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UNIQUE 3β-0-METHYLSTEROLS FROM THE PACIFIC SPONGE JEREICOPSIS GRAPHIDIOPHORA

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ABSTRACT.—The lipid fraction of the Pacific sponge *Jereicopsis graphidiophora* has been investigated for its sterol composition. Although the conventional 3β-hydroxysteroids were totally absent, the extracts contained unique 3β-methoxysteroids. Fifteen 3β-methoxysteroids with six different nuclei [$\Delta^{7,9(11)}, \Delta^{8,14}, \Delta^8, \Delta^7, \Delta^5$, 5α-saturated] and with five usual C_8–C_{10} side chains, have been identified. In addition, the extracts also contained 3β-methoxysteroids with oxygenated functionalities in the nuclei: Δ^7-9α,11α-epoxy, Δ^7-9-hydroxy, Δ^8-7-one, Δ^8-14-hydroxy, $\Delta^{9(11)}$-8α,14α-epoxy. Characterization was accomplished by gc-ms, hreims, ^{1}H-nmr, ^{13}C-nmr, Ft-ir, and uv spectroscopy.

Sponges have been found to be the most diverse source of sterols, particularly in terms of unique side chain structures and unusual functionalization (1–3). During our ongoing program to isolate novel marine natural products from New Caledonian invertebrates, we undertook an investigation of the extracts of the sponge *Jereicopsis graphidiophora* Lévi & Lévi (family Leiodermatiidae) (4). In a preceding paper (5) we described the occurrence in *J. graphidiophora* of two very unusual 3β-methoxysecosteroids. An analysis of the lipidic fraction showed the absence of conventional 3β-hydroxysteroids, while a number of 3β-methoxysteroids were isolated. Here we report the isolation and structure determination of these compounds.

RESULTS AND DISCUSSION

The sponge was ground and freeze-dried, and the dried material (0.7 kg) was extracted with *n*-hexane. Si gel chromatography of the extract (1.4 g) using increasing concentrations of EtOAc in *n*-hexane provided a fraction (0.23 g) of mixed 3β-0-methylsterols and two very minor more polar fractions, one containing a mixture of compounds **16** and **17** and the other a mixture of compounds **18–20**. The compounds were separated by hplc.

The ^{1}H-nmr spectrum of the less polar fraction, which contained methoxyl singlets at δ 3.23–3.37 and the C-3 methine protons highfield shifted to δ 3.08–3.15 ppm, immediately indicated the presence of mixed 3β-0-methylsterols. Gc-ms analysis revealed a complex mixture with fifteen components. Argentation cc gave seven fractions, which were subjected to reversed-phase hplc on a Whatman M9 10/50 ODS-2 column with MeOH-CH$_2$Cl$_2$ (8:2) to give pure **1**, **2**, **5–10**, **12**, and **15** (Table 1) characterized by ms, 250 MHz nmr spectroscopy, and uv spectroscopic measurements. The remaining 3β-0-methylsterols in Table 1 were identified by their ms data. The configuration at C-24 of the isolated 24-methylsterols was assigned by comparison with ^{1}H-nmr spectra of known compounds. The chemical shift of the C-21 methyl is a diagnostic measure·for differentiating 24*R* and 24*S* epimers of (22*E*)-24-methyl-Δ^{22}-sterols (6,7) (compounds **5** and **6**) and their 27-nor-analogues (8) (compound **1**). In the case of

TABLE 1.   3β-O-Methylsterols from *Jereicopsis graphidiophora*.

| Compound	Side chain	Nucleus	[M]$^+$ m/z	Mobility		%
				gc	hplc	
1		Δ^5	398	0.79	0.81	1.06
2		Δ^5	398	0.81	0.81	0.82
3		Δ^5	400	0.82		3.45
4		Δ^0	402	0.84	0.93	2.94
5		Δ^5	412	0.87	0.85	7.80
6		Δ^5	412	0.87	0.89	
7		$\Delta^{8,14}$	412	0.95	0.84	4.30
8		$\Delta^{8(9)}$	414	0.96	0.93	9.75
9		$\Delta^{7,9(11)}$	412	0.97	0.81	6.93
10		Δ^7	414	1.00	1.00	32.69
11		Δ^5	428	1.03		3.25
12		Δ^0	430	1.04	1.01	1.30
13		$\Delta^{8(9)}$	428	1.06		3.64
14		$\Delta^{7,9(11)}$	426	1.07		1.16
15		Δ^7	428	1.11	1.07	20.83

24-methylsterols with saturated side chains (compounds **8–10, 16–20**), slight differences in chemical shift of the 26- and 27-methyls between epimers create diagnostic patterns (6). In the 24-ethylsteroid series the chemical shift differences of the Me-26
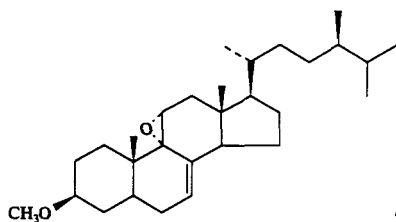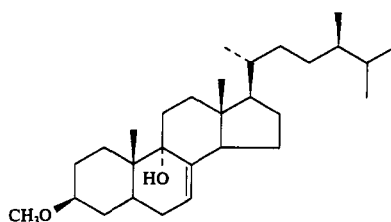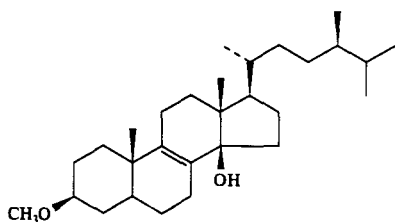
16
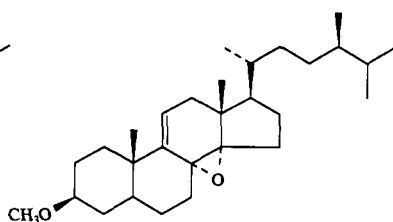
17
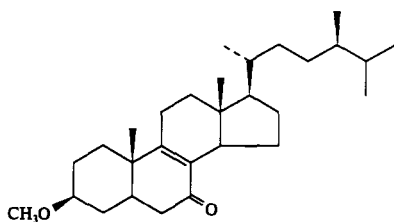
18

19

20

and Me-27 signals between epimers are too small for differentiation; therefore we prefer to leave the stereochemistry at C-24 unassigned. Interestingly the 3β-O-methylsterols mixture also includes the rare $\Delta^{8,14}, \Delta^{8}$, and $\Delta^{7,9(11)}$ nuclei (compounds **7**, **8**, and **9** in Table 1).

Compound **7**, probably an artifact originating from **20** during isolation, exhibited a mass spectrum with molecular ion peak at m/z 412 and fragments at m/z 285 (loss of the side chain) and 253 (loss of the side chain plus MeOH), indicating a di-unsaturated nucleus and a saturated C_9 side chain. The uv spectrum {λ max (MeOH) 248 nm} (9) and the ^{1}H-nmr signals of the angular methyls (δ 0.83 and 0.89) and of the olefinic protons (δ 5.37) were closely comparable with those of synthetic 5α-cholesta-8,14-dien-3β-ol (10). Conclusive support for the existence of the $\Delta^{8,14}$-diene came from a comparison of the ^{13}C-nmr spectrum of **7** (Table 2) with that of the synthetic model compound.

Compound **8**, with the rare Δ^8-unsaturated nucleus, exhibited a molecular ion peak in the ms at m/z 414 and a fragment ion at m/z 287 (loss of the side chain), indicating a mono-unsaturated nucleus and a saturated C_9 side chain (11,12). The existence of Δ^8-unsaturation was confirmed by ^{1}H nmr, since **8** exhibited the angular methyl signals at δ 0.62 and 0.95, virtually identical to those reported for Δ^8-sterols (14), while

TABLE 2. ^{13}C-nmr Data for the Compounds **7**, **18**, **19**, and **20**.

Carbon	Compound			
	7	**18**	**19**	**20**
C-1	34.7	34.9	33.2[a]	34.4
C-2	28.3	28.0	27.3	28.0
C-3	79.6	78.7	79.2	79.8
C-4	34.3	34.7	33.7	33.7
C-5	41.1	41.3	35.8	41.4
C-6	25.6	42.6	29.4	24.9
C-7	26.7	186.6	121.3	26.0[a]
C-8	120.0	133.4	135.5	140.0
C-9	140.4	164.7	73.9	130.3
C-10	37.0	38.7	39.2	37.4
C-11	22.0	24.9	27.7	21.5
C-12	37.1	35.9	30.0[a]	31.5
C-13	45.2	42.6	43.7	45.0
C-14	150.7	48.5	51.2	82.2
C-15	117.3	25.3	23.1	31.1
C-16	35.5	29.4	27.9	25.8[a]
C-17	57.4	53.8	56.1	44.8
C-18	15.8	11.6	11.1	17.9
C-19	18.3	17.1	15.0	17.6
C-20	33.8	36.3	36.3	36.0
C-21	19.0	18.9	19.0	18.6
C-22	34.3	34.0	34.5	34.4
C-23	30.4	30.7	30.5	30.4
C-24	39.0	39.1	38.9	39.1
C-25	32.5	32.5	32.4	32.5
C-26	18.3	18.3	18.3	18.3
C-27	20.2	20.2	20.2	20.2
C-28	15.5	15.5	15.4	15.5
-OMe	55.6	55.5	55.5	55.5

[a]These assignments may be reversed.

signals due to olefinic protons were absent. Δ^8-Marine sterols have been previously reported only from the sponge *Axinella cannabina* (13,14).

For compound **9**, the eims gave the molecular ion peak at m/z 412 and intense fragments at m/z 285 and m/z 253, arising from loss of the side chain and loss of side chain plus MeOH and indicating a di-unsaturated nucleus with a saturated C_9 side chain (11,12). The uv spectrum showing absorption at 242 nm, typical of $\Delta^{7,9(11)}$ sterols (15), and two olefinic signals at δ 5.38 and 5.46 indicated a conjugated system of two double bonds. The structural deduction was confirmed by comparison of ^{1}H-nmr signals of the olefinic protons at C-7 and C-11 and of the angular methyl protons (δ 0.52 and 0.90 ppm) with those of 24-methyl-5α-cholesta-7,9(11),24(28)-trien-3β-ol, the first naturally occurring $\Delta^{7,9(11)}$ unsaturated marine sterol, isolated from the sponge *Haliclona flavescens* (16).

The nmr spectral data indicated that all of the more polar compounds **16–20** possessed a 3β-methoxy group and the same saturated (24R)-methyl side chain. For compounds **18**, **19**, and **20**, obtained in amounts adequate to measure the ^{13}C-nmr spectra, the 24R configuration received further support by comparison of their ^{13}C-nmr spectra with those of known compounds (17).

(24R)-9α,11α-Epoxy-3β-methoxy-24-methyl-5α-cholest-7-ene [**16**], obtained

in 0.8 mg quantities (from 0.7 kg dry wt), gave the molecular ion in hreims at m/z 428.3641, corresponding to a molecular formula $C_{29}H_{48}O_2$, and the major fragment, due to the loss of a saturated C_9 side chain, at m/z 301.2167 ($C_{20}H_{29}O_2$), thereby locating both oxygen functions in the nucleus. An olefinic signal at δ 5.63 along with the high field shift of the C-18 angular methyl signal at δ 0.57 in the ^{1}H-nmr spectrum suggested a Δ^7-unsaturation (18). A resonance at δ 3.24 (1H, d, $J = 5.8$ Hz) indicated the presence of an epoxide moiety. The signal at δ 3.24 has the same doublet appearance reported by Tori *et al.* (19) for the 11β proton of 9α,11α-epoxysteroids while the 11α proton of a 9β,11β-epoxide appears as a triplet ($J = 1.5$ Hz) (19). Furthermore H-[H'] decoupling experiments showed that the epoxy proton was only coupled to a double doublet, at δ 2.14 ($J = 15.0, 5.8$ Hz), assigned to a H-12β and that this latter was coupled to a broad doublet at δ 1.60 attributable to H-12α. Thus the Δ^7-9α,11α-epoxy functionality was established in **16**. The 9α,11α-epoxy functionality has been previously found in two marine polyhydroxysteroids from a sponge *Dysidea* sp. (20,21) and from the marine gastropod *Planaxis sulcatus* (22).

(24R)-8α,14α-Epoxy-3β-methoxy-24-methyl-5α-cholest-9(11)-ene [**17**], obtained in 0.5 mg quantities (from 0.7 kg dry wt) is isomeric with **16**. Peaks observed in the hreims spectrum at m/z 428.3647 [M]$^+$ ($C_{29}H_{48}O_2$) and 301.2154 [M − C_9H_{19}]$^+$ indicated a conventional C_9H_{19} side chain and a tetracyclic nucleus containing both oxygen functions and two formal unsaturations. The ^{1}H-nmr spectrum at 400 MHz contained an olefinic signal at δ 5.14 (1H, t, $J = 6.5$ Hz) coupled with two double doublets observed at δ 3.02 (1H, dd, $J = 13.0, 6.5$ Hz) and δ 2.10 (1H, dd, $J = 13.0, 6.5$ Hz), which are coupled to each other by 13.0 Hz, thus proving the presence of a methylene group next to an olefinic proton and to a blocked position as in the partial structure >C=CH-CH$_2$C<. Incorporation of this partial structure into a sterol nucleus yields a $\Delta^{9(11)}$ structure or a Δ^5 structure with substitution at C-8. This latter could be eliminated because: (a) large [M − MeOH]$^+$ or [M − OH]$^+$ ions are observed for Δ^5 sterols whereas only weak fragment ions are observed in Δ^7 sterols (11), and only a weak [M − MeOH]$^+$ ion was observed for **17**; (b) in the ^{1}H-nmr spectrum the broad 3α-methine proton is observed downfield shifted to δ3.18, whereas in the Δ^5 structures it is systematically observed at δ 3.08 ppm (see Experimental), paralleling the behavior of the corresponding protons in 5α-cholestan-3β-ol and cholesterol (H-3α observed at δ ca. 3.62 and ca. 3.48, respectively).

Product **17** lacked carbonyl absorption in the ir spectrum, suggesting that the second oxygen of the nucleus is incorporated into an epoxide, which must be tetrasubstituted because of the lack of suitable ^{1}H-nmr signals for epoxide protons. The only reasonable location remaining for a tetrasubstituted epoxide in a $\Delta^{9(11)}$ normal steroid skeleton was the 8,14 position. The 8α,14α-stereochemistry was clarified by nOe difference experiments. Irradiation of the H-18 signal (δ 0.84) resulted in the enhancement of the H-12 signal at δ 2.10, while no nOe was detected on the H-12 signal downfield shifted to δ 3.02 ppm. This experiment allowed us to assign the signals at δ 2.10 and 3.02 to H-12β and H-12α, respectively. The downfield position of the H-12α signal in this structure is in good agreement with the 8α,14α orientation of the epoxide function.

To the best of our knowledge this is the first finding of a $\Delta^{9(11)}$-8,14-epoxide functionality in a naturally occurring steroid.

(24R)-3β-Methoxy-24-methyl-5α-cholest-8-en-7-one [**18**], [α]D − 16.5°, was obtained in 2.4 mg amounts (from 0.7 kg dry wt). Hreims spectrum showed a molecular ion peak at m/z 428.3661 ($C_{29}H_{48}O_2$) in full agreement with 29 carbon signals in the ^{13}C-nmr spectrum. Fragmentation peaks were observed at m/z 301.2177 ($C_{20}H_{29}O_2$, loss of a C_9H_{19} side chain from [M]$^+$) and m/z 259.1694 ($C_{17}H_{23}O_2$), cor-

responding to loss of the side chain and ring D (11), thus confirming the two oxygen functions to the A–C rings region.

The ir band at 1655 cm^{-1} and uv absorption at 253 nm (ϵ = 10.000) were indicative of an α,β-unsaturated ketone group, and this found support in the ^{13}C-nmr signals at 186.6, due to the ketone carbon, and at 133.4 and 164.7 ppm, due to quaternary olefinic carbon. The presence of the 3β-methoxy group was indicated by a broad ^{1}H-nmr methine multiplet at δ 3.16 and by a methoxyl singlet at δ 3.36.

The ^{1}H nmr spectrum also contained two double doublets at δ 2.18 (1H, dd, J = 16.2, 4.5 Hz) and 2.42 (1H, dd, J = 16.2, 13.5 Hz), coupled to each other by 16.2 Hz, assigned to a methylene group adjacent to the carbonyl and to a carbon bearing one proton. These data were indicative for the location of the keto group at position 7. The structural deduction of the 8(9)-en-7-one functionality in **18** was conclusively obtained by comparison of the ^{1}H-nmr signals of the angular methyl protons [δ 0.59, 1.17 ppm] with those of the previously synthesized 3β-hydroxy-5α-cholest-8-en-7-one [δ 0.56, 1.18 ppm] (23,24), and by the presence in the mass spectrum of the diagnostic peaks at m/z 288.2463 ($C_{20}H_{32}O$, ring B fragmentation) and at m/z 246.1624 ($C_{16}H_{22}O_2$, loss of $C_{13}H_{26}$ through two consecutive rearrangement processes: retro-Diels-Alder followed by a McLafferty rearrangement). Analogous fragments observed in the mass spectrum of the steroidal $\Delta^{8(9)}$-7-ketones have been found to be of considerable diagnostic importance in differentiating the $\Delta^{8(9)}$-en-7-ones from $\Delta^{8(14)}$-en-7-ones (25).

(24R)-3β-Methoxy-24-methyl-5α-cholest-7-en-9α-ol [**19**], [α]D −36.5°, was obtained in 1.7 mg amounts (from 0.7 kg dry wt). Hreims gave a molecular ion at m/z 430.3801 ($C_{29}H_{50}O_2$) and an intense fragment ion at m/z 412.3692 for [M − H$_2$O]$^+$. The ^{13}C-nmr spectrum and DEPT measurements indicated the presence of 29 carbon atoms and, in addition to the methoxy group at C-3 and the 24-methyl side chain, revealed the presence of one trisubstituted double bond (δ_C 121.3 d, 135.5 s) and one tertiary hydroxyl group (δ_C 73.9 s). An olefinic broad doublet at δ 5.27 along with the upfield shift of the C-18 angular methyl signal to δ 0.57, observed in the ^{1}H-nmr spectrum, suggested a Δ^7 unsaturation (18). Thus the tertiary hydroxyl group could only be located at C-5, C-9, or C-14.

Taking cholest-7-en-3β-ol as a starting structure (26) and using the substituent effects expected for a tertiary axial hydroxy group (27,28), the ^{13}C-nmr spectrum of our steroid could be completely assigned (Table 2) upon introduction of the hydroxyl group at C-9. Strong evidence about the hydroxyl group position was also obtained from ^{1}H-nmr spectral data. The signal of the 3α proton at δ 3.14 was too highfield shifted to assume the presence of a 5α-hydroxyl group (expected value δ 3.64–3.74 ppm) (29,30), and the chemical shifts of both angular methyl group protons (δ 0.57 and 0.90 ppm) were in good agreement with the calculated values using Zürcher's substituent increment parameters (31,32) for Δ^7-3β-methoxy-9α-hydroxycholestane (δ 0.54 and 0.92 ppm) and far away from those calculated for Δ^7-3β-methoxy-14α-hydroxycholestane (δ 0.65 and 0.82 ppm).

The base peak in the mass spectrum at m/z 304.2755 ($C_{21}H_{36}O$), interpreted as due to the ring B fragmentation through retro-Diels-Alder process, is also in strong agreement with the location of the hydroxyl group at C-9, which greatly facilitates such typical fragmentation of Δ^7 sterols (11).

(24R)-3β-Methoxy-24-methyl-5α-cholest-8(9)-en-14α-ol [**20**], obtained in 2.8 mg amounts (from 0.7 kg dry wt), is isomeric with **19**. The molecular ion in the hreims was observed at m/z 430.3796 ($C_{29}H_{50}O_2$), and the ^{13}C-nmr spectrum supported the presence of a tertiary hydroxyl group (δ_C 82.25 ppm) and of a tetrasubstituted double bond [δ_C 130.35 (s) and 140.05 ppm (s)].

On standing in the nmr tube (CDCl$_3$), **20** dehydrated to the $\Delta^{8,14}$ diene **7**. There

are two ways such a tetrasubstituted double bond and a tertiary hydroxyl group can be put into a steroidal skeleton, i.e., $\Delta^{8(9)}$-14-OH or $\Delta^{8(14)}$-9-OH, both affording the $\Delta^{8,14}$ diene. Taking cholest-8(9)-en-3β-ol as a starting structure (26), the ^{13}C-nmr spectrum of the native steroid could be completely assigned on the basis of the expected substituent effects due to the introduction of a hydroxyl group at the C-14α position. Particularly informative about the hydroxyl group's configuration was the resonance position of C-17 at 44.8 ppm, upfield shifted by 10 ppm relative to the starting sterol (γ-gauche interaction) (28).

The process was repeated with a 9α-hydroxy group, taking cholest-8(14)-en-3β-ol as a starting structure (26), and the expected and experimental spectra were significantly different.

EXPERIMENTAL

GENERAL METHODS.—Reversed-phase hplc was performed by using Waters equipment (M 6000 A pump, U6K injector, R 401 refractometer). Gc-ms was performed on a Hewlett-Packard 5890 gas chromatograph with a mass selective detector MSD HP 5970 MS and a split/splitless injector for capillary column, using a fused-silica column, 25 m × 0.20 mm HP-5 (cross linked 15% Ph Me silicone), 0.33 μm film thickness.

Mass spectra were recorded at 70 eV on a Kratos MS 50 mass spectrometer. Ft-ir spectra were recorded on a Bruker IFS-48 spectrometer in KBr pellet and uv spectra on a Beckman DU 70 spectrometer. ^{1}H- and ^{13}C-nmr spectra were determined on a Bruker WM-250 or WM-400 spectrometer in CDCl$_3$. The chemical shifts are given in ppm and referred to the CHCl$_3$ signal observed at 7.27 ppm; the coupling constants are reported in Hertz.

Medium pressure liquid chromatography (mplc) was performed on a Büchi 861 apparatus using a SiO$_2$ (230–400 mesh) column.

The purity of compounds **1**, **2**, **4–10**, **12**, and **15–20** was judged to be >90% by hplc and ^{1}H nmr. For the identification of compounds **7**, **18**, **19**, and **20**, ^{13}C nmr was also used.

COLLECTION AND EXTRACTION.—The sponge was collected in the course of the dredging campaigns of the ORSTOM-CNRS Programme "Substances Marines d'Interest Biologique (SMIB)" by the N/O "Vauban" on the upper slope off New Caledonia in 1985 at a depth of 255 m and has been identified as a new species and new genus by Lévi and Lévi (4). A zoological sample is kept at the Orstom Centre de Nouméa under reference R 1363.

The organisms were freeze-dried, and the lyophilized material (0.7 kg) was extracted in a Soxhlet apparatus with *n*-hexane (3 liters). The *n*-hexane extract was filtered and concentrated under reduced pressure to give 1.4 g of crude material, which was chromatographed by mplc on a SiO$_2$ column using a solvent gradient system from *n*-hexane to *n*-hexane–EtOAc (1:1).

3β-OME STEROLS.—Fractions eluted with *n*-hexane–EtOAc (98:2) afforded a mixture (0.23 g) of 3β-OMe sterols. Gcms analysis of this mixture indicated that it contained fifteen compounds (see Table 1). The mixture was subjected to argentation chromatography on an AgNO$_3$ impregnate Si gel [AgNO$_3$ (10 g)/SiO$_2$ (50 g)] column using solvent system from *n*-hexane to *n*-hexane–EtOAc (9:1) to give seven fractions. Final separation was achieved by hplc on a Whatman M9 10/50 ODS-2 column using CH$_2$Cl$_2$-MeOH (2:8) as eluent to obtain pure **1**, **2**, **4–10**, **12**, and **15**, which were characterized by ms, nmr spectroscopy, and uv measurements. Identification of compounds **3**, **11**, **13**, and **14** was achieved by mass spectral fragmentation, gc retention times, and the published results of Itoh *et al.* (14).

(22E,24S)-27-Norcholesta-5,22-diene [**1**].—Ms *m/z* [M]$^{+}$ 398 (80), 366 (76), 314 (32), 255 (100), 213 (32); ^{1}H nmr (CDCl$_3$) δ 0.70 (3H, s, H$_3$-18), 0.83 (3H, t, *J* = 7.3 Hz, H$_3$-26), 0.93 (3H, d, *J* = 6.9 Hz, H$_3$-27), 1.00 (3H, s, H$_3$-19), 1.02 (3H, d, *J* = 6.7 Hz, H$_3$-21), 3.03 (1H, m, H-3), 3.36 (3H, s, -OMe), 5.16 (2H, m, H-22, -23), 5.36 (1H, m, H-6).

3β-Methoxy-(22E)-cholesta-5,22-diene [**2**].—Ms *m/z* [M]$^{+}$ 398 (80), 366 (76), 314 (32), 255 (100), 213 (32); ^{1}H nmr (CDCl$_3$) δ 0.70 (3H, s, H$_3$-18), 0.87 (6H, d, *J* = 6.6 Hz, H$_3$-26, -27), 1.02 (3H, d, *J* = 6.5 Hz, H$_3$-21), 1.00 (3H, s, H$_3$-19), 3.08 (1H, m, H-3), 3.36 (3H, s, -OMe), 5.21 and 5.30 (2H, further coupled AB system, J_{AB} = 14 Hz, H-23 and H-22, respectively), 5.35 (1H, m, H-6).

3β-Methoxycholest-5-ene [**3**].—Ms *m/z* [M]$^{+}$ 400 (41), 368 (100), 353 (56), 255 (36), 213 (41). The intense peak at *m/z* 368 due to the loss of MeOH from molecular ion is diagnostic for the location of the double bond at position 5.

3β-Methoxy-5α-cholestane [**4**].—Ms *m/z* [M]$^{+}$ 402 (68), 355 (34), 247 (66), 215 (100); ^{1}H nmr

(CDCl$_3$) δ 0.65 (3H, s, H$_3$-18), 0.80 (3H, s, H$_3$-19), 0.85 (6H, d, J = 7.0 Hz, H$_3$-26, -27), 0.90 (3H, d, J = 6.7 Hz, H$_3$-21), 3.13 (1H, m, H-3), 3.35 (3H, s, -OMe).

(22E,24S)-3β-Methoxy-24-methylcholesta-5,22-diene [**5**].—Ms m/z [M]$^+$ 412 (66), 380 (37), 337 (17), 314 (20), 255 (100), 253 (36), 213 (33); ^{1}H nmr (CDCl$_3$) δ 0.70 (3H, s, H$_3$-18), 0.82–0.85 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.92 (3H, d, J = 6.7 Hz, H$_3$-28), 1.01 (3H, s, H$_3$-19), 1.01 (3H, d, J = 6.7 Hz, H$_3$-21), 3.07 (1H, m, H-3), 3.36 (3H, s, -OMe), 5.19 (2H, m, H-22, -23), 5.36 (1H, m, H-6).

(22E,24R)-3β-Methoxy-24-methylcholesta-5,22-diene [**6**].—Ms m/z [M]$^+$ 412 (64). The fragmentation pattern was essentially the same as that of the epimer **5**. ^{1}H nmr (CDCl$_3$) all signals superimposable with those of compound **5** except the resonance ascribable to H$_3$-21: δ 1.03 (3H, d, J = 6.5 Hz, H$_3$-21).

(24R)-3β-Methoxy-24-methyl-5α-cholesta-8,14-diene [**7**].—Ms m/z [M]$^+$ 412 (100), 397 (45), 365 (92), 285 (18), 253 (25); uv (CHCl$_3$) 248 nm (ε = 10000); ^{1}H nmr (CDCl$_3$), δ 0.79 (3H, d, J = 6.8 Hz, H$_3$-28), 0.81 and 0.86 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.83 (3H, s, H$_3$-18), 0.94 (3H, d, J = 6.5 Hz, H$_3$-21), 0.99 (3H, s, H$_3$-19), 3.16 (2H, m, H-3), 3.37 (3H, s, -OMe), 5.37 (1H, m, H-15); ^{13}C nmr (CDCl$_3$) see Table 2.

(24R)-3β-Methoxy-24-methyl-5α-cholest-8(9)-ene [**8**].—Ms m/z [M]$^+$ 414 (100), 367 (19), 287 (14), 255 (17), 229 (33), 213 (31); ^{1}H nmr (CDCl$_3$) δ 0.62 (3H, s, H$_3$-18), 0.78 (3H, d, J = 6.8 Hz, H$_3$-28), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.93 (3H, d, J = 6.5 Hz, H$_3$-21), 0.95 (3H, s, H$_3$-19), 3.15 (1H, m, H-3), 3.36 (3H, s, -OMe).

(24R)-3β-Methoxy-24-methyl-5α-cholesta-7,9(11)-diene [**9**].—Ms m/z [M]$^+$ 412 (100), 365 (38), 285 (38), 253 (36), 211 (55); ^{1}H nmr (CDCl$_3$) δ 0.52 (3H, s, H$_3$-18), 0.79 (3H, d, J = 6.8 Hz, H$_3$-28), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.93 (3H, d, J = 6.5 Hz, H$_3$-21), 0.90 (3H, s, H$_3$-19), 3.12 (1H, m, H-3), 3.36 (3H, s, -OMe), 5.37 (1H, br s), 5.46 (1H, br s, H-11).

(24R)-3β-Methoxy-24-methyl-5α-cholest-7-ene [**10**].—Ms m/z [M]$^+$ 414 (100), 367 (14), 255 (59), 213 (25); ^{1}H nmr (CDCl$_3$), δ 0.54 (3H, s, H$_3$-18), 0.78 (3H, d, J = 6.8 Hz, H$_3$-28), 0.80 (3H, s, H$_3$-19), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.92 (3H, d, J = 6.5 Hz, H$_3$-21), 3.13 (1H, m, H-3), 3.36 (3H, s, -OMe), 5.17 (1H, br s, H-7).

3β-Methoxy-24-ethylcholest-5-ene [**11**].—Ms m/z [M]$^+$ 428 (46), 396 (100), 381 (61), 255 (41), 213 (38). The intense peak at m/z 396 due to the loss of MeOH from molecular ion is diagnostic for the location of the double bond at position 5.

3β-Methoxy-24-ethyl-5α-cholestane [**12**].—Ms m/z [M]$^+$ 430 (83), 383 (50), 215 (100); ^{1}H nmr (CDCl$_3$), δ 0.65 (3H, s, H$_3$-18), 0.80 (3H, s, H$_3$-19), 0.81 and 0.83 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.84 (3H, t, J = 7.2 Hz, H$_3$-29), 0.93 (3H, d, J = 6.5 Hz, H$_3$-21), 3.13 (1H, m, H-3), 3.35 (3H, s, -OMe).

3β-Methoxy-24-ethyl-5α-cholest-8(9)-ene [**13**].—Ms m/z [M]$^+$ 428 (100), 381 (20), 287 (21), 255 (38), 229 (21), 213 (25). The peak at m/z 229 indicates the location of the double bond at position 8 taking into account the gc correlations with other sterols in Table 1.

3β-Methoxy-24-ethyl-5α-cholesta-7,9(11)-diene [**14**].—Ms m/z [M]$^+$ 426 (100), 379 (45), 285 (73), 253 (71), 211 (72). The Δ$^{7,9(11)}$ unsaturation is supported by gc correlation with other sterols in Table 1.

3β-Methoxy-24-ethyl-5α-cholest-7-ene [**15**].—Ms m/z [M]$^+$ 428 (100), 381 (8), 287 (25), 255 (65), 213 (30); ^{1}H nmr (CDCl$_3$) δ 0.54 (3H, s, H$_3$-18), 0.80 (3H, s, H$_3$-19), 0.81 and 0.83 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.84 (3H, t, J = 7.2 Hz, H$_3$-29), 0.93 (3H, d, J = 6.5 Hz, H$_3$-21), 3.13 (1H, m, H-3), 3.36 (3H, s, -OMe), 5.17 (1H, br s, H-7).

ISOLATION OF **16** AND **17**.—Fractions eluted with n-hexane–EtOAc (96:4) (6 mg) were further purified by hplc with a Whatman Partisil 10/25 ODS-2 column using MeOH-CH$_2$Cl$_2$ (9:1) as eluent to give pure compounds **16** and **17**.

(24R)-9α,11α-Epoxy-3β-methoxy-24-methyl-5α-cholest-7-ene [**16**].—Compound **16** (0.8 mg): hreims m/z (assignment, rel. int.) [M]$^+$ 428.3641 (C$_{29}$H$_{48}$O$_2$) (70), 301.2167 (C$_{20}$H$_{29}$O$_2$) (43), 262.1930 (C$_{17}$H$_{26}$O$_2$) (100); ^{1}H nmr (CDCl$_3$) δ 0.57 (3H, s, H$_3$-18), 0.78 (3H, d, J = 6.8 Hz, H$_3$-28), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.89 (3H, d, J = 6.5 Hz, H$_3$-21), 0.99 (3H, s, H$_3$-19), 1.60 (1H, bd, J = 15 Hz, H-12α), 2.14 (1H, dd, J = 15.0, 5.8 Hz, H-12β), 3.13 (1H, m, H-3), 3.24 (1H, d, J = 5.8 Hz, H-11β), 3.35 (3H, s, -OMe), 5.63 (1H, bd, H-7).

(24R)-8α,14α-Epoxy-3β-methoxy-24-methyl-5α-cholest-9(11)-ene [**17**].—Compound **17** (0.5 mg): hreims m/z (assignment, rel. int.) [M]$^+$ 428.3650 (C$_{29}$H$_{48}$O$_2$) (42), 301.2156 (C$_{20}$H$_{29}$O$_2$) (100); ^{1}H nmr (CDCl$_3$) δ 0.78 (3H, d, J = 6.8 Hz, H$_3$-28), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H$_3$-26, -27), 0.84

(3H, s, H_3-18), 0.96 (3H, d, J = 6.5 Hz, H_3-21), 1.12 (3H, s, H_3-19), 2.10 (1H, overlapped signal, H-7α), 2.11 (1H, dd, J = 13, 6.5 Hz, H-12β), 2.21 (1H, dt, J = 13, 3.5 Hz, H-7β), 3.02 (1H, dd, J = 12.5, 6.5 Hz, H-12α), 3.18 (1H, m, H-3), 3.35 (3H, s, -OMe), 5.14 (1H, t, J = 6.5 Hz, H-11).

ISOLATION OF **18–20**.—Fractions eluted with *n*-hexane–EtOAc (85:15) gave 48 mg of an oily residue that was subjected to hplc with a Whatman Partisil M9 10/50 ODS-2 column and MeOH as eluent, thus obtaining pure compounds **18–20**.

(24R)-3β-Methoxy-5α-cholest-8-en-7-one [**18**].—Compound **18** (2.4 mg): ir (KBr) ν max 1655 cm^{-1}; uv (EtOAc) λ max 253 (ε = 10000), hreims *m/z* (assignment, rel. int.) $[M]^+$ 428.3661 ($C_{29}H_{48}O_2$) (100), 301.2177 ($C_{20}H_{29}O_2$) (17), 288.2463 ($C_{20}H_{32}O$) (29), 246.1624 ($C_{16}H_{22}O_2$) (30); ^1H nmr ($CDCl_3$) δ 0.59 (3H, s, H_3-18), 0.78 (3H, d, J = 6.8 Hz, H_3-28), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H_3-26, -27), 0.94 (3H, d, J = 6.5 Hz, H_3-21), 1.17 (3H, s, H_3-19), 2.18 (1H, dd, J = 16.2, 4.5 Hz, H-6α), 2.42 (1H, dd, J = 16.2, 13.5 Hz, H-6β), 3.16 (1H, m, H-3), 3.36 (3H, s, -OMe).

(24R)-3β-Methoxy-24-methyl-5α-cholest-7-en-9α-ol [**19**] —Compound **19** (1.7 mg): hreims *m/z* (assignment, rel. int.) $[M]^+$ 430.3801 ($C_{29}H_{50}O_2$) (37), 412.3692 ($C_{29}H_{48}O$) (15), 397.3463 ($C_{28}H_{45}O$) (9), 304.2755 ($C_{21}H_{36}O$) (100); ^1H nmr ($CDCl_3$) δ 0.57 (3H, s, H_3-18), 0.78 (3H, d, J = 6.8 Hz, H_3-28), 0.82 and 0.86 (each 3H, d's, J = 6.8 Hz, H_3-26, -27), 0.90 (3H, s, H_3-19), 0.93 (3H, d, J = 6.5 Hz, H_3-21), 3.14 (1H, m, H-3), 3.37 (3H, s, -OMe), 5.27 (1H, bd, H-7).

(24R)-3β-Methoxy-24-methyl-5α-cholest-8(9)-en-14α-ol [**20**].—Compound **20** (2.8 mg): hreims *m/z* (assignment, rel. int.) $[M]^+$ 430.3796 ($C_{29}H_{50}O_2$) (50), 412.3690 ($C_{29}H_{48}O$) (100), 397.3461 ($C_{28}H_{45}O$) (42); ^1H nmr ($CDCl_3$) δ 0.78 (3H, d, J = 6.8 Hz, H_3-28), 0.81 and 0.85 (each 3H, d's, J = 6.8 Hz, H_3-26, -27), 0.89 (3H, s, H_3-18), 0.93 (3H, d, J = 6.5 Hz, H_3-21), 0.95 (3H, s, H_3-19), 3.18 (1H, m, H-3), 3.36 (3H, s, -OMe).

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