26,27-Cyclosterols and Other Polyoxygenated Sterols from a Marine Sponge *Topsentia* sp.

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Thirty sterols (**1**−**30**) were isolated from bioactive fractions of a marine sponge *Topsentia* sp., of which 16 were new (**1**, **2**, **8**, **10**−**14**, **16**, **17**, **19**, **21**, **24**, **25**, **27**, and **30**). They were characterized as sterols with 10 different side chains and as having various functionalities including 5α,8α-epidioxy (**1**−**9**), 5α,6α-epoxy-7-ol (**10**−**15**), 5,8-dien-7-one (**24**−**28**), 5-en-3*β*-ol (**29**), and 1(10→6)abeo-5,7,9-triene-3α,11α-diol (**30**) units and included polyoxygenated sterols (**16**−**23**). One of the key features of these new sterols is the presence of the (24*R*,25*R*,27*R*)-26,27-cyclo-24,27-dimethylcholestane side chain, whose absolute stereochemistry was defined by an acid-catalyzed ring-opening method and by comparison with the four synthetic isomers of known absolute stereochemistry. The occurrence of several known fungal sterols and relevant new sterols in this sponge suggested their possible origin from symbiotic fungi. Selected compounds were tested against a panel of five human solid tumor cell lines and displayed moderate to marginal cytotoxicity.

Sponges have been recognized as one of the most plentiful sources of diverse sterols, and the number of novel and bioactive sterols from sponges is still increasing each year.[1] A previous search for cytotoxic components from a marine sponge *Topsentia* sp. has led to the isolation of seven new cytotoxic oxylipins.[2] The gross ^{1}H NMR spectra of the less polar bioactive fractions from this sponge indicated the presence of sterols with upfield cyclopropane signals and oxygenated methylene or methine signals. These suggested the presence of both a 26,27-cyclo sterol side chain and polyoxygenated functionalization in their structures. Therefore, it seemed worthwhile to continue the investigation of these 26,27-cyclo and polyoxygenated sterols, which have not been encountered in the sponge *Topsentia* sp. previously.[3] The occurrence of 26,27-cyclo sterols is still uncommon, and earlier research has shown that they only appear in marine sponges.[4−11] The present report describes the isolation, structure elucidation, and cytotoxicity evaluation of these new and known sterols.

Results and Discussion

Topsentisterol A_1 (**1**) was isolated as a white, amorphous solid. Its molecular formula was established as $C_{29}H_{46}O_3$ on the basis of FABMS and by NMR analysis. In addition to the $[M + Na]^+$ ion at *m*/*z* 465 in the LRFABMS, an intense $[M - O_2]^+$ ion was also observed at *m*/*z* 410, which is typical for the loss of O_2 from the epidioxy moiety.[12] The exact mass of the $[M + Na]^+$ ion (*m*/*z* 465.3339, Δ −0.6 mmu) matched well with the expected molecular formula of $C_{29}H_{46}O_3Na$. In the ^{1}H NMR spectrum, the upfield methyl signals at *δ* 0.84 (H_3-18), 0.89 (H_3-19), and 0.93 (H_3-21) supported a steroidal skeleton. The 5α,8α-epidioxy functionality in the B ring was identified by the characteristic signals of H-6 and H-7 at *δ* 6.25 (d, *J* = 8.5 Hz) and 6.53 (d, *J* = 8.5 Hz), respectively.[13] This was corroborated by the HMBC correlations observed from H-6 and H-7 to C-5 (*δ* 82.5) and C-8 (*δ* 79.0). The broad oxymethine proton signal at *δ* 3.77 ($W_{1/2}$ = 16.5 Hz) indicated an OH-3*β* group at C-3. Therefore, the nucleus of compound **1** was established as 5α,8α-epidioxycholest-6-en-3*β*-ol. The four quite upfield shifted multiplets at *δ* 0.09 (H-26), 0.13 (H-25), 0.15 (H-26), and 0.45 (H-27) suggested a disubstituted cyclopropane ring in the side chain. COSY correlations from H-26 to H-27 and H-25, from H-27 to H_3-27a (*δ* 1.01), and from H-24 (*δ* 0.58) to H_3-24^1 (*δ* 0.91) and H-25 were observed. In the HMBC spectrum, the correlations from H_3-24^1 to C-24 (*δ* 39.6), C-23 (*δ* 36.5), and C-25 (*δ* 28.2) and from H_3-27a to C-25, C-27 (*δ* 14.0), and C-26 (*δ* 12.8) confirmed the presence of a 26,27-cyclo-24,27-dimethylcholestane side chain.

The *trans* configuration of the 26,27-cyclopropane ring was deduced by correlation from H_3-27a to H-25 in the NOESY experiment. The absolute stereochemistry of the side chain was defined as 24*R*,25*R*,27*R* by comparison of the ^{1}H NMR data of the ring cleavage product (**31**) with those of four synthetic stereoisomers (**34**, **35**, **36**, and **37**).[14] The cyclopropane ring of compound **1** was cleaved by catalytic hydrogenolysis (see Experimental Section) to afford 24,25-dimethylcholest-8(14)-ene-3*β*,5α-diol (**31**) as the major product (Scheme 1), which showed an almost identical ^{1}H NMR pattern for the three methyls (H_3-24^1, -25^1, and -27) with those of the model isomer **34** (Table 4). For further confirmation, topsentisterol D_1 (**24**) with the same side chain was also cleaved and afforded **32** and **33**, respectively, in the ratio of 2:1. The ^{1}H NMR pattern of their side chain methyls again matched well with those of **34**. Naturally occurring sterols with the same side chain were also reported to possess the 24*R*,25*R*,27*R* configuration.[4−10] Therefore, the structure of compound **1** was defined as (24*R*,25*R*,27*R*)-5α,8α-epidioxy-26,27-cyclo-24,27-dimethylcholest-6-en-3*β*-ol. Other topsentisterols (**10**−**12**, **16**, **17**, **21**, and **24**) with identical ^{1}H and ^{13}C NMR data for the 26,27-cyclopropane ring are presumed to share the same configuration of the side chain. The 26,27-cyclopropane side chain is uncommon, and it has been reported only from marine sponges.

Topsentisterol A_2 (**2**) was isolated as a white, amorphous solid. Its molecular formula was determined as $C_{29}H_{48}O_3$ on the basis of FABMS and by NMR analysis. In the LRFABMS, both $[M + Na]^+$ and $[M - O_2]^+$ ions were observed at *m*/*z* 467 and 412, respectively. The HRFABMS showed a $[M + Na]^+$ ion at *m*/*z* 467.3517 (Δ +1.6 mmu), corresponding to the molecular formula $C_{29}H_{48}O_3Na$. Comparison of its NMR data with those of **1** revealed that it possesses the same 5α,8α-epidioxy nucleus, with the only difference being in the side chain. The ^{1}H NMR spectrum showed two singlet signals at *δ* 0.84 (H_3-18) and 0.89 (H_3-19) and a doublet signal at *δ* 0.95 (H_3-21), which showed correlations to C-20, C-17, and C-22

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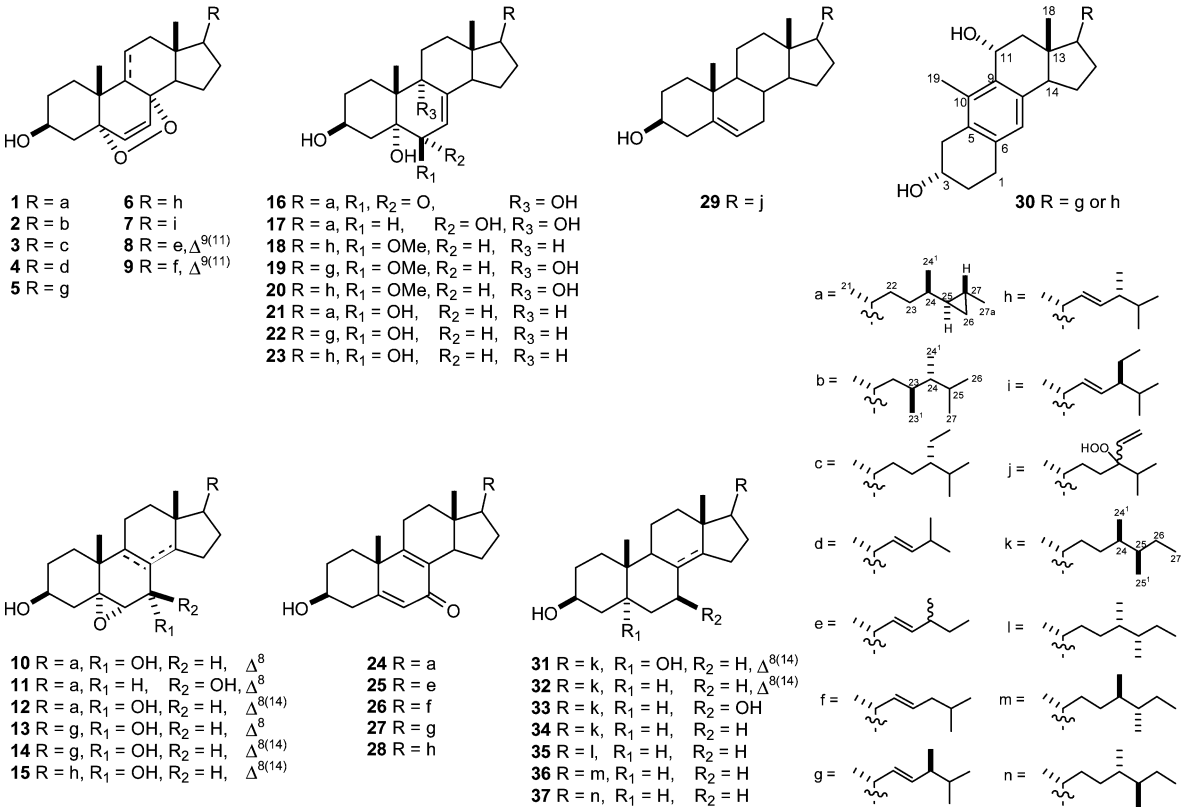
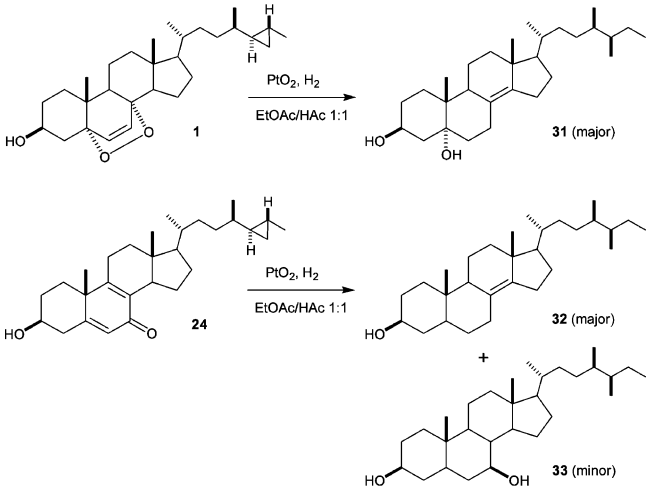
Chart 1



Scheme 1. Reductive Cleavage of the Cyclopropane Ring of Compounds **1** and **24**[14]



in the HMBC spectrum. The other four doublets at δ 0.79, 0.82, 0.90, and 0.91 were attributed to H_3-23[1], H_3-27, H_3-24[1], and H_3-26, respectively, on the basis of COSY and HMBC NMR spectroscopic analysis. The stereochemistry at C-23 and C-24 was assigned as 23*S* and 24*R*, respectively, by comparison of the ^{1}H NMR data of the side chain methyl signals (H_3-21, -23[1], -24[1], -26, and -27) with those of model stereoisomers, i.e., (23*R*,-24*R*), (23*S*,24*S*), (23*R*,24*S*), and (23*S*,24*R*).[15] This is the first report of a 5α,8α-epidioxy sterol with a 23,24-dimethylcholestane side chain.

Various 5α,8α-epidioxy sterols have been encountered frequently in marine organisms.[12,13] In the present study, by comparison with previously reported NMR data, compounds **3**−**7** as well as **9** were identified as known 5α,8α-epidioxy sterols.[12,16,17] However, topsentisterol A_3 (**8**) was defined as a new 5α,8α-epidioxy $\Delta^{6,9(11)}$ sterol with a 24-methyl-27-norcholest-22-ene side chain. The molecular formula of **8** was determined as $C_{27}H_{40}O_3$ on the basis of the analysis of its FABMS and NMR data. In the LRFABMS, the $[M + Na]^+$ and $[M - O_2]^+$ ions were observed at *m*/*z* 435 and 380, respectively. The HRFABMS ($[M + Na]^+$, *m*/*z* 435.2876, Δ +0.1 mmu) also supported this molecular formula. Compared to **1**, the signals of H-6 and H-7 were shifted downfield to δ 6.33 (d, J = 8.0 Hz) and 6.67 (d, J = 8.0 Hz), respectively. An additional olefinic proton signal at δ 5.50 (dd, J = 6.5, 2.0 Hz) attributed to H-11 was observed. The above features were consistent with a 5α,8α-epidioxy $\Delta^{6,9(11)}$ sterol nucleus.[13] Two doublet methyls at δ 0.96 (d, J = 6.5 Hz) and 1.03 (d, J = 6.5 Hz) were assigned to H_3-24[1] and H_3-21 on the basis of the HMBC spectrum. A triplet methyl at δ 0.88 (t, J = 7.0 Hz) was attributed to a terminal methyl (H_3-26), and its HMBC correlations to C-25 and C-24 were observed. The stereochemistry at C-24 could not be defined using the ^{1}H NMR data because only a single epimer was isolated from this sponge.[18] Thus, the structure of compound **8** was defined as (22*E*)-5α,8α-epidioxy-24ξ-methyl-27-norcholesta-6,9(11),22-trien-3β-ol.

The investigation of bioactive sterols from this same sponge has also resulted in the isolation of 5α,6α-epoxy-7-ol sterols, of which five (**10**−**14**) are new and one (**15**) is known. Topsentisterol B_1 (**10**) was isolated as a white, amorphous solid and gave a pseudomolecular ion at *m*/*z* 465.3345 ($[M + Na]^+$, Δ 0.0 mmu) in its HRFABMS, suggesting a molecular formula of $C_{29}H_{46}O_3Na$. In the ^{1}H NMR spectrum, the downfield signals at δ 3.20, 3.75, and 4.25 were attributed to three oxymethine protons. The COSY spectrum showed correlations between H-6 (δ 3.20) and H-7 (δ 4.25), which indicated the presence of an epoxy methine and its neighboring oxymethine proton, respectively. In the HMBC spectrum, H-6 showed correlations to C-7 (δ 67.6) and C-8 (δ 128.0), and H_3-19 showed correlations to C-5 (δ 65.6) and C-9 (δ 135.5), suggesting a 5,6-epoxycholest-8-ene-3,7-diol nucleus for compound **10**. The relative configuration was defined as epoxy-5α,6α and OH-7α by comparison of its ^{1}H and ^{13}C NMR data with those of known compounds[19,20] and **13** (vide infra). The upfield signals in the ^{1}H NMR spectrum displayed an identical pattern to that of **1**, which

Table 1. ^{1}H NMR Data for Compounds **1**, **8**, **10**−**14**, and **16** (CD_3OD, 500 MHz)[a]

position	1	8	10	11	12	13	14	16
1	1.70 (dt, 13.5, 3.0)	2.00 (m)	1.58 (m)	1.58 (m)	1.68 (ddd, 12.5, 3.5, 2.5)	1.57 (m)	1.40 (dd, 13.0, 3.5)	1.52 (m)
	1.85 (m)		1.78 (m)	1.78 (m)	1.96 (ddd, 12.5, 4.0, 2.0)	1.75 (m)	1.68 (ddd, 13.0, 3.5, 3.5)	2.29 (td, 13.5, 3.5)
2	1.52 (m)	1.55 (m)	1.55 (m)	1.62 (m)	1.55 (m)	1.60 (m)	1.55 (m)	1.45 (m)
	1.77 (m)	1.85 (m)	1.90 (m)	1.90 (m)	1.90 (m)	1.90 (m)	1.88 (m)	1.86 (m)
3	3.77 (m)	3.83 (m)	3.75 (m)	3.74 (m)	3.75 (m)	3.78 (m)	3.74 (m)	3.92 (m)
4	1.88 m	1.95 (m)	1.35 (m)	1.37 (m)	1.32 (m)	1.35 (m)	1.32 (dd, 13.0, 7.5)	1.63 (dd, 14.0, 11.5)
	2.00 (m)		2.13 (m)	2.16 (dd, 13.0, 11.5)	2.11 (dd, 13.0, 12.0)	2.15 (m)	2.10 (m)	2.02 (dd, 14.0, 2.0)
6	6.25 (d, 8.5)	6.33 (d, 8.0)	3.20 (br s)	3.02 (d, 3.0)	3.05 (d, 3.0)	3.19 (d, 2.0)	3.04 (d, 2.5)	
7	6.53 (d, 8.5)	6.67 (d, 8.0)	4.25 (br s)	4.28 (d, 3.0)	4.42 (d, 3.0)	4.24 (d, 2.0)	4.40 (d, 2.5)	5.58 (d, 2.0)
9	1.44 (m)				2.41 (m)		2.42 (m)	
11	1.51 (m)	5.50 (dd, 6.5, 2.0)	2.05 (m)	2.05 (m)	1.45 (m)	2.06 (m)	1.45 (m)	1.74 (m)
								1.95 (m)
12	1.22 (m)	2.05 (m)	1.18 (m)	1.18 (m)	1.20 (m)	1.38 (m)	1.20 (m)	1.28 (m)
	1.98 (m)	2.32 (dd, 17.0, 6.5)			1.93 (m)	1.98 (dd, 12.0, 5.5)	1.95 (ddd, 13.5, 3.5, 3.5)	1.56 (m)
14	1.49 (m)	1.82 (m)	2.16 (m)	2.12 (m)		2.18 (m)		2.74 (ddd, 12.0, 7.0, 2.0)
15	1.24 (m)	1.24 (m)	1.90 (m)	1.65 (m)	2.28 (m)	1.28 (m)	2.25 (m)	1.52 (m)
	1.52 (m)	1.55 (m)			2.68 (ddd, 12.5, 8.5, 8.0)	2.15 (m)	2.66 (ddd, 15.5, 8.5, 8.0)	1.69 (m)
16	1.40 (m)	1.38 (m)	1.47 (m)	1.38 (m)	1.45 (m)	1.35 (m)	1.45 (m)	2.01 (m)
	1.94 (m)			1.98 (m)	1.90 (m)	1.78 (m)	1.74 (m)	
17	1.20 (m)	1.36 (m)	1.16 (m)	1.15 (m)	1.24 (m)	1.19 (ddd, 19.0, 9.0, 9.0)	1.25 (m)	1.41 (m)
18	0.84 (s)	0.79 (s)	0.62 (s)	0.66 (s)	0.89 (s)	0.68 (s)	0.90 (s)	0.65 (s)
19	0.89 (s)	1.12 (s)	1.13 (s)	1.28 (s)	0.87 (s)	1.13 (s)	0.87 (s)	0.99 (s)
20	1.35 (m)	2.05 (m)	1.46 (m)	1.38 (m)	1.46 (m)	2.04 (m)	2.10 (m)	1.38 (m)
21	0.93 (d, 7.0)	1.03 (d, 6.5)	0.95 (d, 6.5)	0.95 (d, 7.0)	0.95 (d, 6.5)	1.02 (d, 6.5)	1.02 (d, 6.5)	0.97 (d, 7.0)
22	1.25 (m)	5.21 (m)	1.40 (m)	1.45 (m)	1.24 (m)	5.18 (m)	5.21 (m)	1.23 (m)
			1.56 (m)	1.55 (m)	1.46 (m)			1.52 (m)
23	1.37 (m)	5.18 (m)	1.28 (m)	1.28 (m)	1.28 (m)	5.20 (m)	5.22 (m)	1.30 (m)
			1.35 (m)	1.35 (m)	1.35 (m)			1.38 (m)
24	0.58 (m)	1.92 (m)	0.60 (m)	0.60 (m)	0.60 (m)	1.82 (m)	1.82 (m)	0.62 (m)
24[1]	0.91 (d, 7.0)	0.96 (d, 6.5)	0.91 (d, 7.0)	0.90 (d, 6.5)	0.90 (d, 6.5)	0.92 (d, 7.0)	0.92 (d, 7.0)	0.91 (d, 6.5)
25	0.13 (m)	1.18 (m) 1.25 (m)	0.13 (m)	0.12 (m)	0.13 (m)	1.45 (m)	1.45 (m)	0.11 (m)
26	0.09 (m)	0.88 (t, 7.0)	0.09 (m)	0.09 (m)	0.09 (m)	0.83 (d, 7.5)	0.84 (d, 7.0)	0.09 (m)
	0.15 (m)		0.15 (m)	0.15 (m)	0.15 (m)			0.15 (m)
27	0.45 (m)		0.45 (m)	0.45 (m)	0.45 (m)	0.85 (d, 7.5)	0.86 (d, 7.0)	0.46 (m)
27a	1.01 (d, 6.0)		1.01 (d, 6.0)	1.01 (d, 6.5)	1.01 (d, 6.0)			1.01 (d, 7.0)

[a] Multiplicities and coupling constants are in parentheses.

indicated that **10** possesses the same cyclopropane side chain. Therefore, the structure of topsentisterol B_1 was established as (24*R*,25*R*,27*R*)-5α,6α-epoxy-26,27-cyclo-24,27-dimethylcholest-8-ene-3*β*,7α-diol.

Topsentisterol B_2 (**11**) was isolated as a white, amorphous solid. Its molecular formula was determined as $C_{29}H_{46}O_3$ on the basis of HRFABMS and NMR experiments. The HRFABMS showed a $[M + Na]^+$ ion at *m*/*z* 465.3342 (Δ −0.3 mmu). Its ^{1}H NMR data were almost the same as those of compound **10** except for the obvious differences at H-6 and H-7 (Table 1), which were attributed to the different configuration at C-7. For compound **11**, the OH-7*β* configuration was assigned by a pyridine-induced deshielding effect.[21,22] In the ^{1}H NMR spectrum, which was measured in pyridine-d_5, the H_3-19 and H_3-18 methyl signals were shifted downfield to *δ* 1.57 and 0.88, respectively, compared to *δ* 1.28 and 0.66 in methanol-d_4 (Figure 1). Thus, topsentisterol B_2 was defined as the 7*β*-epimer of **10**.

Topsentisterol B_3 (**12**) was also isolated as a white, amorphous solid. Its HRFABMS showed a $[M + Na]^+$ ion at *m*/*z* 465.3350 (Δ +0.5 mmu), establishing the molecular formula of $C_{29}H_{46}O_3$. By comparing its ^{1}H NMR data with those of **10**, differences were again noted for H-6 and H-7 (Table 1). The allylic proton signals at *δ* 2.41, 2.28, and 2.68 were assigned to H-9, Ha-15, and Hb-15 by interpretation of the HSQC and HMBC spectra. In addition, the HMBC spectrum also showed correlations from H-6 (*δ* 3.05) to C-7 (*δ* 65.6) and C-8 (*δ* 126.2), from H-7 (*δ* 4.42) to C-8, C-9 (*δ* 40.5), and C-14 (*δ* 153.0), and from H_3-18 (*δ* 0.89) to C-14. These data indicated that a tetrasubstituted double bond was located between C-8 and C-14. By further comparison of its NMR data with literature values,[20] the structure of compound **12** was elucidated as (24*R*,25*R*,27*R*)-5α,6α-epoxy-26,27-cyclo-24,27-dimethylcholest-8(14)-ene-3*β*,7α-diol.

Topsentisterol B_4 (**13**) was isolated as a white, amorphous solid with a molecular formula of $C_{28}H_{44}O_3$, which was established by HRFABMS of the $[M + Na]^+$ ion at *m*/*z* 451.3192 (Δ +0.4 mmu). The ^{1}H NMR data of compound **13** were almost identical with those of **10** and displayed differences only in the side chain. The NOESY correlations from H-7 (*δ* 4.24) to H_3-19 (*δ* 1.13) and H-4*β* (*δ* 1.35) suggested that H-7 is on the same *β* face of the ring. The OH-7α configuration was further corroborated by the negligible shifts of H_3-19 (Δ*δ* +0.08) and H_3-18 (Δ*δ* +0.06) of the ^{1}H NMR spectrum run in pyridine-d_5. The side chain of **13** was found to be a common 24-methylcholest-22-ene functionality, and the stereochemistry at C-24 was defined as *S* by comparison of the H_3-21 chemical shift with **14** and its 24*R*-epimer (**15**) (vide infra). The skeleton of **13** has been reported from the mushroom *Grifola frondosa*[22] as its 24*R*-epimer. However, this is the first occurrence of the 24*S*-epimer.

Table 2. ^{1}H NMR Data for Compounds **17**, **19**, **21**, **24**, **25**, **27**, and **30** (CD_3OD, 500 MHz)[a]

position	17	19	21	24	25	27	30
1	1.38 (m)	1.34 (m)	1.45 (m)	1.25 (m)	1.25 (m)	1.25 (m)	2.75 (m)
	2.22 (m)	2.17 (dt, 13.5, 4.0)	1.55 (m)	2.15 (m)	2.15 (m)	2.15 (m)	2.86 (dt, 17.0, 6.0)
2	1.44 (m)	1.48 (m)	1.77 (m)	1.71 (ddd, 25.0, 13.5, 4.0)	1.71 (ddd, 25.0, 13.5, 4.5)	1.70 (ddd, 25.0, 14.0, 4.0)	1.68 (m)
	1.85 (m)	1.84 (m)		1.92 (m)	1.91 (m)	1.92 (m)	1.98 (m)
3	3.89 (m)	3.97 (m)	3.96 (m)	3.54 (m)	3.54 (m)	3.54 (m)	4.05 (m)
4	1.46 (m)	1.65 (ddd, 13.5, 5.0, 2.0)	1.67 (ddd, 13.5, 5.0, 2.0)	2.55 (m)	2.55 (m)	2.56 (m)	2.47 (dd, 16.5, 8.0)
	2.12 (m)	2.10 (dd, 13.5, 12.0)	2.05 (m)				3.00 (dd, 16.5, 4.5)
6	3.87 (br s)	3.22 (dd, 5.0, 2.0)	3.54 (m)	6.00 (br s)	6.00 (br s)	6.00 (br s)	
7	5.05 (br s)	5.47 (dd, 5.0, 2.0)	5.26 (m)				6.60 (s)
9			1.97 (m)				
11	1.28 (m)	1.58 (m)	1.55 (m)	2.50 (m)	2.50 (m)	2.50 (m)	5.09 (dd, 9.0, 6.5)
	2.12 (m)						
12	1.58 (m)	1.55 (m)	1.30 (m)	1.51 (m)	1.51 (m)	1.51 (m)	1.75 (dd, 13.5, 6.5)
	1.82 (m)	1.80 (m)	2.05 (m)	2.14 (m)	2.12 (m)	2.12 (m)	2.63 (dd, 13.5, 9.0)
14	2.49 (m)	2.50 (m)	1.94 (m)	2.25 (m)	2.26 (m)	2.26 (m)	2.92 (dd, 11.0, 8.0)
15	1.55 (m)	1.49 (m)	1.52 (m)	1.40 (m)	1.39 (m)	1.39 (m)	1.50 (m)
		1.52 (m)	1.57 (m)	2.50 (m)	2.52 (m)	2.53 (m)	2.00 (m)
16	1.55 (m)		1.42 (m)	1.37 (m)	1.36 (m)	1.36 (m)	1.87 (m)
	1.82 (m)			2.02 (m)	1.82 (m)	1.82 (m)	
17	1.33 (m)	1.35 (m)	1.25 (m)	1.26 (m)	1.26 (m)	1.26 (m)	1.48 (m)
18	0.60 (s)	0.63 (s)	0.63 (s)	0.67 (s)	0.68 (s)	0.68 (s)	0.44 (s)
19	1.05 (s)	1.02 (s)	1.05 (s)	1.37 (s)	1.38 (s)	1.38 (s)	2.26 (s)
20	1.34 (m)	2.05 (m)	1.35 (m)	1.40 (m)	2.06 (m)	2.06 (m)	2.05 (m)
21	0.95 (d, 6.5)	1.02 (d, 6.5)	0.95 (d, 6.0)	0.99 (d, 7.0)	1.06 (d, 7.0)	1.06 (d, 6.5)	1.09 (d, 6.5)
22	1.28 (m)	5.18 (m)	1.24 (m)	1.05 (m)	5.22 (dd, 15.0,	5.20 (m)	5.22 (m)
				1.57 (m)	8.0)		
23	1.38 (m)	5.20 (m)	1.33 (m)	1.27 (m)	5.16 (dd, 15.0,	5.22 (m)	5.23 (m)
				1.38 (m)	7.5)		
24	0.60 (m)	1.85 (m)	0.60 (m)	0.60 (m)	1.92 (m)	1.84 (m)	1.85 (m)
24[1]	0.91 (d, 7.0)	0.93 (d, 7.0)	0.91 (d, 7.0)	0.91 (d, 7.0)	0.94 (d, 6.5)	0.93 (d, 6.5)	0.94 (d, 7.0)
25	0.12 (m)	1.45 (m)	0.11 (m)	0.13 (m)	1.24 (m)	1.45 (m)	1.48 (m)
					1.32 (m)		
26	0.09 (m)	0.84 (d, 7.5)	0.09 (m)	0.09 (m)	0.86 (t, 7.0)	0.84 (d, 6.5)	0.85 (d, 7.0)
	0.15 (m)		0.15 (m)	0.15 (m)			
27	0.45 (m)	0.86 (d, 7.0)	0.45 (m)	0.45 (m)		0.86 (d, 7.0)	0.87 (d, 7.0)
27a	1.01 (d, 6.5)		1.01 (d, 6.5)	1.01 (d, 6.5)			
OCH_3		3.38 (s)					

[a] Mutiplicities and coupling constants are in parentheses.

Therefore, **13** was defined as a new derivative with the structure (22*E*,24*S*)-5α,6α-epoxy-24-methylcholesta-8,22-diene-3β,7α-diol.

Topsentisterol B_5 (**14**) was also isolated as a white, amorphous solid. The molecular formula was established as $C_{28}H_{44}O_3$ by HRFABMS analysis ($[M + Na]^+$, *m*/*z* 451.3188, Δ 0.0 mmu). A comparison of its NMR data revealed that **14** shares the same nucleus as compound **12** and the same side chain as **13**. The stereochemistry at C-24 was defined as *S* by comparison of the H_3-21 chemical shift with its 24*R*-epimer (**15**) (vide infra). Therefore, **14** was established as a new derivative with the structure (22*E*,24*S*)-5α,6α-epoxy-24-methylcholesta-8(14),22-diene-3β,7α-diol.

Compound **15** was identified as the known compound 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol, which has been found previously in an edible mushroom.[22] It showed almost the same NMR data as **14**, with the only difference being in the side chain. In its ^{1}H NMR spectrum, the chemical shift of H_3-21 (δ 1.03) was shifted downfield compared to the 24*S*-epimer (**14**), which showed the signal for H_3-21 at δ 1.02.[23,24] Therefore, compound **15** was identified as the 24*R*-epimer of **14**.

The characteristic feature of compounds **16**−**23** was polyoxygenation of the B ring. Topsentisterol C_1 (**16**) was isolated as a white, amorphous solid. The HRFABMS gave a $[M + Na]^+$ ion at *m*/*z* 481.3294 (Δ +0.1 mmu), in accordance with a molecular formula of $C_{29}H_{46}O_4Na$. The ^{13}C NMR spectrum indicated the presence of 29 carbons including an α,β-unsaturated ketone carbonyl carbon signal at δ 200.2 (C-6), two olefinic carbons at δ 120.9 (C-7) and 165.1 (C-8), and three oxygenated carbons at δ 67.8 (C-3), 76.1 (C-9), and 80.2 (C-5). In the COSY spectrum, a long-range correlation from δ 5.58 (d, J = 2.0 Hz, H-7) to 2.74 (ddd, J = 12.0, 7.0, 2.0 Hz, H-14) was observed, which suggested the location of the trisubstituted double bond between C-7 and C-8. In the HMBC spectrum, correlations from H_3-19 (δ 0.99) to C-5 and C-9 and from H-7 (δ 5.58) to C-5, C-9, and C-14 (δ 52.8) were observed. These data suggested a 3,5,9-trihydroxycholest-7-en-6-one nucleus. The OH-3β,5α configuration was established by the downfield shifted broad oxymethine multiplet at δ 3.92 ($W_{1/2}$ = 16.0 Hz, H-3).[25,26] Since all natural steroids have a *trans* B/C fusion,[27] the configuration of the hydroxyl group at C-9 was presumed as α.[28] The pyridine-induced deshielding effect was also used to confirm the OH-9α configuration, and the signal of H-14 was shifted downfield from δ 2.74 to 3.03. By comparison of the upfield signals, it was recognized that **16** shares the same cyclopropane side chain as **1**. Thus, the structure of **16** was established as (24*R*,25*R*,27*R*)-3β,5α,9α-trihydroxy-26,27-cyclo-24,27-dimethylcholest-7-en-6-one.

Topsentisterol C_2 (**17**) was isolated as a white, amorphous solid and obtained as a trace component. The sodiated molecular ion $[M + Na]^+$ at *m*/*z* 483.3453 (Δ +0.3 mmu) was observed in its HRFABMS, supporting a molecular formula of $C_{29}H_{48}O_4Na$. Its ^{1}H NMR data showed an olefinic proton signal at δ 5.05 (br s, H-7) and two oxymethine signals at δ 3.89 (m, $W_{1/2}$ = 16.5 Hz, H-3) and 3.87 (br s, H-6). The COSY spectrum displayed

Table 3. ^{13}C NMR Data for Compounds **1**, **8**, **10**−**14**, **16**, **17**, **19**, **21**, **24**, **25**, **27**, and **30** (CD_3OD)

position	**1**[a]	**8**[a]	**10**[a]	**11**[a]	**12**[a]	**13**[a]	**14**[a]	**16**[b]	**17**[a]	**19**[a]	**21**[a]	**24**[b]	**25**[a]	**27**[a]	**30**[a]
1	35.5	33.8	31.2	31.8	33.2	31.0	33.2	27.2	27.8	27.8	31.6	35.8	35.7	35.5	28.5
2	30.6	31.0	31.6	31.6	31.6	31.5	31.6	30.9	30.6	31.0	31.4	31.3	31.1	31.0	32.0
3	66.6	66.8	69.0	69.0	69.0	69.0	69.0	67.8	67.8	68.0	68.0	72.7	72.6	72.2	68.4
4	37.5	36.5	40.0	39.8	40.0	39.8	40.0	37.4	40.5	40.8	40.4	42.8	42.6	42.6	36.5
5	82.5	84.0	65.6	65.0	67.6	65.5	67.6	80.2	77.8	78.0	76.8	166.3	166.5	166.0	131.6
6	136.8	137.5	64.0	62.0	62.4	64.0	62.4	200.2	71.0	83.0	74.0	126.6	125.5	126.2	135.4
7	131.1	132.5	67.6	67.0	65.6	67.5	65.0	120.9	121.6	118.0	119.0	188.5	188.5	188.5	123.5
8	79.0	79.5	128.0	127.2	126.2	128.0	126.2	165.1	143.0	144.4	144.0	134.6	134.0	134.5	139.4
9	52.4	144.0	135.5	137.0	40.5	135.0	40.4	76.1	75.8	76.0	44.0	165.4	165.0	165.0	134.4
10	38.0	39.0	39.0	38.8	37.0	39.0	37.0	42.7	42.0	42.0	38.0	43.7	43.5	43.5	136.8
11	21.0	120.5	24.5	24.5	20.0	24.4	19.5	30.4	30.5	28.8	24.0	25.7	25.7	25.7	67.4
12	40.5	42.0	37.0	37.8	38.0	36.8	37.5	36.2	36.2	36.5	40.2	36.8	36.6	36.3	50.0
13	45.5	44.5	43.0	43.0	44.0	43.0	44.0	46.3	44.8	45.0	44.5	43.6	43.3	43.2	46.0
14	52.6	49.5	50.8	53.8	153.0	50.8	153.0	52.8	51.2	51.8	55.7	49.5	49.2	49.2	51.5
15	24.5	24.4	27.5	27.5	25.5	32.0	25.5	23.4	23.5	24.0	23.2	25.8	25.6	25.8	25.0
16	28.8	30.0	30.7	30.7	31.6	30.5	28.7	28.5	27.6	27.8	31.5	30.4	30.9	30.4	23.8
17	57.5	57.0	54.8	55.5	57.8	54.8	57.8	57.7	57.2	57.0	57.1	54.8	54.4	54.5	57.0
18	12.8	20.5	12.0	11.5	18.2	11.8	18.2	12.3	11.8	11.3	12.6	12.1	12.1	12.1	12.5
19	19.5	21.0	22.6	23.0	16.8	22.8	16.8	19.4	20.3	21.6	18.5	24.2	24.1	24.2	14.7
20	34.3	41.0	35.8	37.5	35.6	42.6	41.0	37.1	37.0	42.0	35.3	37.5	41.5	41.5	42.0
21	19.5	21.0	19.5	19.2	19.5	21.8	21.5	19.4	19.2	21.6	19.4	19.3	21.4	20.6	21.5
22	34.6	136.0	34.5	34.4	35.0	137.0	138.0	35.1	35.2	137.0	35.0	34.6	136.0	136.0	137.0
23	36.5	135.0	35.5	35.0	34.8	133.0	134.0	34.5	35.0	133.2	35.0	35.1	135.0	133.2	133.5
24	39.6	39.8	39.8	40.0	39.8	44.3	44.3	40.1	40.0	44.5	40.0	40.1	39.7	44.2	44.5
24[1]	19.5	20.5	20.2	20.2	20.2	18.0	20.5	20.2	20.2	18.0	20.2	20.2	21.0	17.0	18.0
25	28.2	30.5	28.2	28.2	28.2	34.2	35.4	28.6	28.2	34.5	28.2	28.5	30.6	34.1	34.5
26	12.8	13.0	12.2	12.2	12.2	19.8	19.8	12.3	13.3	19.8	13.0	12.3	12.1	19.5	20.0
27	14.0		13.8	13.5	13.8	19.8	19.8	13.8	13.1	19.8	13.7	13.8		20.2	20.5
27a	19.5		19.5	19.6	19.5			20.5	19.5		19.2	19.4			
OCH_3										57.8					

[a] Signals were assigned by HMBC and HSQC experiments (500 MHz). [b]Spectrum was measured at 75 MHz.

Table 4. Comparison of ^1H NMR Data for Compounds **31**−**37** ($CDCl_3$)[a]

compound	H_3-24[1]	H_3-25[1]	H_3-27	$\delta_{H_3-24^1} - \delta_{H_3-25^1}$
31	0.751	0.733	0.862	+0.018
32	0.750	0.732	0.860	+0.018
33	0.750	0.728	0.859	+0.022
34 (24*R*,25*R*)	0.747	0.724	0.857	+0.023
35 (24*S*,25*S*)	0.726	0.726	0.856	0
36 (24*R*,25*S*)	0.790	0.805	0.855	−0.015
37 (24*S*,25*R*)	0.796	0.811	0.856	−0.015

[a] The ^1H NMR data of **34**−**37** are cited from ref 14.

correlations from H-7 to H-6 and H-14 (long-range correlations). In the HMBC spectrum, correlations from H-6 to C-7 (δ 121.6) and C-8 (δ 143.0) and from H_3-19 (δ 1.05) to C-5 (δ 77.8) and C-9 (δ 75.8) were observed. Thus, the above data indicated a 3,5,6,9-tetrahydroxycholest-7-ene nucleus.[25] The OH-3β,5α configuration was suggested by the broad oxymethine signal at δ 3.89 ($W_{1/2}$ = 16.5 Hz, H-3). The configuration of the OH-6α group was defined by a NOESY experiment (Figure 1), which showed correlations from H_3-19 to H-6 and in turn from H-6 to H-4β (δ 1.46). The ^1H NMR data revealed that **17** possesses the same side chain as **1**. Therefore, topsentisterol C_2 was defined as (24*R*,25*R*,-27*R*)-26,27-cyclo-24,27-dimethylcholest-7-ene-3β,5α,6α,9α-tetrol.

By comparison of the NMR data, **18** was identified as the known compound 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene, which has been reported from the fruiting bodies of *Agaricus blazei*.[29]

Topsentisterol C_3 (**19**) was isolated as a white, amorphous solid. The molecular formula was assigned as $C_{29}H_{48}O_4$ by HRFABMS analysis ($[M + Na]^+$, *m*/*z* 483.3477, Δ +2.7 mmu). The ^1H NMR spectroscopic data showed three olefinic proton signals at δ 5.47 (H-7), 5.20 (H-23), and 5.18 (H-22), two oxymethine proton signals at δ 3.97 ($W_{1/2}$ = 16.0 Hz, H-3) and 3.22 (H-6), and a methoxy proton signal at δ 3.38. In the COSY spectrum, correlations from H-7 to H-6 and H-14 (long-range correlations) were observed. The methoxy signal showed a correlation to C-6 (δ 83.0) in the HMBC

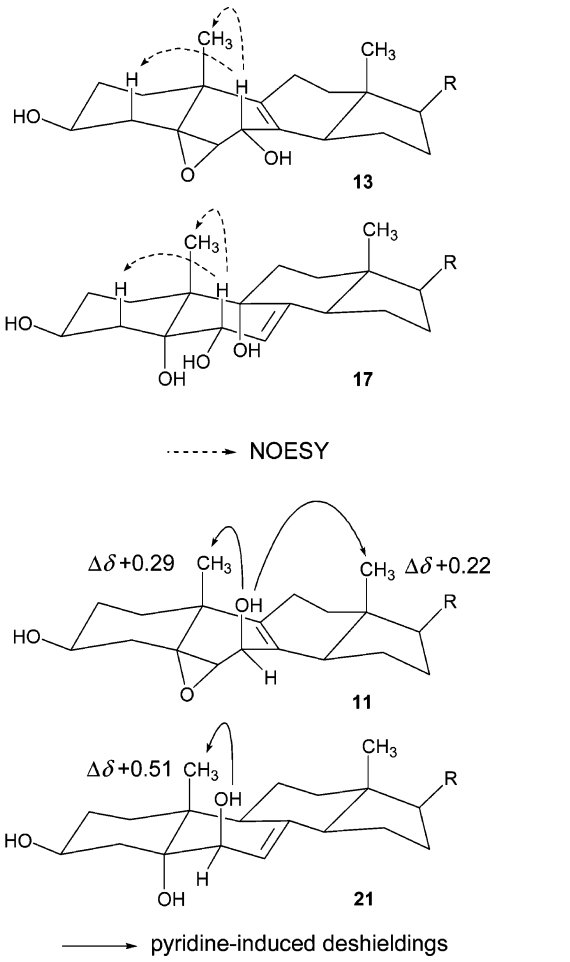


Figure 1. Key NOESY correlations and pyridine-induced deshieldings for compounds **11**, **13**, **17**, and **21**.

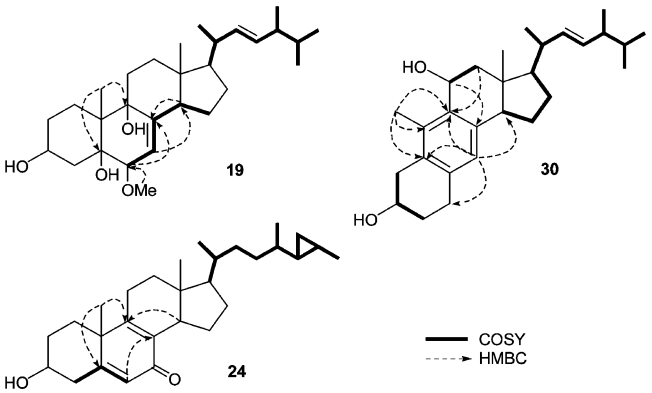


Figure 2. Key COSY and HMBC correlations for compounds **19**, **24**, and **30**.

spectrum. With the aid of COSY and HMBC experiments, the 3β,5α,9α-trihydroxy-6β-*O*-methylcholest-7-ene nucleus was established, and the key correlations are shown in Figure 2. The OMe-6β configuration was determined from the large coupling constant (J = 5.0 Hz) between H-6 and H-7.[16] Compound **19** was found to possess a 24-methylcholest-22-ene side chain.[22] The stereochemistry at C-24 was determined as *S* by comparison of the chemical shift of H_3-21 (δ 1.02) with that of its 24*R*-epimer (**20**) (δ 1.03). Thus, the structure of **19** was established as (22*E*,24*S*)-6-*O*-methyl-24-methylcholesta-7,22-diene-3β,5α,6β,9α-tetrol. Although the 24*R*-epimer (**20**) has previously been reported from a European wood-rotting fungus, *Polyporus versicolor*,[30] compound **19** is unprecedented.

Topsentisterol C_4 (**21**) was isolated as a white, amorphous solid, and the molecular formula was established as $C_{29}H_{48}O_4$ by HRFABMS ($[M + Na]^+$, *m*/*z* 467.3499, Δ −0.2 mmu). The ^{1}H NMR data showed an olefinic proton signal at δ 5.26 (m, H-7) and two oxymethine proton signals at δ 3.96 (m, H-3) and 3.54 (m, H-6). The COSY spectrum displayed a long-range correlation from H-7 to H-14, indicating a trisubstituted double bond located between C-7 and C-8. In the HMBC spectrum, correlations from H-6 to C-7 (δ 119.0) and C-8 (δ 144.0) and from H_3-19 (δ 1.05) to C-5 (δ 76.8) and C-9 (δ 44.0) were observed. The above data suggested that compound **21** possesses a 3β,5α,6β-trihydroxycholest-7-ene nucleus.[31] The OH-6β configuration was defined by a pyridine-induced shift in the ^{1}H NMR spectrum (Figure 1). The signal of H_3-19 was shifted downfield from δ 1.05 to 1.56. Moreover, a 3β,5α,6β-trihydroxycholest-7-ene nucleus and a 3β,5α,6α-trihydroxycholest-7-ene nucleus could be differentiated by the different chemical shifts of H-6 and H-7. It has been reported that in the OH-6β epimer these resonances would be δ 3.57 and 5.30, whereas in the OH-6α epimer they would be δ 3.93 and 5.00, respectively.[31] The side chain of **21** was determined to be the same as **1**. Therefore, the structure of **21** was established as (24*R*,25*R*,-27*R*)-26,27-cyclo-24,27-dimethylcholest-7-ene-3β,5α,6β-triol.

Compounds **22** and **23** were identified by comparison with reported data as epimers at C-24 with the skeleton (22*E*)-24-methylcholesta-7,22-diene-3β,5α,6β-triol.[32]

The close similarity of NMR spectra revealed that compounds **24**−**28** share the same 3β-hydroxycholesta-5,8-dien-7-one nucleus, of which compounds **24**, **25**, and **27** were defined as new derivatives. The nucleus of 3β-hydroxycholesta-5,8-dien-7-one is uncommon and has previously been encountered mostly in sponges.[33,34]

Topsentisterol D_1 (**24**) was isolated as a white, amorphous solid. Its molecular formula was established as $C_{29}H_{44}O_2$ on the basis of the sodiated molecular ion at *m*/*z* 447.3239 ($[M + Na]^+$, Δ 0.0 mmu) in its HRFABMS. Its ^{13}C NMR spectrum indicated the presence of 29 carbons including a ketone carbonyl carbon at δ 188.5 (C-7), four olefinic carbons at δ 166.3 (C-5), 165.4 (C-9), 134.6 (C-8), and 126.6 (C-6), and an oxygenated carbon at δ 72.7 (C-3). The above data suggested a 3β-hydroxycholesta-5,8-dien-7-one nucleus,[35] and this was further corroborated by HMBC correlations (Figure 2). The H_3-19 (δ 1.37) signal showed correlations to C-5 (δ 166.3) and C-9 (δ 165.4), and the olefinic proton signal at δ 6.00 (br s, H-6) correlated with C-8 (δ 134.6), C-4 (δ 42.8), and C-10 (δ 43.7). A long-range correlation from H-6 to H-4 (δ 2.55) was also observed in the COSY spectrum. The quite upfield shifted multiplet signals indicated that compound **24** possesses the same side chain as **1**. Thus, the structure of **24** was determined as (24*R*,25*R*,27*R*)-3β-hydroxy-26,27-cyclo-24,27-dimethylcholesta-5,8-dien-7-one.

Topsentisterol D_2 (**25**) was isolated as a white, amorphous solid, and its molecular formula was established as $C_{27}H_{40}O_2$ by HR-FABMS analysis ($[M + Na]^+$, *m*/*z* 419.2912, Δ −1.4 mmu). Comparison of the ^{1}H NMR data revealed that **25** shares the same nucleus as **24** and the same 24-methyl-27-norcholest-22-ene side chain as **8**. These were confirmed from the COSY and HMBC spectra. The stereochemistry at C-24 could not be defined because only a single epimer was isolated from this sponge.[18] Thus, the structure of **25** was defined as (22*E*)-3β-hydroxy-24ξ-methyl-27-norcholesta-5,8,22-trien-7-one.

Compound **26** was identified as a known compound with the skeleton (22*E*)-3β-hydroxycholesta-5,8,22-trien-7-one. It has previously been reported from Mediterranean sponges *Clathrina clathrus*[33] and *Psammocinia* sp.[35]

Topsentisterol D_3 (**27**) was isolated as a white, amorphous solid. The molecular formula was determined as $C_{28}H_{42}O_2$ by HRFABMS analysis ($[M + Na]^+$, *m*/*z* 433.3093, Δ +1.0 mmu). Analysis of its NMR data revealed that **27** shares the same nucleus as **24** and the same side chain as **13**. The configuration at C-24 was deduced as *S* by comparing the H_3-21 (δ 1.06) chemical shift with its known 24*R*-epimer (**28**), for which the H_3-21 signal shifted relatively downfield to δ 1.07. Therefore, the structure of compound **27** was determined as (22*E*,24*S*)-3β-hydroxy-24-methylcholesta-5,8,22-trien-7-one. The configurations at C-24 of the five epimeric pairs (**5**/**6**, **14**/**15**, **19**/**20**, **22**/**23**, and **27**/**28**), isolated in the present study, were all defined on the basis of the H_3-21 chemical shift values. Moreover, the HPLC retention times were also in good agreement with a previous report[16] that *S*-epimers showed shorter retention times in reversed-phase HPLC as compared to their *R*-epimers.

Compound **29** was identified as a known allylic hydroperoxide sterol by comparison of its NMR data with those of reported compounds.[36,37] Its ^{1}H NMR spectrum showed two sets of olefinic proton signals of H-24^1 (δ 5.771 and 5.768, dd, J = 18.0, 12.0 Hz) and H-24^2 (δ 5.207 and 5.204, dd, J = 12.0, 1.0 Hz) and two sets of methyl signals of H_3-26 (δ 0.881 and 0.874, d, J = 7.0 Hz) and H_3-27 (δ 0.895 and 0.889, d, J = 7.0 Hz). These data implied that this 24-hydroperoxy-24-ethylcholesta-5,24^1-dien-3β-ol is a mixture of 24*R*- and 24*S*-epimers.[37]

Topsentisterol E_1 (**30**) was isolated as a white, amorphous solid. The molecular formula was assigned as $C_{28}H_{42}O_2$ by HRFABMS, which showed a $[M + Na]^+$ ion at *m*/*z* 433.3085 (Δ +0.2 mmu). The ^{1}H NMR spectrum showed three olefinic proton signals at δ 6.60 (s, H-7), 5.22 (m, H-22), and 5.23 (m, H-23), two oxymethine proton signals at δ 5.09 (dd, J = 9.0, 6.5 Hz, H-11) and 4.05 (m, H-3), and five allylic proton signals at δ 3.00, 2.92, 2.86, 2.75, and 2.47 (Table 2). The HMBC spectrum indicated the presence of eight olefinic carbon signals at δ 139.4 (C-8), 137.0 (C-22), 136.8 (C-10), 135.4 (C-6), 134.4 (C-9), 133.5 (C-23), 131.6 (C-5), and 123.5 (C-7), and key correlations were observed as shown in Figure 2. Thus, the aromatic B ring and 24-methylcholest-22-ene side chain in **30** were established. The configuration of the OH-3α,11α groups was defined by a NOESY experiment, which showed correlations from H-3 (δ 4.05) to H-2β (δ 1.98) and H-4β (δ 3.00) and from H-11 to H_3-18 (δ 0.44). The stereochemistry at C-24 could not be defined from the ^{1}H NMR data because only a single epimer was

Table 5. Cytotoxicity Data for Compounds **4**, **6**, **8**, **9**, **13**−**16**, **19**, and **25**−**27**[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
4	30.0	19.4	25.9	>30.0	21.4
6	17.5	17.8	19.4	24.7	21.1
8	5.4	7.1	4.6	6.2	4.2
9	6.3	6.3	5.2	11.4	4.7
13	9.7	7.8	11.3	19.0	14.1
14	13.9	7.9	5.5	>30.0	18.4
15	>30.0	17.9	>30.0	>30.0	17.9
16	7.7	4.2	11.3	17.7	6.1
19	5.6	7.8	11.5	4.7	4.3
25	8.5	13.5	6.3	4.6	8.4
26	>30.0	>30.0	>30.0	>30.0	38.4
27	>30.0	>30.0	24.5	>30.0	33.6
doxorubicin	0.08	0.19	0.08	0.07	0.13

[a] Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer.

isolated from this sponge. Therefore, the structure of compound **30** was defined as 1(10→6)abeo-(22*E*)-24ξ-methylcholesta-5,7,9,-22-tetraene-3α,11α-diol. Anthrasteroids resembling compound **30** have been previously isolated only from terrestrial fungi,[38,39] and **30** has been postulated as an intermediate in the biosynthesis of other anthrasteroids.[39] Although its 3-acetate form has been synthesized,[39] compound **30** has never been reported from a natural source.

In the present study, the sterol composition of the sponge *Topsentia* sp. was defined for 30 compounds (**1**−**30**) with various nuclei and side chains. Many of the known sterols (**6**, **15**, **18**, **20**, **22**, **23**, and **28**)[22,29,40−42] have been previously reported as fungal metabolites, which suggests the possible symbiotic origin of these sterols and related substances (**8**, **13**, **14**, **19**, **25**, **27**, and **30**).

All isolated compounds except the minor components were evaluated for cytotoxicity against a panel of five human solid tumor cell lines (Table 5), and compounds **8**, **9**, **13**, **16**, **19**, and **25** exhibited weak cytotoxicity. No clear correlations between structure and cytotoxicity could be delineated due to diverse variations in the structure of the nucleus and the side chain.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO P-1020 digital polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity INOVA 300, 400, and 500 MHz spectrometers. Chemical shifts are reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD; δ_H 7.26 for $CDCl_3$; δ_H 7.22, 7.58, 8.74 for pyridine-d_5). LRFABMS data were obtained on a JEOL JMS SX-102A spectrometer. HRFABMS data were obtained on a JEOL JMS SX-101A spectrometer. HPLC was performed with a C_{18}-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) and an YMC packed ODS column (250 × 10 mm, 5 μm, 120 Å) using Shodex RI-101 and Shodex RI-71 detectors.

Animal Material. The sponge was collected by hand using scuba (20 m depth) in October 2002 off the coast of Jeju Island, Korea. The collected sample was frozen immediately. This specimen was identified as *Topsentia* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen (registry No. Spo. 46) was deposited in the Natural History Museum, Hannam University, Daejon, Korea, and has been described elsewhere.[2]

Extraction and Isolation Procedure. The frozen sponge (8.2 kg) was exhaustively extracted with MeOH at room temperature to afford a MeOH extract, which was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 extract was further partitioned between aqueous MeOH and *n*-hexane to yield a bioactive aqueous MeOH extract, which showed lethality to brine shrimp larvae (LD_{50} 30 μg/mL). The aqueous MeOH extract was subjected to stepped gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 400/500 mesh), eluting with 50 to 100% MeOH/H_2O, to afford 23 fractions. Fraction 19 was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with a solvent system of MeOH−CH_3CN−H_2O (9:10:1), to afford three fractions. Compounds **1** (15.8 mg) and **7** (7.0 mg) were obtained by purifying subfractions 1 and 2 using reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 95% and 97% MeOH, respectively. Fraction 20 was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with a solvent system of MeOH−CH_3CN−H_2O (5:14:1), to yield four fractions. Compounds **2** (1.2 mg) and **3** (4.0 mg) were obtained by purifying subfractions 3 and 4 with reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 97% MeOH. Fraction 16, one of the bioactive fractions (LD_{50} 31.6 μg/mL), was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with a solvent system of MeOH−CH_3CN−H_2O (13:70:17), to afford 11 fractions. Compounds **22** (2.7 mg) and **23** (1.3 mg) were obtained by purifying subfraction 1 using reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), with 83% MeOH as mobile phase. Subfractions 2 and 3 were combined and subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 80% CH_3CN, to yield compounds **13** (3.6 mg), **16** (1.7 mg), and **17** (0.4 mg). Compounds **14** (2.8 mg) and **19** (1.7 mg) were obtained by purification of subfraction 4 by reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 83% MeOH. Compounds **11** (0.6 mg), **15** (1.2 mg), **20** (0.5 mg), **25** (1.1 mg), and **26** (0.7 mg) were obtained by purification of subfraction 5 by reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 83% MeOH. Subfraction 6 was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 85% MeOH, to yield compounds **4** (4.8 mg), **21** (1.9 mg), and **30** (0.4 mg). Subfractions 7 and 8 were combined and subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 85% MeOH, to afford compounds **8** (1.9 mg) and **9** (1.6 mg). Compounds **10** (0.5 mg) and **12** (0.8 mg) were obtained by purification of subfraction 9 by reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 85% MeOH, and subsequently subjecting subfraction 9-4 to reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 75% CH_3CN. Compounds **27** (2.3 mg) and **28** (0.5 mg) were obtained by purifying subfraction 10 by reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 88% MeOH. Fraction 17 (LD_{50} 31.6 μg/mL) was subjected to reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with the same mobile phase as fraction 16, to yield 10 fractions. Compound **29** (6.6 mg) was obtained by purifying subfraction 4 using reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 75% CH_3CN. Compound **24** (1.5 mg) was obtained by purifying subfraction 8 with reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 88% MeOH and subsequently with 92% MeOH. Compound **18** (3.0 mg) was obtained by purifying subfraction 9 using reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 83% CH_3CN. Compounds **5** (12.1 mg) and **6** (1.6 mg) were obtained by purifying subfraction 10 with reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 85% CH_3CN.

Hydrogenation of Topsentisterols A_1 (1) and D_1 (24). The catalyst platinum oxide (10 mg) was added to an ethyl acetate/acetic acid (1:1, 1.0 mL) solution of compound **1** (2.0 mg). The solution was stirred at room temperature with a continuous supply of H_2 gas for 12 h. Filtration through Celite and evaporation of the solution gave the crude product, which was purified by reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with MeOH, to afford **31** (0.8 mg) as a major product. Compound **24** was treated under the same conditions to afford compounds **32** (0.6 mg) and **33** (0.3 mg), respectively.

Topsentisterol A_1 (1): white, amorphous solid; $[\alpha]^{24}_D$ −15 (*c* 0.16, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m*/*z* 465 $[M + Na]^+$, 410 $[M - O_2]^+$; HRFABMS *m*/*z* 465.3339 (calcd for $C_{29}H_{46}O_3Na$, 465.3345).

Topsentisterol A_2 (2): white, amorphous solid; ^{1}H and ^{13}C NMR data for the nucleus were identical to those of compound **1**; ^{1}H NMR (CD_3OD, 500 MHz) of the side chain, δ 1.65 (1H, m, H-25), 1.56 (3H, m, H-22, 24), 1.40 (1H, m, H-20), 1.10 (1H, m, H-23), 0.95 (3H, d, J = 6.5 Hz, H_3-21), 0.91 (3H, d, J = 7.0 Hz, H_3-26), 0.90 (3H, d, J = 6.5 Hz, H_3-24[1]), 0.82 (3H, d, J = 6.5 Hz, H_3-27), 0.79 (3H, d, J = 7.0 Hz, H_3-23[1]); ($CDCl_3$, 400 MHz) of the doublet methyls, δ 0.921 (3H,

d, $J = 6.4$ Hz, H_3-21), 0.886 (3H, d, $J = 6.8$ Hz, H_3-26), 0.877 (3H, d, $J = 6.8$ Hz, H_3-24[1]), 0.789 (3H, d, $J = 6.4$ Hz, H_3-27), 0.759 (3H, d, $J = 7.2$ Hz, H_3-23[1]); ^{13}C NMR (CD_3OD, assigned by HMBC and HSQC, 500 MHz) of the side chain, δ 45.0 (CH, C-23), 41.0 (CH_2, C-22), 36.5 (CH, C-20), 35.6 (CH, C-24), 29.8 (CH, C-25), 22.8 (CH_3, C-26), 20.5 (CH_3, C-21), 19.2 (CH_3, C-27), 18.0 (CH_3, C-24[1]), 12.5 (CH_3, C-23[1]); FABMS *m/z* 467 $[M + Na]^+$, 412 $[M - O_2]^+$; HRFABMS *m/z* 467.3517 (calcd for $C_{29}H_{48}O_3Na$, 467.3501).

Topsentisterol A_3 (8): white, amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 435 $[M + Na]^+$, 380 $[M - O_2]^+$; HRFABMS *m/z* 435.2876 (calcd for $C_{29}H_{48}O_3Na$, 435.2875).

Topsentisterol B_1 (10): white, amorphous solid; $[\alpha]^{24}_D$ −157 (*c* 0.02, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 465 $[M + Na]^+$; HRFABMS *m/z* 465.3345 (calcd for $C_{29}H_{48}O_3Na$, 465.3345).

Topsentisterol B_2 (11): white, amorphous solid; $[\alpha]^{24}_D$ −61 (*c* 0.02, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 425 $[MH - H_2O]^+$; HRFABMS *m/z* 465.3342 (calcd for $C_{29}H_{48}O_3Na$, 465.3345).

Topsentisterol B_3 (12): white, amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 465 $[M + Na]^+$; HRFABMS *m/z* 465.3350 (calcd for $C_{29}H_{48}O_3Na$, 465.3345).

Topsentisterol B_4 (13): white, amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 451 $[M + Na]^+$; HRFABMS *m/z* 451.3192 (calcd for $C_{29}H_{48}O_3Na$, 451.3188).

Topsentisterol B_5 (14): white, amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 451 $[M + Na]^+$; HRFABMS *m/z* 451.3188 (calcd for $C_{29}H_{48}O_3Na$, 451.3188).

Topsentisterol C_1 (16): white, amorphous solid; $[\alpha]^{24}_D$ −28 (*c* 0.05, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; FABMS *m/z* 481 $[M + Na]^+$; HRFABMS *m/z* 481.3294 (calcd for $C_{29}H_{48}O_3Na$, 481.3294).

Topsentisterol C_2 (17): white, amorphous solid; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 483 $[M + Na]^+$; HRFABMS *m/z* 483.3453 (calcd for $C_{29}H_{48}O_4Na$, 483.3450).

Topsentisterol C_3 (19): white, amorphous solid; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 483 $[M + Na]^+$; HRFABMS *m/z* 483.3477 (calcd for $C_{29}H_{48}O_4Na$, 483.3450).

Topsentisterol C_4 (21): white, amorphous solid; $[\alpha]^{24}_D$ −83 (*c* 0.02, MeOH); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 467 $[M + Na]^+$; HRFABMS *m/z* 467.3499 (calcd for $C_{29}H_{48}O_3Na$, 467.3501).

Topsentisterol D_1 (24): white, amorphous solid; $[\alpha]^{24}_D$ −20 (*c* 0.03, MeOH); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 425 $[M + H]^+$; HRFABMS *m/z* 447.3239 (calcd for $C_{29}H_{48}O_3Na$, 447.3239).

Topsentisterol D_2 (25): white, amorphous solid; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 419 $[M + Na]^+$; HRFABMS *m/z* 419.2912 (calcd for $C_{29}H_{48}O_3Na$, 419.2926).

Topsentisterol D_3 (27): white, amorphous solid; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 433 $[M + Na]^+$; HRFABMS *m/z* 433.3093 (calcd for $C_{28}H_{42}O_2Na$, 433.3083).

Topsentisterol E_1 (30): white, amorphous solid; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 433 $[M + Na]^+$; HRFABMS *m/z* 433.3085 (calcd for $C_{28}H_{42}O_2Na$, 433.3083).

(24*R*,25*R*)-24,25-Dimethylcholest-8(14)-ene-3β,5α-diol (31): white, amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 4.079 (1H, m, H-3), 0.927 (3H, d, $J = 6.4$ Hz, H_3-21), 0.875 (3H, s, H_3-18), 0.862 (3H, t, $J = 6.0$ Hz, H_3-27), 0.847 (3H, s, H_3-19), 0.751 (3H, d, $J = 7.2$ Hz, H_3-24[1]), 0.733 (3H, d, $J = 6.8$ Hz, H_3-25[1]); FABMS *m/z* 430 $[M]^+$.

(24*R*,25*R*)-24,25-Dimethylcholest-8(14)-en-3β-ol (32): white, amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 3.608 (1H, m, H-3), 0.926 (3H, d, $J = 6.4$ Hz, H_3-21), 0.860 (3H, t, $J = 6.8$ Hz, H_3-27), 0.838 (3H, s, H_3-19), 0.750 (3H, d, $J = 7.2$ Hz, H_3-24[1]), 0.732 (3H, d, $J = 6.8$ Hz, H_3-25[1]), 0.687 (3H, s, H_3-18); FABMS *m/z* 414 $[M]^+$.

(24*R*,25*R*)-24,25-Dimethylcholestane-3β,7β-diol (33): white, amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 3.589 (1H, m, H-3), 3.360 (1H, m, H-7), 0.909 (3H, d, $J = 6.4$ Hz, H_3-21), 0.859 (3H, t, $J = 6.8$ Hz, H_3-27), 0.832 (3H, s, H_3-19), 0.750 (3H, d, $J = 7.2$ Hz, H_3-24[1]), 0.728 (3H, d, $J = 6.8$ Hz, H_3-25[1]), 0.680 (3H, s, H_3-18); FABMS *m/z* 432 $[M]^+$.

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