α,25-tetrol (II, **Fig. 2**) was synthesized from methyl cholate by a Grignard reaction. Formation of the tetrol (II) was monitored by its infrared spectrum (**Fig. 3**). The reaction was complete when the ester carbonyl absorption at 1730 cm^{-1} of methyl cholate completely disappeared. The product had a strong (OH) absorption at 3400 cm^{-1} as compared to that at 3320 cm^{-1} in 5β-cholestane-3α,7α,12α,25-tetrol (C_{27}-series) (7). The NMR spectrum of the C_{26}-tetrol (**Fig. 4**; **Table 2**) was very similar to that of its C_{27}-analog (15), except for the chemical shift differences in the resonance of the C-18, C-19, C-21 and C-26/27 methyls. The mass spectrum of the TMSi derivative of the C_{26}-sterol was very similar to that of its C_{27}-analog, 5β-cholestane-3α,7α,12α,25-tetrol (6), except for a shift of 14 amu for the high field fragments containing the entire side chain. The base peak of both sterols at *m/e* 131 results from the scission of the carbon–carbon bond α to the terminal trimethylsiloxyl group (6). Although the molecular ion, M^+, was not detected in the spectrum of the C_{26}-tetrol, a peak at *m/e* 695 (M − 15) arising from the loss of a methyl group was observed. A different fragmentation process visible in the 24-nor-5β-cholestane-3α,7α,12α,25-tetrol, and which was not observed in its C_{27}-analog, resulted in the following peaks: *m/e* 564 (M − 146), 474 [M − (90 + 146)], 384 [M − (2 × 90 + 146)] and 294 [M − (3 × 90 + 146)].

When the C_{26}-tetrol (II, Fig. 2) was refluxed for 12

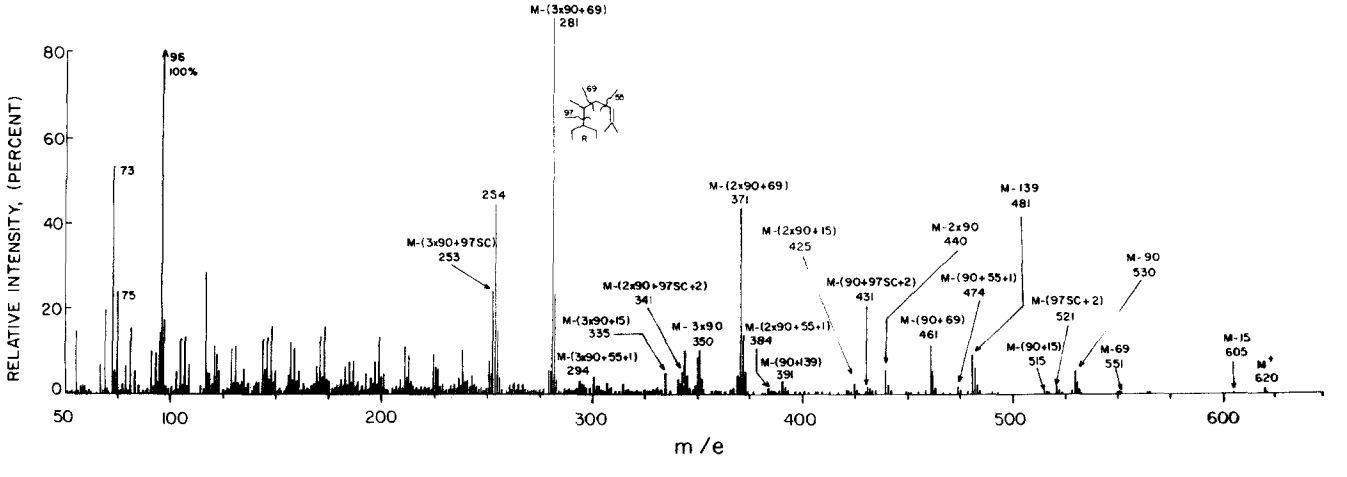

Fig. 5. Mass spectrum of 24-nor-5β-cholest-23-ene-3α,7α,12α-triol (III) TMSi-ether. SC = side chain.

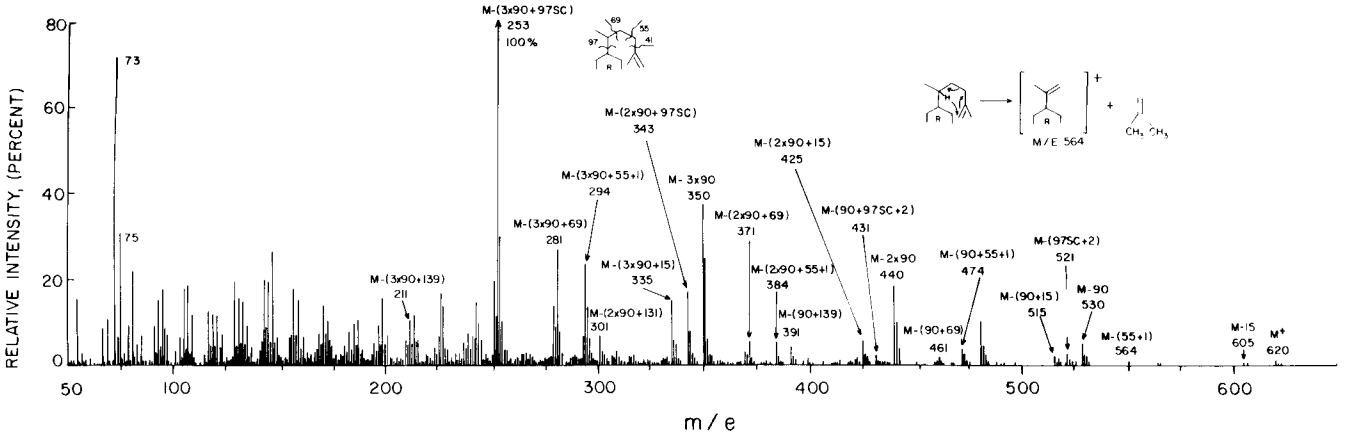

Fig. 6. Mass spectrum of 24-nor-5β-cholest-25-ene-3α,7α,12α-triol (IV) TMSi-ether. SC = side chain.

hr with glacial acetic acid and acetic anhydride (3:2, v/v) (7) a mixture of 24-nor-5β-cholest-23-ene-3α,7α,12α-triol and 24-nor-5β-cholest-25-ene-3α,7α,-12α-triol was obtained. On the other hand, when the C_{26}-tetrol was refluxed for 20 hr with a different ratio of glacial acetic acid to acetic anhydride (10:1, v/v) only the Δ^{23}-triacetoxy-nor-triol was obtained (14). This product was utilized for the preparation of the 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol without further purification.

According to Scallen and Krueger (16), introduction of a Δ^{24}-bond in C_{27}-steroids produces a weakening or disappearance of one of the three major peaks in the 1365–1395 cm^{-1} region of the IR spectrum. This was attributed to restriction of the free rotation at C-24. Similarly, in the infrared spectrum of the 24-nor-5β-cholest-23-ene-3α,7α,12α-triol the peaks at 1365–1395 cm^{-1} were absent as compared to the 24-nor-5β-cholestane-3α,7α,12α-triol (Fig. 3). The infrared spectrum of the Δ^{25}-nor-triol had a peak at 883 cm^{-1}, which is characteristic of the end methylene group. Nuclear magnetic resonance spectra allowed unambiguous assignment of the potentially important Δ^{23}-bond. Introduction of this double bond caused the appearance of peaks at δ 1.60 and 1.68 associated with C-26, C-27 isopropylidene methyls, while C_{26}-saturated steroids of the series possess peaks at δ 0.84 and 0.92 associated with the C-26 and C-27 gem-dimethyls. Small peaks due to the protons attached to the rings at C-12, C-7 and C-3 were noted at δ 3.9, 3.75 and 3.4, signifying that under the reaction conditions used there was no dehydration at the 3, 7 or 12 positions of the steroid nucleus. There were characteristic differences in the mass spectra of the two unsaturated Δ^{23}- and Δ^{25}-nor-triols.

The mass spectrum of the TMSi derivative of 24-nor-5β-cholest-23-ene-3α,7α,12α-triol (**Fig. 5**) is characterized by a base peak at *m/e* 96 and a molecular ion peak at *m/e* 620; while the mass spectrum of the TMSi derivative of 24-nor-5β-cholest-25-ene-3α,7α,12α-triol (**Fig. 6**) has a base peak at *m/e* 253.

The major difference between the two spectra is governed by two important bond fissions. Allylic cleavage dominates the mass spectrum of 24-nor-5β-cholest-23-ene-3α,7α,12α-triol (III), which gives fragments at *m/e* 551, 461, 371, and 281. In the case of the Δ^{25} isomer, the ions *m/e* 564, 474, 384, and 294 are attributed to the rupture of the bond between carbons 22 and 23 (loss of 55 amu) together with the transfer of a hydrogen atom (1 amu) from the charged fragment. Mechanistically, the latter decomposition may be rationalized by a "McLafferty" type of rearrangement (see Fig. 6), in which the transferred hydrogen originates from C-20 (12). The mass spectrum of the saturated 24-nor-5β-cholestane-3α,7α,12α-triol (I, Fig. 1) showed a similar

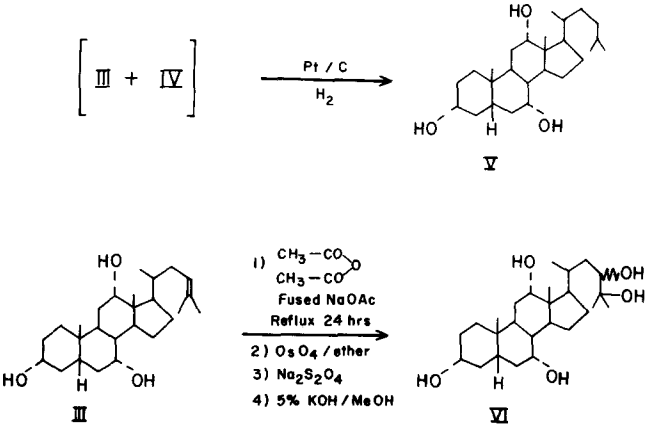

Fig. 7. Synthesis of 24-nor-5β-cholestane-3α,7α,12α-triol and 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol. III, 24-Nor-5β-cholest-23-ene-3α,7α,12α-triol; IV, 24-nor-5β-cholest-25-ene-3α,-7α,12α-triol; V, 24-nor-5β-cholestane-3α,7α,12α-triol; VI, 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol.

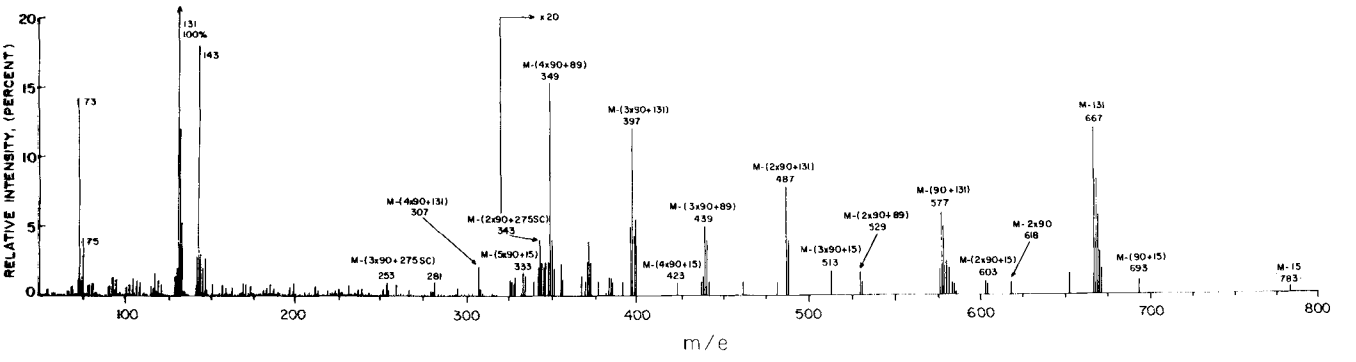

Fig. 8. Mass spectrum of 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol (VI) TMSi-ether. SC = side chain; ×20 indicates change in sensitivity.

fragmentation pattern to that of its C_{27}-analog (17). This C_{26}-saturated triol (I), like the C_{26}-saturated tetrol (II), was more polar on TLC than its C_{27}-analog (Table 1).

24 - Nor - 5β - cholestane - 3α,7α,12α,23ξ,25-pentol (VI, **Fig. 7**) was obtained from 24-nor-5β-cholest-23-ene-3α,7α,12α-triol by oxidation with one equivalent of osmium tetroxide (60% yield). The GLC and TLC characteristics of this compound are shown in Table 1. They differ from those of the C_{27}-analog, but in this case the 24-nor-5β-cholestanepentol is less polar than the 5β-cholestane-3α,7α,12α,24ξ,25-pentol. The mass spectrum of the 24-nor-5β-cholestane-3α,7α,12α,23ξ,25-pentol is shown in **Fig. 8**. The molecular ion peak was not observed, but a peak at *m/e* 784 (M − 15) was seen and the remaining fragmentation pattern was similar to that of 5β-cholestane-3α,7α,12α,24ξ,25-pentol (15).

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REFERENCES

1. Danielsson, H. 1973. Mechanisms of bile acid biosynthesis. *In* The Bile Acids: Chemistry, Physiology, and Metabolism. P. P. Nair and D. Kritchevsky, editors. Plenum Press, New York. **2:** 1–32.
2. Mosbach, E. H. 1972. Hepatic synthesis of bile acids: Biochemical steps and mechanisms of rate control. *Arch. Intern. Med.* **130:** 478–487.
3. Björkhem, I., and J. Gustafsson. 1974. Mitochondrial ω-hydroxylation of cholesterol side chain. *J. Biol. Chem.* **249:** 2528–2535.
4. Masui, T., R. Herman, and E. Staple. 1966. The oxidation of 5β-cholestane-3α,7α,12α,26-tetrol to 5β-cholestane-3α,7α,12α-triol-26-oic acid via 5β-cholestane-3α,7α,12α-triol-26-al by rat liver. *Biochim. Biophys. Acta.* **117:** 266–268.
5. Shefer, S., F. W. Cheng, B. Dayal, S. Hauser, G. S. Tint, G. Salen, and E. H. Mosbach. 1976. A 25-hydroxylation pathway of cholic acid biosynthesis in man and rat. *J. Clin. Invest.* **57:** 897–903.
6. Setoguchi, T., G. Salen, G. S. Tint, and E. H. Mosbach. 1974. A biochemical abnormality in cerebrotendinous xanthomatosis. *J. Clin. Invest.* **53:** 1393–1401.
7. Dayal, B., S. Shefer, G. S. Tint, G. Salen, and E. H. Mosbach. 1976. Synthesis of 5β-cholestane-3α,7α,12α,-25-tetrol, and 5β-cholestane-3α,7α,12α,24ξ,25-pentol. *J. Lipid Res.* **17:** 74–77.
8. Pearlman, W. H. 1947. The preparation of C_{27} steroids from bile acids. I. Coprostanetetrol-3(α),7(α),-12(α),25. *J. Amer. Chem. Soc.* **69:** 1475–1476.
9. Morsman, H., M. Steiger, and T. Reichstein. 1937. Abbau der Cholsäure zu 3,7,12-Trioxy-pregnan-20-on. *Helv. Chim. Acta.* **20:** 3–16.
10. Shimizu, T., and T. Kazuno. 1936. Über die Konstitution der Trioxy-bufosterocholensäure und den systematischen Abbau der Cholsäure. V. *Hoppe-Seyler's Z. Physiol. Chem.* **244:** 167–172.
11. Rosa, M.De., L. Minale, and G. Sodano. 1973. Metabolism in Porifera. II. Distribution of sterols. *Comp. Biochem. Physiol.* **46B:** 823–837.
12. Wyllie, S. G., and C. Djerassi. 1968. Mass spectrometric fragmentations typical of sterols with unsaturated side chains. *J. Org. Chem.* **33:** 305–313.
13. Djerassi, C. 1970. Applications of mass spectrometry in the steroid field. *Pure Appl. Chem.* **21:** 205–225.
14. Ikan, R., A. Markus, and Z. Goldschmidt. 1972. Synthesis of steroidal aziridines. *J. Org. Chem.* **37:** 1892–1894.
15. Shefer, S., B. Dayal, G. S. Tint, G. Salen, and E. H. Mosbach. 1975. Identification of pentahydroxy bile alcohols in cerebrotendinous xanthomatosis (CTX). Characterization of 5β-cholestane-3α,7α,12α,24ξ,25-pentol and 5β-cholestane-3α,7α,12α,23ξ,25-pentol. *J. Lipid Res.* **16:** 280–286.
16. Scallen, T. J., and W. Krueger. 1968. Nuclear magnetic resonance and infrared spectra of Δ^{24}- and C-24 saturated steroids. *J. Lipid Res.* **9:** 120–128.
17. Aringer, L. 1975. Conversion of 7α-hydroxycholesterol and 7α-hydroxy-β-sitosterol to 3α,7α-dihydroxy- and 3α,7α,12α-trihydroxy-5β-steroids in vitro. *J. Lipid Res.* **16:** 426–433.