Haliclostanone Sulfate and Halistanol Sulfate from an Indo-Pacific *Haliclona* Sponge

Sam Sperry and Phil Crews*

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064

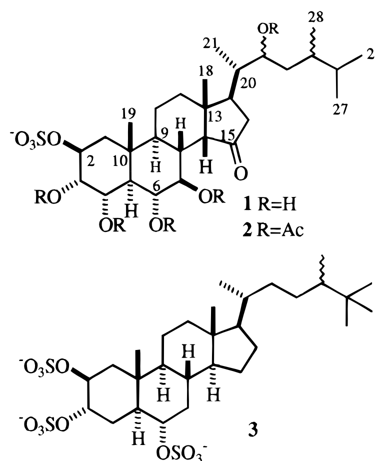
Received August 23, 1996[®]

Two steroids, haliclostanone sulfate (**1**) and halistanol sulfate (**3**), were isolated from a *Haliclona* sp. marine sponge. The structure of the new compound **1** was established based on its spectroscopic data and the properties of a pentaacetate derivative **2**. Compound **1** is unique in that it possesses the rare *cis* C/D ring junction precedented only in marine sterols, contignasterol (**4**) and xestobergsterols A (**5**), B (**6**), and C (**7**).

For the past 20 years marine organisms have become increasingly recognized as an important source of sterol metabolites.[1,2] In fact, some believe that sponges may provide the richest source of sterol diversity in the entire animal kingdom.[3] Recently, we reported on the isolation of halistanol sulfate (**3**) from two different Halichondrid sponges in the genus *Topsentia*.[4] During the course of chemically examining a *Haliclona* sp. (order Haplosclerida) collected in Malaysian waters, we again encountered **3** accompanied by what appeared to be a new steroid. The genus *Haliclona* has a history of providing diverse structures including polycyclic amines,[5] sequiterpenoid quinols,[6] glycosphingolipids,[7] resorcinols,[8] and a tetrahydropyranone.[9] Its sponges are also a source of at least eight new sterol metabolites, as shown by previous chemical studies of *H. permolis* (C_{27}−C_{29} unsaturated),[3a] *H. oculata* (highly oxygenated C_{27}−C_{28} analogues),[10] *H. chilensis* (keto sterols),[11] *H. flavescens* (C_{28} $\Delta^{7,9}$-unsaturated),[12] and *H. rubens* (pregnanes).[13] In this report we describe the structural features of the new sterol, haliclostanone sulfate (**1**) and also offer comments on its novel features.

The initial processing of the *Haliclona* sp. (coll. no. 92228) was conducted according to procedures described previously.[14] The aqueous MeOH partition fraction (80 mg) was selected for further fractionation based on the presence of several resonances of atoms attached to heteroatoms in both ^{1}H- and ^{13}C-NMR spectra. Subjecting this crude extract to Si gel flash chromatography [$CHCl_3$−MeOH−H_2O (7:3:1), lower phase] yielded 11 fractions. The sixth fraction contained pure **1** (6.8 mg), and the tenth fraction contained pure **3** (13 mg).

Halistanol sulfate (**3**) was quickly recognized by its spectroscopic properties, which were consistent with those previously reported.[4,15,16] It must be noted that **3** as well as its analogues have been previously isolated only from sponges belonging to the orders Halichondrida or Axinellida[17] (genera *Halichondria*,[15] *Epipolasis*,[16] *Topsentia*,[4] *Pseudoaxinissa*,[18] *Trachyopsis*,[19] and *Cymbastela*[19]). Our isolation of **3** constitutes the first case of this compound to be obtained from a Haplosclerid sponge. Because characterization and related bioactivity data of **3** have already been extensively explored,[4,15,16,18] it will not be discussed further in this report.



The structure characterization of **1** began with the negative ion HRFABMS spectrum in which a [M]$^-$ of 575.2889 (Δ −0.6 mmu of calcd) was dominant. This *m/z* peak established the molecular formula as $C_{28}H_{47}$-SO_{10} for the anionic form of **1**. Further analysis of the oxygen functionality of **1** proceeded with the ^{13}C-APT-NMR data, which gave a count (Table 1) of $C_{28}H_{42}$. Comparison to the MS formula indicated that five OH groups were present, which was also supported by ^{13}C- and ^{1}H-NMR signals at δ 76.5/δ 3.71, δ 75.1/δ 4.44, δ 71.6/δ 3.97, δ 71.5/δ 4.00, δ 71.1/δ 4.22, while the ^{13}C δ 223 (s) meant that a non-six-membered ring ketone could be specified. Another oxygen residue was evident from the NMR signals at δ 78.1/δ 4.56, and a sulfate anion group was envisioned to account for this CH−O attached to the remaining three oxygens. The ketone noted above plus four additional rings accounted for the unsaturation equivalence of five. Furthermore, the carbon count, the ring count, and the coelution of **1** with the known steroid **3** suggested it was also steroidal in nature. In further support of this supposition was that the ^{1}H-NMR spectrum contained two methyl singlets (δ 1.16 and 1.02) and four methyl doublets (δ 0.94, 0.89, 0.86, and 0.81). An HMQC[20] experiment allowed all resonances to be assigned (Table 1), which then enabled five substructures to be drawn. Three partial structures, **a**−**c**, were rapidly constructed through interpretation of the ^{1}H−^{1}H COSY NMR data (Table 1), while the HMBC[21] correlations (Table 1) led to fragments **d** and **e**. The remaining carbonyl fragment **f**, completed our analysis of all NMR resonances.

* To whom correspondence should be addressed. Phone: (408) 459-2603. FAX: (408) 459-2935. E-mail: phil@hydrogen.ucsc.edu.

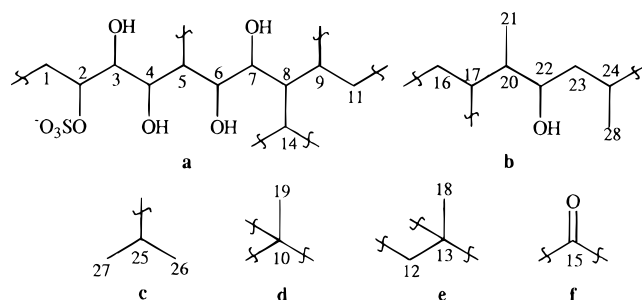
[®] Abstract published in *Advance ACS Abstracts,* December 15, 1996.

S0163-3864(96)00592-7 CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognogy

Table 1. NMR Data for **1** at 62.5 MHz (^{13}C) and 500 MHz (^{1}H) in CD_3OD

carbon		proton			
atom #	δ_C, mult	atom #	δ_H (mult, J in Hz)	**COSY** ($^1H-^1H$)	**HMBC** (C→H)
1	37.7 t	1α	1.42 (dd, 14.5, 3.5)	1β, 2	3, 19
		1β	2.03 (dd, 14.5, 2.0)	1α, 2	
2	78.1 d	2	4.56 (q, 3.5)	1α, 1β, 3	1β, 3
3	71.6 d	3	3.97 (t, 3.0)	2, 4	1β
4	71.1 d	4	4.22 (dd, 11.0, 3.0)	3, 5	2, 5
5	45.0 d	5[a]	1.67 (t, 11.0)	4, 6	1β, 3, 6, 19
6	76.5 d	6	3.71 (dd, 11.0, 8.5)	5, 7	5, 7
7	75.1 d	7	4.44 (dd, 11.0, 8.5)	6, 8	6, 8
8	40.0 d	8[a]	1.68 (t, 11.0)	7, 9, 14	7, 14
9	47.9 d	9	1.05 (m)	8, 11β	1α, 5, 12β, 14, 19
10	38.8 s				1α, 1β, 2, 8, 19
11	22.2 t	11α[b]	1.49 (m)	11β	12α
		11β[c]	1.28 (m)	9, 11α	
12	37.9 t	12α[c]	1.27 (m)	12β	14, 17, 18
		12β	1.39 (m)	12α, 14	
13	42.7 s				12β, 14, 16β, 17, 18
14	52.6 d	14	2.70 (br s)	8, 12β	7, 8, 12β, 18
15	223.0 s				14, 16α, 16β, 17
16	39.5 t	16α	2.31 (dd, 19.5, 10.5)	16β, 17	
		16β	2.81 (dt, 19.5, 2.0)	16α	
17	49.1 d	17	1.76 (ddd, 10.5, 4.5, 2.5)	16α, 20	16α, 16β, 18, 20, 21
18	19.1 q	18	1.16 (s)		12α
19	16.6 q	19	1.02 (s)		1α, 1β, 5
20	37.3 d	20	1.94 (m)	17, 21	16α, 16β, 21
21	13.1 q	21	0.94 (d, 7.0)	20	20, 22
22	71.5 d	22	4.00 (m)	23, 23′	17, 21, 23, 23′
23	42.3 t	23[b]	1.50 (m)	22, 23′	28
		23′[c]	1.30 (m)	22, 23	
24	35.9 d	24[b]	1.47 (m)	28	22, 23, 23′, 26, 27, 28
25	32.2 d	25	1.65 (m)	26, 27	23, 23′, 26, 27, 28
26	17.4 q	26	0.81 (d, 6.5)	25	27
27	20.4 q	27	0.89 (d, 7.0)	25	26
28	16.3 q	28	0.86 (d, 6.5)	24	23, 23′

[a,b,c] These ^1H-NMR shifts are overlapping.



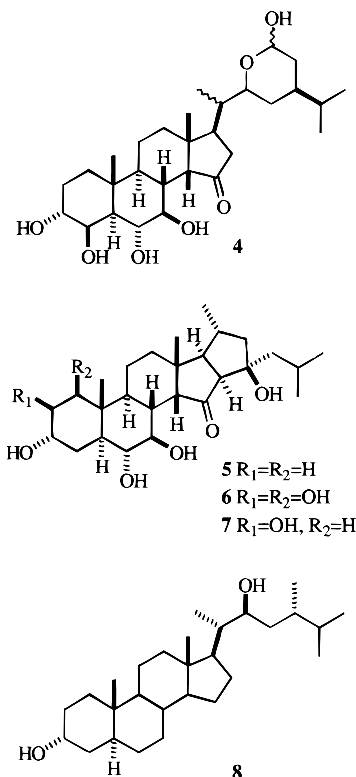
The entire gross structure of **1** was elucidated as follows. HMBC correlations from δ 37.7 (C1), δ 45.0 (C5), and δ 47.9 (C9) to δ 1.02 ($H_3$19) allowed connection of fragments **a** and **d**, through two six-membered rings as shown. Correlations from δ 49.1 (C17) to δ 1.16 (H_3-18) further confirmed that C13 was connected to C17. Correlations from δ 52.6 (C14) to δ 1.16 ($H_3$18) and δ 47.9 (C9) to δ 1.39 (H12β) allowed fragments **a** and **e** to be joined in another six-membered ring. The correlations from δ 223.0 (C15) to δ 2.70 (H14) and δ 1.76 (H17) justified adding fragments **b** and **f** to **a**, forming the final five-membered ring. Lastly, correlations from δ 35.9 (C24) to δ 0.89 ($H_3$27) and δ 0.81 ($H_3$26) allowed the remaining isopropyl fragment **c** to be attached to the end of subunit **b**.

The regiochemistry of the sulfate moiety assumed to be associated with the ^{13}C and ^1H resonances at δ 78.1 (C2) and δ 4.56 (H2), respectively, was addressed next. This involved preparing the pentaacetate **2** by reaction of **1** with Ac_2O in pyridine and a catalytic amount of DMAP. The ^1H-NMR spectrum of **2** displayed five acetate methyls (δ 2.10, 2.02, 1.93, 1.89, and 1.86), and a molecular formula of $C_{38}H_{57}SO_{15}$ was established for the anionic form by a negative HRFABMS molecular ion $[M]^-$ of 785.3418 (Δ 0.5 mmu of calcd). Additionally, in the ^1H-NMR spectrum of **1** vs. **2** the five oxymethine protons H3, H4, H6, H7, and H22 each moved downfield, while the H2 oxymethine proton remained nearly unchanged. Thus, the sulfate was unequivocally assigned to position 2.

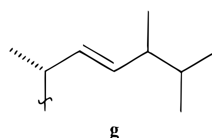
The relative stereochemistry features in **1** were set by employing a combination of ^1H-NMR J values, ^{13}C chemical shifts, and NOE difference data. Characteristic J values (Table 1) indicated that H2 and H3 were equatorial and that H4, H5, H6, H7, and H8 were all axial. Although the coupling pattern for H9 in **1** could not be unambiguously interpreted, its axial orientation was established by the J values for H9 in **2** (δ 1.14, $J=$ 3, 12 Hz). The relative upfield ^{13}C shifts of the ring methyls Me18 (δ 19.1) and Me19 (δ 16.6) were diagnostic of both groups as axial. Furthermore, these shifts were nearly identical to those of the corresponding axial methyls in contignasterol (**4**): Me18 (δ 19.3) and Me19 (δ 14.5),[22] which indicated that the tetracyclic ring fusion stereochemistry of **1** was identical to that deduced for **4**. Further support that the six- and five-membered rings of **1** were *cis*-fused and that various substituents in this part of the molecule were *syn* to one another was provided by irradiation of the CH_3 signal at δ 1.16 (Me18), which induced NOEs at H8, H14, H20, and H22. Likewise, NOEs induced from Me21 to H16α and to H17 and from H20 to H14, along with biogenetic considerations, justified the relative stereochemistry assigned at C20. Although it is tempting to assign the stereochemistry at C22 based on comparison of the ^{13}C-Me21-

NMR shifts between **1** and sterol **8** isolated from the wood of *Abies pinsapo* plus that of other sterol models, this is too risky because the limiting values of the models are too close,[23] and the stereochemistries at C22 and C24 are thus unassigned.

Compound **1** is the fifth member of a rare class of naturally occurring sterols sharing a *cis* C/D ring junction, 14β proton, and a C15 ketone. To the best of our knowledge, sponge metabolites contignasterol (**4**), isolated from a *Petrosia contignata* specimen;[22] xestobergsterol A (**5**) and B (**6**) from *Xestospongia berquistia*;[24] and xestobergsterol A (**5**), B (**6**), and C (**7**) from an *Ircinia* sp.,[25] represent the only other natural sterols containing these three features. Equilibration studies



conducted on semisynthetic 15-keto steroids concluded that 14β steroids are more stable than their 14α epimers.[26] This may in part account for why the five natural 15-keto sterols isolated to date have conserved this feature. Another interesting feature of **1** is the unusual substitution present in the side chain attached at C17. Sterol **8**, from the wood *Abies pinsapo*,[23a] constitutes the single literature example of a sterol bearing this exact side chain. The most obvious biosynthetic precursor to this oxygenated side chain is represented by substructure **g**, which is found in such steroids as brassicasterol or epibrassicasterol,[27] halistanol sulfate A,[16] and stoloniferone B.[28] The unusual structural features of haliclostanone sulfate allow it to join the list of novel steroids continuing to emerge from sponges which are both highly oxygenated and carry modified side chains.[29,30,31]



Experimental Section

General Experimental Procedures. NMR spectra were recorded at 500 MHz (^{1}H) on a Varian Unity Plus NMR spectrometer and at 62.5 MHz (^{13}C) and 250 MHz (^{1}H) on a Bruker AC-250 NMR spectrometer in CD_3OD. Carbon multiplicities were determined using DEPT-135 data. Atom connectivities were determined using HMQC, HMBC, and COSY data. LRFABMS and HRFABMS were measured on VG ZAB-SE and VG 70-SE-4F mass spectrometers, respectively. Optical rotations were determined on a JASCO DIP-370 digital polarimeter in MeOH. IR spectra were measured on a Varian 1600 series FTIR spectrometer. Flash chromatography was carried out on Si gel (200−400 mesh) obtained from Aldrich. Preparatory HPLC was performed using an Econosil C-18 10-μm column (250 × 10 mm) with RI detection.

Animal Materials. The sponge *Haliclona* sp. (coll. no. 92228) was collected from Tioman Is., Malaysia, and was identified as an undescribed species of the genus *Haliclona* (family Chalinidae; order Haplosclerida)[32] by Dr. M. C. Diaz (UCSC). The sponge was massive—amorphous to subglobular in shape, up to 6 cm in thickness, with a smooth but irregular surface, riddled with oscules 2−3 mm wide. The sponge was compressible and crumbly in consistency, and its color was reddish brown externally and tan internally. The skeleton consists of an isotropic unispicular to multispicular (2−3 spicules in cross section) reticulation, made up of small hastate oxeas (140−160 × 6−8 μm in length and width) and very small, thin oxeas (< 100 × 1 μm in size). A voucher, as well as an underwater photograph, are in the UCSC sponge collection archives and are available from P. C.

Extraction and Isolation. The sponge (0.26 kg, dry wt) was processed according to our standard procedures.[14] The aqueous MeOH partition fraction afforded a crude extract (80 mg) that was flash chromatographed on Si gel [$CHCl_3$−MeOH−H_2O (7:3:1) lower phase] yielding sterols **1** (6.8 mg) and **3** (13 mg).

Haliclostanone sulfate (1): amorphous yellow solid; $[\alpha]_D$ +15.6° (*c* 0.18, MeOH); IR (neat) ν 3377, 2919, 1731, 1595, 1249, 1055, 961 cm^{-1}; negative-ion HRFABMS *m/z* $[M]^-$ 575.2889 ($C_{28}H_{47}SO_{10}$, Δ −0.6 mmu of calcd); ^{13}C- and ^{1}H-NMR data are shown in Table 1, and assignments are based on HMQC, DEPT-135, COSY, and HMBC data.

Conversion of 1 to 2. Approximately 20 mg of crude **1** was dissolved in dry Ac_2O−pyr (1:1). A catalytic amount of DMAP was added, and the mixture was stirred overnight at room temperature. Following rotoevaporation, 15 mg of crude product was subjected to reversed-phase HPLC purification in MeOH−H_2O (97:3). Pure **2** (1.3 mg) eluted with the column wash (MeOH).

Haliclostanone sulfate pentaacetate (2): amorphous amber solid; $[\alpha]_D$ +56.9° (*c* 0.13, MeOH); IR (neat) ν 2954, 1737, 1367, 1237, 1038, 750 cm^{-1}; negative HRFABMS *m/z* $[M]^-$ 785.3418 ($C_{38}H_{57}SO_{15}$, Δ 0.5 mmu of calcd); ^{1}H NMR (CD_3OD, 500 MHz) 6.04 (1H, dd, *J* = 10, 11 Hz, H7), 5.40 (1H, t, *J* = 3.5 Hz, H3), 5.38 (1H, dd, *J* = 12.5, 3.5 Hz, H4), 5.15 (1H, m, H22), 5.11 (1H, t, *J* = 12.5 Hz, H6), 4.47 (1H, q, *J* = 3.5 Hz, H2), 2.53 (1H, bd, *J* = 20 Hz, H16β), 2.40 (1H, dd, *J* = 20, 10 Hz, H16α), 2.29 (1H, br s, H14), 2.25 (1H, br d, *J* = 16 Hz,

H1β), 2.19 (1H, m, H20), [five acetate methyls, 2.10 (3H, s), 2.02 (3H, s), 1.93 (3H, s), 1.89 (3H, s), 1.86 (3H, s)], 2.04 (1H, m, H5), 1.98 (1H, m, H8), 1.84 (1H, m, H17), 1.62 (1H, m, H25), 1.57 (1H, t, J = 10 Hz, H23) 1.45 (1H, dd, J = 16, 2 Hz, H1α), 1.32 (1H, m, H23′), 1.21 (3H, s, Me18), 1.20 (3H, s, Me19), 1.14 (1H, dt, J = 3, 12 Hz, H9), 1.04 (3H, d, J = 7.0 Hz, Me21), 0.89 (3H, d, J = 6.0 Hz, Me28), 0.85 (3H, d, J = 6.5 Hz, Me26), 0.83 (3H, d, J = 7.0 Hz, Me27); assignments are based on interpretation of ^{1}H−^{1}H COSY NMR data and comparison to the ^{1}H NMR spectrum of **1**.

Halistanol sulfate (3): amorphous amber solid; $[\alpha]_D$ +19.6° (*c* 0.45, MeOH); ^{13}C NMR (CD_3OD, 62.5 MHz) δ 78.6 (C6), 75.6 (C2, C3), 57.5 (C17), 57.2 (C14), 55.6 (C9), 45.2 (C5, C24), 43.7 (C13), 41.0 (C12), 39.9 (C1), 39.2 (C7), 37.5 (C10, C20), 36.5 (C22), 35.0 (C8), 33.9 (C25), 29.1 (C16, C23), 27.7 (C26, C27, C29), 25.0 (C4, C15), 21.7 (C11), 19.4 (C21), 15.1 (C19), 14.9 (C28), 12.7 (C18); ^{1}H NMR (CD_3OD, 250 MHz) 4.73 (1H, br s, H2), 4.70 (1H, br s, H3), 4.13 (1H, dt, J = 10, 4.1 Hz, H6), 0.98 (3H, s, Me19), 0.89 (3H, d, J = 6.2 Hz, Me21), 0.80 (9H, s, Me26, Me27, Me29), 0.77 (3H, d, 7.0 Hz, Me28), 0.63 (3H, s, Me 18); assignments are based on literature values.[15,16]

Acknowledgment. Financial support was from NIH Grant CA47135. We appreciate assistance in the collection of sponges by Ms. Lisa Hunter and Mr. Leif Abrell. Taxonomic analysis was conducted by Dr. M. Cristina Diaz (UCSC, Institute of Marine Sciences). Equipment funds from the Elsa Pardee Foundation and NSF-CHE-93-22464 supported the purchase of a 500 MHz NMR.

References and Notes

(1) Giner, J.-L. *Chem. Rev.* **1993**, *93*, 1735−1752.
(2) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839−1895.
(3) (a) Sheikh, Y. M.; Djerassi, C. *Tetrahedron* **1974**, *30*, 4095−4103. (b) Djerassi, C.; Silva, C. J. *Acc. Chem. Res.*, **1991**, *24*, 371−378.
(4) Slate, D. L.; Lee, R. H.; Rodríguez, J.; Crews, P. *Biochem. Biophys. Res. Commun.* **1994**, *203*, 260−264.
(5) Jaspars, M.; Pasupathy, V.; Crews, P. *J. Org. Chem.* **1994**, *59*, 3253−3255.
(6) Jaspars, M.; Horton, P. A.; Madrid, L. H.; Crews, P. *J. Nat. Prod.* **1995**, *58*, 609−612.
(7) Hirsch, S.; Kashman, Y. *Tetrahedron* **1989**, *45*, 3897−3906.
(8) Barrow, R. A.; Capon, R. J. *Aust. J. Chem.* **1991**, *44*, 1393−1405.
(9) Ciminiello, P.; Costantino, V.; Fattorusso, E.; Magno, S.; Mangoni, A. *Heterocycles* **1992**, *34*, 765−770.
(10) Findlay, J. A.; Patil, A. D. *Can. J. Chem.* **1985**, *63*, 2406−2410.
(11) Seldes, A. M.; Rovirosa, J.; San Martin, A.; Gros, E. G. *Experientia* **1985**, *41*, 34−35.
(12) Zielinski, J.; Milkova, T.; Popov, S.; Marekov, N.; Djerassi, C. *Steroids* **1982**, *39*, 675−680.
(13) Ballantine, J. A.; Williams, K. *Tetrahedron Lett.* **1977**, 1547−1550.
(14) Rodríguez, J.; Nieto, R. M.; Crews, P. *J. Nat. Prod.* **1993**, *56*, 2034−2040.
(15) Fusetani, N.; Matsunaga, S.; Konosu, S. *Tetrahedron Lett.* **1981**, *22*, 1985−1988.
(16) Kanazawa, S.; Fusetani, N.; Matsunaga, S. *Tetrahedron*, **1992**, *48*, 5467−5472.
(17) Van Soest, R. W. M.; Diaz, M. C.; Pomponi, S. A. *Beaufortia* **1990**, *40*, 15−62.
(18) Bitulco, G.; Bruno, I.; Minale, L.; Riccio, R. *J. Nat. Prod.* **1994**, *57*, 164−167.
(19) Makarieva, T. N.; Stonik, V. A.; Dmitrenok, A. S.; Krasokhin, V. B.; Svetashev, V. I.; Vysotskii, M. V. *Steroids* **1995**, *60*, 316−320.
(20) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565−569.
(21) Bax, A.; Sparks, S. W.; Torchin, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 7926−7927.
(22) Burgoyne, D. L.; Andersen, R. J. *J. Org. Chem.* **1992**, *57*, 525−528.
(23) (a) Barrero, A. F.; Sanchez, J. F.; Alvarez-Manzaneda, E. J.; Dorado, M. M.; Haidour, A. *Phytochemistry* **1993**, *32*, 1261−1265. (b) In **8**, the sidechain stereochemistry of *S* at C-20 and C-24 (ref 23a) was assigned using a biogenetic assumption. The C-17, C-20, and Me-21 shifts vary as a function of differing C-22−OH stereochemistry in model sterols. Respective model shifts at C-17, C-20, and Me-21 for 22*S* (δ 52.6, 40.3, and 11.6) and for 22*R* (δ 53.2, 42.6, and 12.5) were used to designate *S* stereochemistry at C-22 in **8** (δ 52.7, 39.4, and 11.3). The data we observed in CD_3OD for 1 (δ 49.1, 37.3, and 13.1) did not correlate well with the respective model shifts measured in $CDCl_3$.
(24) Shoji, N.; Umeyama, A.; Shin, K.; Takeda, K.; Arihara, S. *J. Org. Chem.* **1992**, *57*, 2996−2997.
(25) Kobayashi, J.; Shinonaga, H.; Shigemori, H. *J. Nat. Prod.* **1995**, *58*, 312−318.
(26) See Burgoyne and Andersen,[22] refs. 9 and 10 therein.
(27) Schmitz, F. J. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; pp 261−262, and references cited therein.
(28) Kobayashi, M.; Lee, N. K.; Son, B. W.; Yamayi, K.; Kyoyoku, Y; Kitagawa, I. *Tetrahedron Lett.* **1984**, *25*, 5925−5928.
(29) Kong, F.; Andersen, R. J. *J. Nat. Prod.* **1996**, *59*, 379−385.
(30) Gunasekera, S. P.; Sennett, S. H.; Kelly-Borges, M.; Bryant, R. W. *J. Nat. Prod.* **1994**, *57*, 1751−1774.
(31) Makarieva, T. N.; Stonik, V. A.; D'yachuk, G.; Dmitrenok, A. S. *Tetrahedron Lett.* **1995**, *36*, 129−132.
(32) De Weerdt, W. H. *Beaufortia* **1987**, *36*, 81−165.

NP960592S