Reverse cross-coupling in the synthesis of 3α,7α-dihydroxy-5β-cholestanoic acid

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Abstract The present report describes the characterization of (24*R* and 24*S*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acids obtained in considerable amounts during the synthesis of (25*RS*)-3α,7α-dihydroxy-5β-cholestan-26-oic acid by the electrolytic coupling of chenodeoxycholic acid and the half ester of methylsuccinic acid. The mixture of 24*R* and 24*S* diastereomers was resolved by analytical and preparative thin-layer chromatography and characterized by gas–liquid chromatography, proton magnetic resonance, and molecular rotation differences. For reference, the model compound, 27-nor-3α,7α-dihydroxy-5β-cholestan-26-oic acid, was synthesized by electrolytic coupling of chenodeoxycholic acid and the half ester of succinic acid.—**Kihira, K., A. K. Batta, E. H. Mosbach, and G. Salen.** Reverse cross-coupling in the synthesis of 3α,7α-dihydroxy-5β-cholestanoic acid. *J. Lipid Res.* 1979. **20:** 421–427.

Supplementary key words Kolbe electrolysis · (24*R* and 24*S*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acids · bile alcohols · diastereomers

The most commonly accepted mechanism whereby cholesterol is converted into bile acids involves 26-hydroxylated intermediates (1), and C_{27}-bile acids are postulated as the obligatory intermediates in this biosynthetic pathway (2). A number of methods have been used for the synthesis of the 5β-cholestanoic acids (3–5), but the most generally employed is electrolytic coupling of a C_{24}-bile acid with the half ester of methylsuccinic acid (3). However, methylsuccinic acid is an asymmetric compound and two half esters exist. It has been assumed that only the β-methyl (β to carboxyl end) half ester undergoes electrolytic coupling (6), but previous work suggests certain anomalous results during the synthesis of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid. Briggs, in 1970 (5), suggested that these anomalies were probably due to the coupling of the undesirable α-methyl half ester with cholic acid. In an attempt to prepare 3α,7α-dihydroxy-5β-cholestan-26-oic acid for metabolic studies, we obtained as much as 40% of the reverse cross-coupled products. Since the 5β-cholestanoic acids are often used as substrates for biological experiments and are usually prepared by the electrolytic process, we present this report as a caution against the use of incompletely defined substrates. The report deals with the thin-layer chromatographic separation, identification, and tentative configuration of the two diastereomeric (24*R* and 24*S*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acids.

METHODS

Melting points were determined on a Thermolyne apparatus, model MP-12600, and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer model 421 grating spectrophotometer in chloroform solution. Absorption frequencies are quoted in reciprocal centimeters.

NMR spectra were obtained in deuterated methanol solution using a Varian HA-220 spectrometer equipped with Fourier Transform capability.

Optical rotations were determined at 25°C in methanol on a Cary 60 ORD/CD spectrometer.

GLC. The bile alcohols and the methyl esters of bile acids, as the trimethylsilyl ether derivatives, were analyzed on a 180 cm × 4 mm column packed with 3% QF-1; column temperature 225°C (Hewlett-Packard model 7610 gas chromatograph). The retention times are given relative to the trimethylsilyl ether of methyl cholate (1.00).

Mass spectra (electron impact and high resolution) were obtained with a JEOL D-300 mass spectrometer us-

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; IR, infrared; PMR, proton magnetic resonance; RRT, relative retention time; TMSi, trimethylsilyl.

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ing the following operating conditions: ion source temperature 250°C, ionizing voltage 70 eV, and ionizing current 300 μA.

TLC. The bile acids and bile alcohols were separated on silica gel G plates (Brinkmann, 0.25 mm thickness). The spots were detected by spraying first with phosphomolybdic acid (3.5% in isopropanol) and then with sulfuric acid (10%) and heating at 110°C. During preparative TLC the bands were made visible with iodine. Two solvent systems, isooctane–ethyl acetate–acetic acid 5:25:0.2 (A), and chloroform–acetone–methanol 70:30:1.5 (B), were used.

EXPERIMENTAL

Preparation of (24*R* and 24*S*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acids (I and II; Fig. 1)

Methyl hydrogen methylsuccinate. Methylsuccinic anhydride (5 g, Aldrich Chemicals, Milwaukee, WI) was refluxed with methanol (1.8 ml) in an oil bath for 2 hr, and the solution was used without further purification.

Electrolysis. Chenodeoxycholic acid (10 g) and methyl hydrogen methylsuccinate, as obtained above, were added to a solution of Na (0.6 g) in methanol (300 ml). The solution was electrolyzed for 8 hr in a 600-ml beaker with two platinum electrodes, using direct current from a 50 V source. The current was maintained at 1.5 A and the current polarity was reversed every 15 min. The solution was stirred constantly and kept at 15–25°C by external cooling.

Isolation of products. The methanolic solution containing the products of electrolysis was poured into water (1 l), made alkaline (NaOH) to pH 10 and extracted with ethyl acetate (3 × 200 ml). The ethyl acetate extract was washed with water (3 × 50 ml), dried over anhydrous Na_2SO_4 and evaporated. The oily product obtained was refluxed with methanolic KOH (10%, 50 ml) for 2 hr. After cooling, the methanolic solution was acidified to pH 2 with dilute HCl and extracted with ethyl acetate (3 × 100 ml). The ethyl acetate extract was washed with water (25 ml), with saturated NaCl solution (2 × 25 ml), and then was dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 2.7 g of a semisolid product. TLC of this product in solvent system A showed two spots, R_f 0.43 and 0.50, whereas TLC in solvent system B (developed twice) showed the presence of four spots, R_f 0.41, 0.51, 0.56, and 0.60.

Isolation of pure compounds. A portion (120 mg) of the

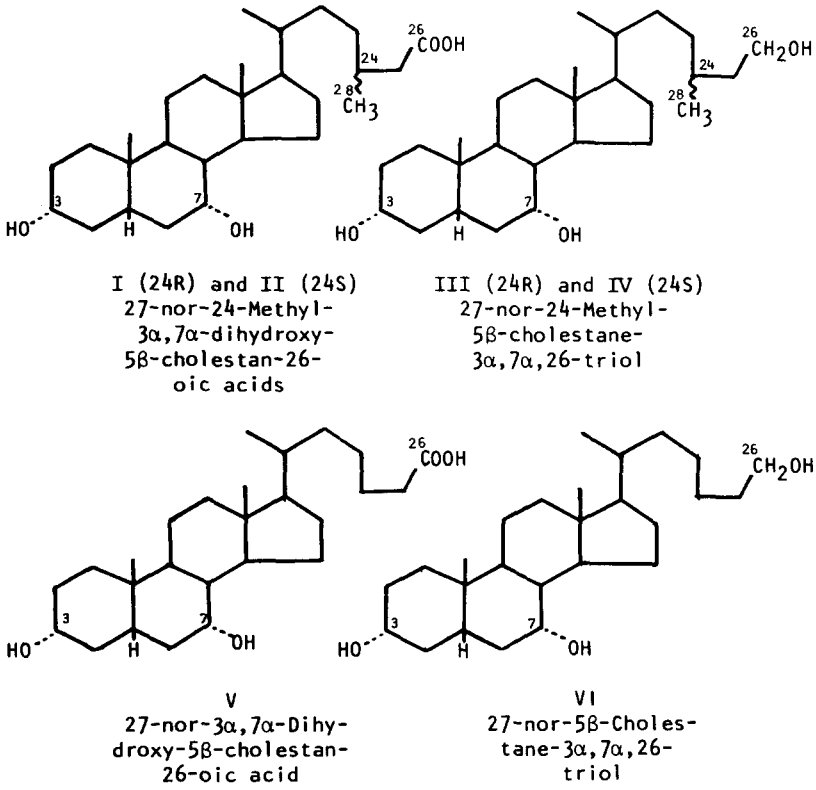

Fig. 1. Structures of compounds I–VI.

TABLE 1. 220 MHz PMR spectra of compounds I–VI[a]

Compound	C-18	C-19	C-21	C-28[b]
27-Nor-24*R*-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid (I)	151.5s	203.5s	206.8d J = 6.6	205.1d J = 6.5
27-Nor-24*S*-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid (II)	150.9s	203.6s	207.5d J = 6.6	207.5d J = 6.6
27-Nor-3α,7α-dihydroxy-5β-cholestan-26-oic acid (V)	151.1s	203.4s	207.1d J = 7.5	
27-Nor-24*R*-methyl-5β-cholestane-3α,7α,26-triol (III)	151.0s	203.5s	206.9d J = 6.8	193.9d J = 6.2
27-Nor-24*S*-methyl-5β-cholestane-3α,7α,26-triol (IV)	151.0s	203.6s	207.4d J = 7.5	196.3d J = 6.4
27-Nor-5β-cholestane-3α,7α,26-triol (VI)	150.2s	203.1s	206.9d J = 7.7	

[a] Values given as displacements in Hz from the signal for tetramethylsilane (s = singlet, d = doublet). For discussion, see text.
[b] Methyl group on C-24.

product obtained above was dissolved in methanol (1 ml) and the solution was applied as a thin streak on TLC plates. The plates were developed twice in solvent system B and the bands were made visible with iodine vapor; four bands were seen corresponding to R_f values of 0.60 (band 1), 0.56 (band 2), 0.51 (band 3), and 0.41 (band 4). The upper two bands [band 1 (31 mg) and band 2 (29 mg)] were separately scraped from the silica gel and eluted with ethyl acetate–methanol 85:15. These were identified by direct comparison with authentic material (5) as (25*S* and 25*R*)-3α,7α-dihydroxy-5β-cholestan-26-oic acids.

The two lower bands (band 3 and band 4) were eluted from the silica gel with ethyl acetate–methanol 85:15. Band 3 yielded 19 mg of a colorless semisolid (I; **Fig. 1**), which crystallized from aqueous methanol as colorless needles. This compound (I) could also be obtained in pure form by repeated crystallization of the crude mixture. Crystallization of the mixture containing the above four compounds (2.5 g) from ethyl acetate yielded crude I as colorless needles (350 mg). Two crystallizations from aqueous methanol yielded an analytically pure compound; single spot on TLC (R_f 0.50, system A, and 0.51, system B); the TMSi ether of the methyl ester gave a single peak on GLC (RRT = 1.59); mp 179–181°C; $[\alpha]_D^{25}$ + 18.5°; IR: 3610 and 3440 (hydroxyl), 1725 (carboxyl), 1455, 1430, 1375, 1160, 1070, 970, and 890 cm^{-1}. The PMR spectral data are shown in **Table 1.** High resolution mass spectrum: M^+, 434.3392 (calc. for $C_{27}H_{46}O_4$, 434.3396); electron impact mass spectrum: 434 (M^+, 5%), 416 (40), 398 (99), 383 (98), 344 (36), 304 (12), 290 (14), 283 (13), 273 (6), 264 (16), 255 (100), 246 (23), 228 (60), 213 (80), and 201 (81); electron impact mass spectrum of methyl ester: 448 (M^+, 3%), 430 (17), 412 (40), 397 (31), 381 (14), 359 (14), 273 (24), 264 (10), 255 (51), 246 (20), 228 (40), 213 (100), and 201 (43). Analysis: calculated for $C_{27}H_{46}O_4$: C, 74.65 and H, 10.60%; found: C, 74.19 and H, 10.60%.

Elution of band 4 with ethyl acetate–methanol 85:15 yielded 21 mg of acid II. Crystallization from aqueous methanol gave colorless needles; single spot on TLC (R_f 0.43, system A, and 0.41, system B); the TMSi ether of the methyl ester gave a single peak on GLC (RRT = 1.59); mp 197–199°C; $[\alpha]_D^{25}$ + 8.7°. Its IR spectrum was superimposable with that of acid I. The PMR data are shown in Table 1. High resolution mass spectrum: M^+, 434.2420 (calc. for $C_{27}H_{46}O_4$: 434.3396); electron impact mass spectrum: 434 (M^+, 5%), 416 (38), 398 (100), 383 (40), 344 (15), 304 (6), 290 (5), 283 (2), 273 (3), 264 (17), 255 (31), 246 (19), 228 (40), 213 (41), and 201 (25); electron impact mass spectrum of methyl ester: 448 (M^+, 3%), 430 (13), 412 (38), 397 (30), 381 (10), 359 (13), 273 (30), 264 (18), 255 (75), 246 (30), 228 (58), 213 (100), and 201 (43). Analysis: calculated for $C_{27}H_{46}O_4$: C, 74.65 and H, 10.60%; found: C, 74.74 and H, 10.62%.

Preparation of (24*R* and 24*S*)-27-nor-24-methyl-5β-cholestane-3α,7α,26-triols (III and IV; Fig. 1)

(24R)-27-nor-24-methyl-5β-cholestane-3α,7α,26-triol (III). Compound I (15 mg) in anhydrous ether was treated with an ethereal solution of diazomethane. Evaporation of the solvent yielded the corresponding methyl ester, which gave a single spot on TLC. This methyl ester was dissolved in anhydrous THF (3 ml) and $LiAlH_4$ (10 mg) was added. The mixture was refluxed for 3 hr. After cooling, ethyl acetate (2 ml) was added with external cooling to destroy excess $LiAlH_4$, followed by a dilute solution of H_2SO_4 (3%, w/w). The

product was extracted with ethyl acetate (2 × 25 ml), and the ethyl acetate layer was washed with 5% $NaHCO_3$ solution and then with water to neutrality. Evaporation of solvent yielded 13 mg of the alcohol III. Crystallization from aqueous methanol yielded colorless needles; there was a single spot on TLC (R_f 0.40, system A); the TMSi ether gave a single peak on GLC (RRT = 0.97); mp 154–156°C; $[\alpha]_D^{25}$ + 19.5°; IR: 3610 and 3440 (hydroxyl), 1455, 1370, 1160, 1100, 1070, 970 and 890 cm^{-1}; the PMR data are shown in Table 1. High resolution mass spectrum: M^+, 420.3517 (calc. for $C_{27}H_{48}O_3$: 420.3603); electron impact mass spectrum: 420 (M^+, 11%), 402 (100), 384 (98), 370 (86), 351 (13), 330 (28), 290 (7), 283 (10), 273 (10), 264 (30), 255 (75), 246 (39), 228 (82), 213 (78), and 201 (50).

(24S)-27-nor-24-methyl-5β-cholestane-3α,7α,26-triol (IV). Compound II (15 mg) was methylated and reduced with $LiAlH_4$ as described above. Crystallization of the reduction product from aqueous acetone yielded colorless needles of the alcohol IV; single spot on TLC (R_f 0.33, system A); the TMSi ether gave a single peak on GLC (RRT = 0.95); mp 170–171°C; $[\alpha]_D^{25}$ + 10.4°. Its IR spectrum was superimposable with that of III. The PMR data are shown in Table 1. High resolution mass spectrum: M^+, 420.3627 (calc. for $C_{27}H_{48}O_3$: 420.3603); electron impact mass spectrum: 420 (M^+, 10%), 402 (100), 384 (95), 370 (78), 351 (11), 330 (28), 290 (5), 283 (8), 273 (7), 264 (33), 255 (65), 246 (37), 228 (90), 213 (85), and 201 (47).

Preparation of 27-nor-3α,7α-dihydroxy-5β-cholestan-26-oic acid (V; Fig. 1)

Methyl hydrogen succinate. Succinic anhydride (5 g) was refluxed with methanol (2 ml) for 2 hr and the solution was used without purification.

Electrolysis. Chenodeoxycholic acid (10 g) and methyl hydrogen succinate, obtained above, were added to a solution of Na (0.6 g) in methanol (300 ml). The solution was electrolyzed as described for the preparation of compounds I and II.

Isolation of product. The electrolysis product was worked up in the same way as described above for compounds I and II. After hydrolysis of the methyl ester, 2.5 g of a pale yellow solid was obtained. Crystallization of this solid from aqueous methanol yielded colorless needles of the acid V; single spot on TLC (R_f 0.43, system A); the TMSi ether of the methyl ester gave a single peak on GLC (RRT = 1.59); mp 191–192°C; $[\alpha]_D^{25}$ + 15.2°; IR: 3610 and 3440 (hydroxyl), 1725 (carboxyl), 1455, 1430, 1375, 1160, 1105, 1070, 970, 890, and 850 cm^{-1}. The PMR data are shown in Table 1. High resolution mass spectrum: M^+, 420.3207 (calc. for $C_{26}H_{44}O_4$: 420.3240); electron impact mass spectrum: 420 (M^+, 5%), 402 (28), 384 (100), 369 (45), 330 (12), 290 (7), 283 (1), 273 (1), 264 (16), 255 (32), 246 (19), 228 (38), 213 (40), and 201 (30). Analysis: calculated for $C_{26}H_{44}O_4$: C, 74.28 and H, 10.48%; found: C, 73.89 and H, 10.52%.

Preparation of 27-nor-5β-cholestane-3α,7α,26-triol (VI; Fig. 1)

Twenty mg of acid V were esterified with diazomethane and reduced as described for the preparation of compound III. The resulting alcohol VI could not be crystallized, but showed a single spot on TLC, R_f 0.33 in solvent system A; the TMSi ether gave a single peak on GLC (RRT = 0.93); $[\alpha]_D^{25}$ + 14.4°; IR: 3610 and 3440 (hydroxyl), 1455, 1370, 1160, 1100, 1070, 970, 890, and 865 cm^{-1}. The PMR data are shown in Table 1. High resolution mass spectrum: M^+, 406.3491 (calc. for $C_{26}H_{46}O_3$: 406.3447); electron impact mass spectrum: 406 (M^+, 12%), 388 (100), 370 (99), 355 (92), 316 (25), 290 (5), 283 (7), 276 (7), 273 (10), 264 (31), 255 (70), 246 (45), 228 (90), 213 (88), and 201 (50).

RESULTS AND DISCUSSION

This report describes the production of unusual cross-coupling products, (24*R* and 24*S*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acids (I and II), during the synthesis of 3α,7α-dihydroxy-5β-cholestan-26-oic acid by electrolytic coupling of chenodeoxycholic acid and the half ester of methylsuccinic acid. In order to define the configuration at C-24 in the acids I and II, a model compound, 27- nor-3α,7α-dihydroxy-5β-cholestan-26-oic acid (V), was synthesized in a similar way from chenodeoxycholic acid and methyl hydrogen succinate.

Chenodeoxycholic acid was electrolyzed with the half ester of methylsuccinic acid according to Briggs (5), and the unreacted starting material was separated from the neutral products by extraction with ethyl acetate and washing with alkali. The C_{27}-acids were obtained from the neutral products by saponification. TLC of the free acids in solvent system A showed two spots, whereas TLC in solvent system B (developed twice) showed the presence of four spots with R_f values of 0.41, 0.51, 0.56, and 0.60. The four compounds separated by preparative TLC in solvent system B were obtained in the ratio of approximately 20:20:30:30. Both compounds I and II showed identical IR spectral behavior, and the retention times of the methyl esters of their TMSi ethers on 3% QF-1 were too similar to effect a separation. The compound I with R_f 0.51 could also be obtained in pure form by

TABLE 2. Molecular rotations[a] of compounds I–VI[b]

Compound	$[\alpha]_D^{25}$	M_D	ΔM_D
27-Nor-24*R*-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid (I)	+18.5°	+80.29	+16.45[c]
27-Nor-24*S*-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid (II)	+8.7°	+37.76	−26.08[d]
27-Nor-3α,7α-dihydroxy-5β-cholestan-26-oic acid (V)	+15.2°	+63.84	
27-Nor-24*R*-methyl-5β-cholestane-3α,7α,26-triol (III)	+19.5°	+83.79	+25.33[e]
27-Nor-24*S*-methyl-5β-cholestane-3α,7α,26-triol (IV)	+10.4°	+43.68	−14.78[f]
27-Nor-5β-cholestane-3α,7α,26-triol (VI)	+14.4°	+58.46	

[a] For discussion of the contribution of molecular rotation at C-24, see text.
[b] I, 2.12 mg/ml; II, 1.65 mg/ml; III, 1.03 mg/ml; IV, 0.46 mg/ml; V, 1.52 mg/ml; VI, 4.11 mg/ml.
[c] M_D of I − M_D of V.
[d] M_D of II − M_D of V.
[e] M_D of III − M_D of VI.
[f] M_D of IV − M_D of VI.

repeated crystallization of the mixture of electrolysis products. Direct comparison with authentic samples proved that the two compounds with R_f values of 0.56 and 0.60 were identical with the 25*R*- and 25*S*-isomers of 3α,7α-dihydroxy-5β-cholestan-26-oic acid. Compound I had mp 180–181°C, $[\alpha]_D^{25}$ + 18.5° and compound II had mp 197–199°C and $[\alpha]_D^{25}$ + 8.7°.

The model compound V, which has no asymmetric center at C-24, was synthesized by electrolytic coupling of chenodeoxycholic acid and methyl hydrogen succinate. This compound had mp 191–192°C and $[\alpha]_D^{25}$ + 15.2°.

These three acids (I, II, and V) were reduced with $LiAlH_4$ to the corresponding alcohols (III, IV, and VI, respectively). Compound III had mp 154–156°C, $[\alpha]_D^{25}$ + 19.5° and compound IV had mp 170–171°C, $[\alpha]_D^{25}$ + 10.4°. Both alcohols III and IV showed identical IR spectral behavior. Compound VI could not be crystallized, but showed a single spot on TLC and had $[\alpha]_D^{25}$ + 14.4°.

The configuration at C-24 in compounds I and II was tentatively assigned on the basis of molecular rotation differences. Acids of the type $RCH(CH_3)$-CH_2COOH show a (+) rotation for *R*-isomers and a (−) rotation for the *S*-isomers (7). Also, campesterol, 24*R*-methylcholesterol, has a higher rotation than cholesterol (8), while 22-dihydrobrassicasterol, 24*S*-methylcholesterol, has a lower rotation than cholesterol (9). Applying these results to our compounds, it is clear from **Table 2** that the isomer with a rotation higher than compound V must be 24*R*-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid and the one with a rotation lower than compound V must be 24*S*-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid. When the above acids were reduced to the corresponding alcohols, the optical rotation relationships were similar (Table 2). This assignment of configuration must be considered tentative. For rigorous assignments, either the side chain should be cleaved from the molecule and related to a compound of known absolute configuration, or the compound itself should be converted chemically to a steroid of established chirality at C-24.

Comparison of the methyl resonances in the 220 MHz PMR spectra of the two diastereomeric acids gave some interesting results. As seen from Table 1, the PMR signal for C-28 was downfield by 2.4 Hz for the 24*S*-isomer compared to the 24*R*-isomer. These values are opposite to those obtained by Nes, Krevitz, and Behzandan (10) for the C-28 methyl signal in case of compesterol and 22-dihydrobrassicasterol. The reason may lie in the fact that the C-26 carboxylic acid can have weak hydrogen bonding with the 3α- and 7α-hydroxyl groups, so that the side chain is in the proximity of the ring system and its configuration is fixed. On making molecular models, it was seen that the C-26 carboxyl group could approach quite close to the 3α-hydroxyl group and was fairly close to the 7α-hydroxyl group. This is not the case with campesterol and 22-dihydrobrassicasterol, where no such hydrogen bonding can exist. A similar, though weaker, hydrogen bonding of the C-26 hydroxyl group with hydroxyl groups at C-3 and C-7 could account for the shift of the C-28 methyl PMR signal downfield by 2.4 Hz for the 24*S*-isomer compared to the 24*R*-isomer.

The mass spectra of compounds I, II, III, and IV are shown in **Fig. 2** and those of compounds V and VI in **Fig. 3.** The base peak in the mass spectrum of the free acid I appeared at *m/e* 255 [$M^+ - (2 \times H_2O$ + side chain)] and in that of the free acid II appeared at *m/e*

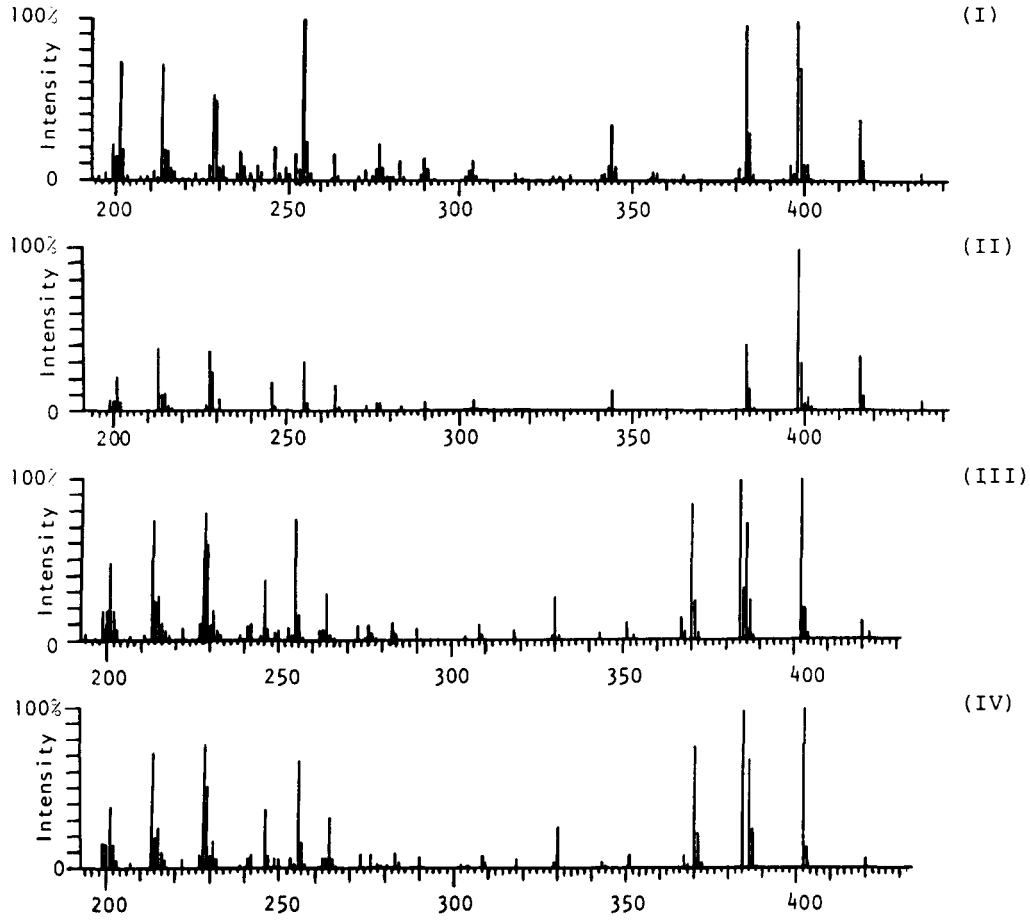

Fig. 2. Mass spectra of (24*R*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid (I), (24*S*)-27-nor-24-methyl-3α,7α-dihydroxy-5β-cholestan-26-oic acid (II), (24*R*)-27-nor-24-methyl-5β-cholestane-3α,7α,26-triol (III), and (24*S*)-27-nor-24-methyl-5β-cholestane-3α,7α,26-triol (IV). For discussion, see text.

398 [$M^+ - (2 \times H_2O)$]. The relative intensity of the peak at *m/e* 398 was 99% in the mass spectrum of compound I, but the peak at *m/e* 255 had an intensity of only 31% in the spectrum of compound II. The spectra of compounds I and II both showed a sequential loss of two H_2O molecules from the molecular ion at *m/e* 416 ($M^+ - H_2O$) and *m/e* 398 ($M^+ - 2 \times H_2O$). The same fragmentation pattern was observed in

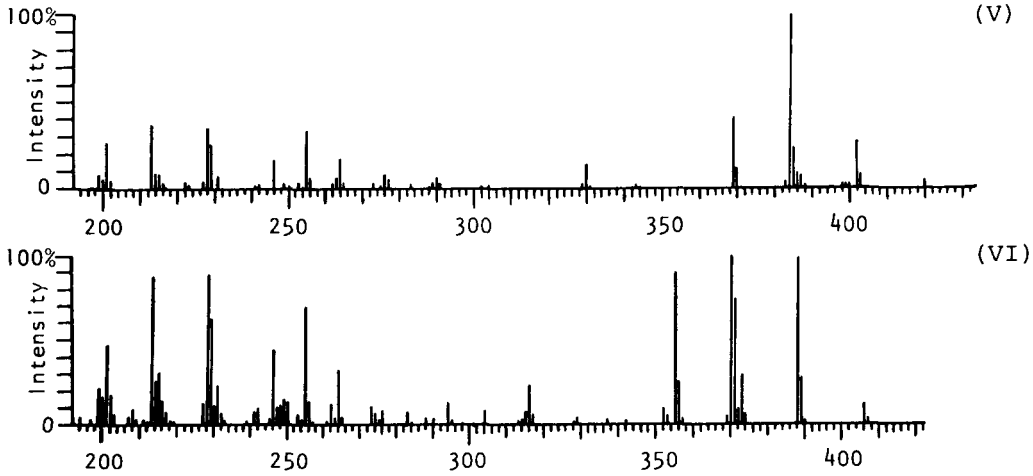

Fig. 3. Mass spectra of 27-nor-3α,7α-dihydroxy-5β-cholestan-26-oic acid (V), and 27-nor-5β-cholestane-3α,7α,26-triol (VI). For discussion, see text.

compounds III–VI. Subsequent loss of $\cdot CH_3$ then gave peaks at *m/e* 383 (compounds I and II), *m/e* 369 (compound V), and *m/e* 355 (compound VI). In the case of alcohols III and IV, the corresponding peak was observed at *m/e* 370, probably with retention of one proton. This fragment could also arise via the loss of CH_3OH (32 mass units) from the fragment at *m/e* 402 ($M^+ - H_2O$). The peak at *m/e* 344 in the spectra of compounds I and II was probably due to retro Diels–Alder fragmentation of ring A [$M^+ - (2 \times H_2O + 54)$]. Similar fragmentation was observed with compounds III, IV, and V (at *m/e* 330) and compound VI (at *m/e* 316). The fragments at *m/e* 304 (compounds I and II), *m/e* 290 (compounds III, IV, and V), and *m/e* 276 (compound VI) could be due to the loss of the entire ring A (scission at C-5, C-6 and C-9, C-10) subsequent to the loss of two water molecules. The peak at *m/e* 283 observed in the spectra of all compounds, I–VI, probably arose by the cleavage of the side chain between C-20 and C-22 accompanied by the loss of two water molecules. The spectra of acids I, II, and V showed a similar series of fragments at *m/e* 273, 264, 255, 246, 228, 213, and 201 due to the loss of the complete side chain followed by other nuclear fragmentations, a pattern also observed in the mass spectra of the methyl esters of chenodeoxycholic acid and 3α,7α-dihydroxy-5β-cholestan-26-oic acid (11). These series of fragments were also seen in the mass spectra of the alcohols III, IV, and VI. The mass spectrum of the acid V showed a fragmentation pattern similar to that obtained for acids I and II in the mass region of *m/e* 420 (M^+) to *m/e* 290, but the fragments were 14 mass units less. The alcohols III and IV showed identical fragmentation patterns and the alcohol VI bore the same relationship to alcohols III and IV as was observed for acids I, II, and V.

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