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Fibrosterol Sulfates from the Philippine Sponge *Lissodendoryx* (*Acanthodoryx*) *fibrosa*: Sterol Dimers that Inhibit PKCζ

Emily L. Whitson,[†,∥] Tim S. Bugni,[†] Priya S. Chockalingam,[‡] Gisela P. Concepcion,[§] Xidong Feng,[‡] Guixian Jin,[‡] Mary Kay Harper,[†] Gina C. Mangalindan,[§] Leonard A. McDonald,[‡] and Chris M. Ireland*,[†]

[†]*Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112,* [‡]*Wyeth Research, Pearl River, New York 10965, and* [§]*Marine Science Institute, University of the Philippines, Diliman 1101, Quezon City, Philippines.* [∥]*Present address: National Cancer Institute - Frederick, Frederick, MD 21702.*

cireland@pharm.utah.edu

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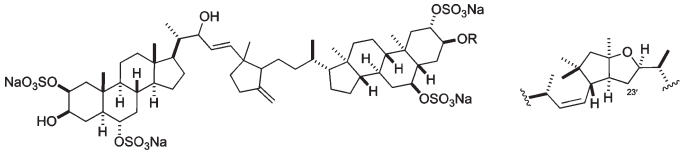

Fibrosterol Sulfate A R = H
Fibrosterol Sulfate B R = SO_3Na

Fibrosterol Sulfate C

Three new sulfated sterol dimers, fibrosterol sulfates A−C (**1**−**3**), have been isolated from the sponge *Lissodendoryx* (*Acanthodoryx*) *fibrosa*, collected in the Philippines. The structures were assigned on the basis of extensive 1D and 2D NMR studies as well as analysis by HRESIMS. Compounds **1** and **2** inhibited PKCζ with IC_{50} values of 16.4 and 5.6 μM, respectively.

Introduction

Polyoxygenated[1] and polysulfated steroids with atypical, modified side chains[2] are prevalent among compounds isolated from marine organisms. Sponge-derived sulfated sterols have a wide array of reported biological activities in a variety of therapeutic areas. Of particular note is their activity against HIV-1.[3−5] In addition, the spheciosterol sulfates A−C, isolated from a *Spheciospongia* sp. sponge, were recently shown to inhibit protein kinase C ζ (PKCζ) as well as downstream NF-κB activation.[6] PKCζ has been implicated as an integral factor in several types of cancer,[7−11] obesity,[12] and osteoarthritis.[8,13] Consequently, the detection and identification of PKCζ-specific inhibitors could have a potentially profound impact on the treatment of a number of diseases and disorders.

As part of an ongoing search for bioactive marine metabolites, crude extracts from our marine invertebrate library were screened for PKCζ inhibition. The methanol extract of a *Lissodendoryx* (*Acanthodoryx*) *fibrosa* sample, collected from Coron Island, Philippines, showed promising PKCζ inhibition in the initial screening. No natural products had been reported from this sponge, suggesting *L.* (*A.*) *fibrosa* would be an attractive source for chemical investigation. As a result, fibrosterol sulfates A−C (**1**−**3**), three new sulfated bis-steroids, were isolated from the sponge. Data from

*To whom correspondence should be addressed. Tel: (801) 581-8305. Fax: (801) 585-6208.

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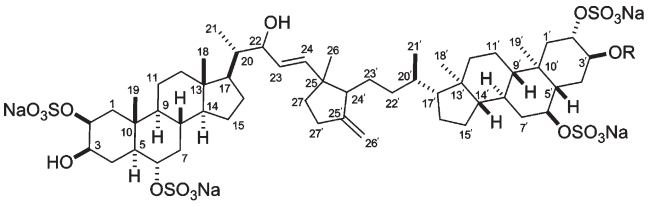
TABLE 1. NMR Data for Fibrosterol Sulfate A (1) (600 MHz, CD_3OD)

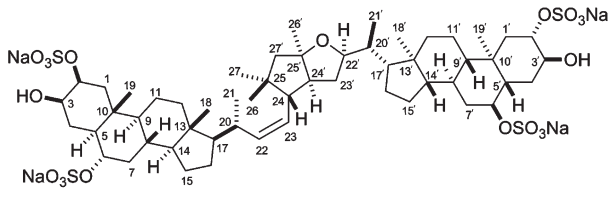
position	δ_C	δ_H mult (*J*, Hz)	HMBC	position	δ_C	δ_H mult (*J*, Hz)	HMBC
1α	42.8	1.14,[a] m	5, 9, 10, 19	1α′	38.8	1.47, dd (14.8, 3.5)	9′, 10′, 19′
1β		2.42,[a] m	2, 3, 5, 6, 19	1β′		2.06,[a] m	2′, 3′, 10′, 19′
2	78.2	4.64, m	1, 3, 4, 10	2′	77.8	4.46, m	3′, 4′, 10′
3	72.1	3.58, ddd (11.8, 4.0, 4.0)	2, 4, 5	3′	68.4	4.06, m	1′, 2′, 5′
4α	27.7	2.10, m	2, 3, 5, 6, 10	4α′	26.3	2.04,[a] m	2′, 3′, 5′, 6′
4β		1.62,[a] m	2, 3, 5, 6, 10	4β′		1.72,[a] m	5′, 6′, 10′
5	51.7	1.19,[b] m	1, 3, 4, 6, 7, 9, 10	5′	44.5	1.66,[a] m	1′, 3′, 4′, 6′, 7′, 9′, 10′
6	78.0	4.19,[a] m	4, 5, 10	6′	78.6	4.19,[a] m	4′, 5′, 10′
7α	39.8	0.98,[b] m	5, 6, 8, 9, 14	7α′	39.8	1.06,[b] m	6′, 8′, 14′
7β		2.36,[a] m	5, 6, 8, 9, 14	7β′		2.36,[a] m	5′, 6′, 8′, 9′, 14′
8	35.0	1.53,[a] m	7, 9, 13, 14	8′	35.0	1.53,[a] m	7′, 9′, 13′, 14′
9	55.7	0.69,[b] m	8, 10, 11, 19	9′	55.6	0.75,[b] m	8′, 10′, 11′, 19′
10	37.8			10′	38.1		
11α	22.1	1.55,[a] m	8, 9, 12, 13	11α′	21.7	1.56,[a] m	8′, 9′, 12′, 13′
11β		1.38, m	9, 10, 12	11β′		1.33,[a] m	9′, 10′, 13′
12α	41.0	1.16,[b] m	9, 11, 13, 18	12α′	41.3	1.18,[b] m	9′, 11′, 13′, 18′
12β		2.01,[a] m	9, 11, 13, 14, 18	12β′		2.02,[a] m	9′, 11′, 13′, 14′, 18′
13	43.9			13′	43.8		
14	56.9	1.07,[b] m	8, 12, 13, 15, 18	14′	57.4	1.12,[b] m	8′, 13′, 15′, 18′
15α	25.0	1.12,[a] m	13, 14	15α′	25.0	1.12,[b] m	13′, 14′
15β		1.56,[a] m	13, 14	15β′		1.62,[a] m	13′, 14′
16α	28.1	1.30,[a] m	13, 15, 17	16α′	29.1	1.30,[a] m	13′, 15′, 17′
16β		1.74,[a] m	13, 15, 17	16β′		1.87,[a] m	13′, 15′, 17′
17	54.3	1.03,[b] m	13, 16, 20, 21, 22	17′	57.3	1.15,[b] m	13′, 15′, 16′, 18′, 20′, 21′
18	12.4	0.71, s	12, 13, 14, 17	18′	13.1	0.72, s	12′, 13′, 14′, 17′
19	15.6	1.08, s	1, 5, 9, 10	19′	15.1	1.05, s	1′, 5′, 9′, 10′
20	43.1	1.69, m (6.6, 3.4)	13, 16, 17, 21, 22, 23	20′	37.7	1.42,[a] m	16′, 17′, 22′
21	12.7	0.97, d (6.6)	17, 20, 22	21′	19.8	0.99, d (6.5)	17′, 20′, 22′
22	76.3	3.98, dd (8.7, 3.4)	17, 20, 21, 23, 24	22a′	36.9	1.69,[a] m	17′, 20′, 21′, 23′, 24′
				22b′		0.99,[b] m	20′, 21′, 23′, 24′
23	126.2	5.35, dd (15.7, 8.7)	20, 22, 24, 25, 27, 24′	23a′	26.6	1.42,[a] m	25, 20′, 22′, 24′, 25′
				23b′		1.07,[b] m	25, 22′, 24′, 25′
24	138.6	5.54, d (15.7)	22, 23, 25, 26, 27, 24′	24′	56.8	1.84,[a] m	
25	47.8			25′	157.5		
26	24.0	1.15, s	23, 24, 25, 27, 24′	26a′	105.1	4.85[c]	24′, 25′, 27′
				26b′		4.81[c]	24′, 25′, 27′
27a	38.5	1.68,[a] m	24, 25, 26, 24′, 25′, 27′	27′	29.8	2.43,[a] m	27, 25′
27b		1.57,[a] m	24, 25, 26, 24′, 25′, 27′				

[a]Signals overlapped. [b]Signal buried under overlapping methyl. [c]Overlapped with HOD signal.

several experiments verified that fibrosterol sulfates A (**1**) and B (**2**) were, in fact, PKCζ inhibitors.



1 R = H
2 R = SO_3Na



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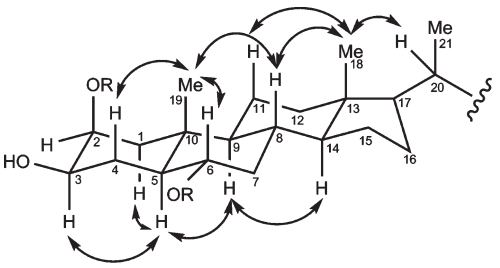
Results and Discussion

The *L.* (*A.*) *fibrosa* specimen (PC00-04-56) was exhaustively extracted with MeOH and the crude extract separated on an HP20SS resin using a step gradient of H_2O to 2-propanol (IPA) (25% steps, five fractions). Bioassay-guided fractionation of the second (75/25 H_2O/IPA) and third fractions (50/50 H_2O/IPA), utilizing reversed-phase column chromatography and reversed-phase HPLC, resulted in the isolation of fibrosterol sulfates A−C (**1**−**3**).

The molecular formula for fibrosterol sulfate A (**1**), $C_{54}H_{84}O_{19}S_4Na_4$, was derived from NMR data and the HRESIMS ion at *m*/*z* 605.2147 ($[M - 2Na]^{-2}$; Δ +0.21 ppm). Utilizing the ultrahigh resolution capabilities of FTMS, the ^{34}S peak could be resolved from the $^{13}C_2$ peak, indicating the presence of four sulfurs in **1**. The presence of multiply charged ions in the mass spectra coupled with characteristic sulfate losses in FT-MS/MS experiments indicated that sulfate esters were present in **1**. The structure of fibrosterol sulfate A (**1**) was established on the basis of extensive 1D and 2D NMR studies. Initial interpretation of the NMR data (Table 1) suggested that **1** was an isoprenoid containing five methyl singlets (δ_H 0.71, 0.72, 1.05, 1.08, 1.15), two methyl doublets (δ_H 0.97, 0.99), seven oxygenated methines (δ_H 3.58, 3.98, 4.06, 4.19 (2), 4.46, 4.64), a *trans* olefin (δ_H 5.35, *J* = 15.7; 5.54, *J* = 15.7), and a terminal olefin (δ_H 4.85, 4.81). The data also indicated that fibrosterol sulfate A (**1**) contained six quaternary carbons, 22 methines, and 19 methylenes. Many of the signals, specifically the methyls, appeared in pairs, indicating that

1 was a pseudosymmetrical bis-steroid. The pseudosymmetrical nature of the molecule created an overlap of spectral resonances which complicated data interpretation at lower field strength (500 MHz). However, the increased sensitivity and resolution, afforded by a 600 MHz instrument equipped with a cryoprobe, allowed for clear interpretation of the overlapping signals in **1**. Analysis of COSY and HMBC data led to the assignment of the ABCD and A′B′C′D′ steroid ring systems in fibrosterol sulfate A (**1**) (Table 1). Rings A and B were assembled based on COSY correlations among all adjacent protons between H-1 and H-9. HMBC correlations from Me-19 to C-1, C-5, C-9, and C-10 completed the structural assignment of the A and B rings. Rings C and D were assigned on the basis of HMBC correlations from Me-18 to C-12, C-13, C-14, and C-17 and H-14 to C-8, C-13, and C-15. COSY correlations between H-11 and H-9 and between H-11 and H-12 and HMBC correlations from H-11 to C-9 and C-12 supported the connectivity of C-9 through C-12. HMBC correlations from H-16 to C-17 and C-15 completed the structural assignment of the C and D rings. The A′B′C′D′ rings were assembled in the same fashion; all of the correlations used to assign the ABCD rings were identical to the correlations used to assign the A′B′C′D′ rings. HMBC correlations from Me-21, Me-26, and Me-21′ were particularly useful in constructing the side chain that fuses rings D and D′ in **1** (HMBC correlations from Me-21 to C-17, C-20 and C-22; Me-21′ to C17′, C-20′ and C-22′; Me-26 to C-24, C-25, C-27 and C-24′). COSY correlations between H-22 and H-23 and between H-23 and H-24, in addition to the methyl correlations, supported all connectivities between C-20 and C-27. HMBC correlations from H-23′ to C-22′ and C-24′ and from H-26′ to C-24′ and C-27′ supported all connectivities between C-20′ and C-27′. COSY correlations between H-27 and H-27′, as well as HMBC correlations from H-27′ to C-27, completed the assignment of the side chain that joins the two steroid units in fibrosterol sulfate A (**1**). Sulfates induce a downfield ^{13}C shift of ~5−9 ppm on the δ_C of C-2, C-3, and C-6 when compared to the corresponding triols.[14−16] As such, the sulfate groups were assigned to C-2, C-6, C-2′, and C-6′, and hydroxyls were placed at C-3, C-3′, and C-22 in fibrosterol sulfate A (**1**).

Because of a significant number of overlapping ^1H signals, few coupling constants could be obtained for fibrosterol sulfate A (**1**), complicating the relative configuration analysis. However, biosynthetic precedents and ROESY data clearly indicated that **1** contained two standard 5α10β steroid nuclei (Figure 1). ROESY data, w-coupling, and ^{13}C chemical shift analysis were all necessary to determine the configuration at C-2, C-3, C-6, C-2′, C-3′, and C-6′. H-2 was given an equatorial assignment based on the observed w-coupling between H-2 and H-4α in the COSY spectrum and the narrow multiplicities for H-2 in the ^1H spectrum (absence of large vicinal coupling constants). H-3 was designated axial based on the presence of a large vicinal coupling constant (J = 11.8 Hz), and the ROE observed between H-3 and H-5. H-6 was given an axial assignment based on the



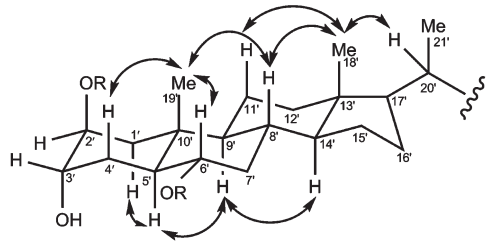


FIGURE 1. Key ROE correlations supporting the relative configuration of the ABCD rings and the A′B′C′D′ rings of fibrosterol sulfate A (**1**).

ROE observed between H-6 and Me-19. The ^{13}C chemical shifts in the AB rings were compared with AB steroid rings containing different configurations at C-2, C-3, and C-6.[16−21] The configuration of the AB rings in fibrosterol sulfate A (**1**) corresponded best to that observed in amaranzole A[19] as 2*S*,3*R*,6*S*. Chemical shift variances observed between the AB and A′B′ rings suggested configuration differences, which were confirmed after careful observation of several parameters. The narrow multiplicities for H-2′ and H-3′ in the ^1H spectrum and the lack of an ROE between H-2′ and Me-19′, and H-3′ and H-5′ implied that H-2′ and H-3′ were equatorial. It is probable that w-coupling between H-2′ and H-4α′ and H-3′ and H-1β′ occurs; however, the signal overlap of H-4α′ and H-1β′ (δ_H 2.04 and 2.06, respectively) precludes discrete observation in the COSY spectrum. H-6′ was given an axial assignment based on the ROE observed between H-6′ and Me-19′. ^{13}C chemical shifts in the A′B′ rings were compared with AB steroid rings containing different configurations at C-2, C-3, and C-6.[16−21] The configuration of the A′B′ rings in fibrosterol sulfate A (**1**) was consistent with the configurations of halistanol sulfate[16] and a semisynthetic sterol,[22] as 2′*S*,3′*S*,6′*S*. *J*-based analysis of **1** was used in an attempt to assign the relative configuration of the C-22 alcohol,[23] but the results were inconclusive (see the Supporting Information). Molecular modeling studies

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TABLE 2. NMR Data for Fibrosterol Sulfate B (2) (500 MHz, CD_3OD)

position	δ_C	δ_H mult (*J*, Hz)	position	δ_C	δ_H mult (*J*, Hz)
1α	43.0	1.16,[a] m	1α′	39.5	1.47, dd (14.7, 3.5)
1β		2.44,[b] m	1β′		2.06,[b] m
2	78.3	4.67, m	2′	75.4	4.83,[c]
3	72.3	3.59, ddd (11.8, 4.0, 4.0)	3′	75.4	4.76, m
4α	27.9	1.63,[b] m	4α′	24.9	1.80, m
4β		2.12, m	4β′		2.28, m
5	51.7	1.19,[a] m	5′	45.2	1.62, m
6	78.4	4.20,[b] m	6′	78.4	4.20, m
7α	40.0	0.98,[a] m	7α′	40.0	1.05, m
7β		2.37, m	7β′		2.37, m
8	35.0	1.53,[b] m	8′	35.0	1.53, m
9	55.9	0.68,[a] m	9′	55.8	0.74, m
10	38.0		10′	37.5	
11α	22.2	1.38,[b] m	11α′	22.1	1.36, m
11β		1.55,[b] m	11β′		1.55, m
12α	41.1	1.16,[a] m	12α′	41.3	1.19, m
12β		2.02,[b] m	12β′		2.04, m
13	44.0		13′	43.9	
14	57.0	1.08,[a] m	14′	57.3	1.15, m
15α	25.2	1.13,[a] m	15α′	25.1	1.13, m
15β		1.58,[b] m	15β′		1.64, m
16α	28.2	1.31,[b] m	16α′	29.3	1.31, m
16β		1.74,[b] m	16β′		1.88, m
17	54.5	1.05,[a] m	17′	57.5	1.13, m
18	12.7	0.71, s	18′	13.2	0.73, s
19	15.8	1.08, s	19′	15.2	1.05, s
20	43.1	1.70,[b] m (6.8, 3.4)	20′	37.9	1.42, m
21	13.0	0.98, d (6.8)	21′	19.8	0.99, d (7.0)
22	76.5	3.99, dd (8.7, 3.4)	22a′	36.9	1.71, m
			22b′		0.99, m
23	126.2	5.37, dd (15.7, 8.7)	23a′	26.7	1.43, m
			23b′		1.08, m
24	138.9	5.57, d (15.7)	24′	56.9	1.85, m
25	47.9		25′	157.5	
26	24.2	1.16, s	26a′	105.3	4.87, m
			26b′		4.82, m
27a	38.8	1.68,[b] m	27′	30.0	2.43,[b] m
27b		1.57,[b] m			

[a]Signal buried under overlapping methyl signal. [b]Signals overlapped. [c]Overlapped with HOD signal.

were conducted on four diastereomers of **1** (25*R*,24′*S*, 25*S*,24′*R*, 25*R*,24′*R*, and 25*S*,24′*S*), and interatomic distances were calculated (Table 1, Supporting Information) in an effort to determine if ROEs could be used to distinguish whether the ring in **1** had a *cis* or *trans* relative configuration. However, no conclusive results could be obtained (see the Supporting Information). Ongoing studies using RDC analysis should aid in the assignment of the C-22 configuration as well as the configuration at C-25 and C-24′. Based on the aforementioned data, fibrosterol sulfate A (**1**) was assigned as 2*S*,3*R*,5*S*,6*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*,20*R*,23*E*,2′*S*,3′*S*,-5′*S*,6′*S*,8′*S*,9′*S*,10′*R*,13′*S*,14′*S*,17′*R*,20′*R*.

The molecular formula for fibrosterol sulfate B (**2**), $C_{54}H_{83}O_{22}S_5Na_5$, was derived from NMR data and the HRESIMS ion at *m*/*z* 656.1843 ($[M - 2Na]^{2-}$, Δ −0.35 ppm). FT-MS analysis indicated five sulfur atoms were present in the molecular formula for **2**. Characteristic sulfate losses in FT-MS/MS experiments combined with multiply charged ions in the mass spectra indicated that **2** also contained sulfate esters. Comparison of the molecular formulas for **1** and **2** revealed that fibrosterol sulfate B (**2**) contained an additional sulfate group. The NMR spectra of fibrosterol sulfates A (**1**) and B (**2**) are very similar, with the major ^1H and ^{13}C chemical shift differentials between the two molecules occurring in the A′ ring (Tables 1 and 2). All of the data suggested that the only difference between the two molecules was an additional sulfate in **2** at C-3′.

The molecular formula for the minor compound fibrosterol sulfate C (**3**), $C_{54}H_{84}O_{19}S_4Na_4$, was derived from NMR data and the HRESIMS ion at *m*/*z* 1233.4167 ($[M - Na]^-$, Δ −1.3 ppm). Compound **3** showed a similar ^1H NMR spectrum and identical molecular formula when compared with **1**, which suggested that **3** was also a sulfated bis-steroid. Comparison of the NMR data for fibrosterol sulfates A (**1**) and C (**3**) indicated that the ABCD and A′B′C′D′ rings are virtually indistinguishable, while the side chains are quite different (Tables 1 and 3). The side chain of fibrosterol sulfate C (**3**) lacked the terminal olefin seen in **1**, but contained a *cis* olefin (δ_H 5.29, *J* = 10.8; 5.02, *J* = 10.8), three singlet methyls, an oxygenated methine (δ_C 82.4; δ_H 4.16), and a deshielded quaternary carbon (δ_C 91.0). HMBC correlations from Me-21, Me-26, Me-27, Me-21′, and Me-26′ were essential for constructing the side chain that fuses rings D and D′ in **3**. HMBC correlations from Me-21 to C-17, C-20 and C-22; Me-26 to C-24, C-25, C-27, and C-27′; and Me-27 to C-24, C-25, C-26, and C-27′ and COSY correlations between H-22 and H-23 and between H-23 and H-24 supported all connectivities between C-20 and C-27. HMBC correlations from Me-21′ to C-17′, C-20′, and C-22′ and Me-26′ to C-24′, C-25′, and C-27′ and COSY correlations

TABLE 3. NMR Data for Fibrosterol Sulfate C (3) (600 MHz, CD_3OD)

position	δ_C	δ_H mult (*J*, Hz)	position	δ_C	δ_H mult (*J*, Hz)
1α	42.4	1.41,[a] m	1α′	38.8	1.46,[a] m
1β		2.34,[a] m	1β′		2.07,[a] m
2	79.1	4.76, m	2′	77.8	4.42, m
3	71.9	3.71, ddd (11.8, 4.0, 4.0)	3′	68.4	4.08, m
4α	27.9	1.59,[a] m	4α′	26.5	1.76,[a] m
4β		2.08, m	4β′		2.04,[a] m
5	51.0	1.33,[b] m	5′	44.6	1.65,[a] m
6	78.4	4.19,[a] m	6′	78.4	4.19,[a] m
7α	39.9	1.04,[b] m	7α′	39.7	1.04,[b] m
7β		2.38,[a] m	7β′		2.34,[a] m
8	35.0	1.50,[a] m	8′	35.0	1.50,[a]
9	55.3	0.74,[b] m	9′	55.7	0.75,[b] m
10	37.7		10′	37.5	
11α	21.7	1.33,[a] m	11α′	22.0	1.35,[b] m
11β		1.56,[a] m	11β′		1.58,[a] m
12α	41.0	1.20,[a] m	12α′	41.4	1.13,[a] m
12β		2.01,[a] m	12β′		2.02,[a] m
13	43.4		13′	43.9	
14	57.6	1.14,[a] m	14′	56.9	1.04,[b] m
15α	25.1	1.11,[a] m	15α′	25.1	1.11,[a] m
15β		1.63,[a] m	15β′		1.63,[a] m
16α	29.8	1.29,[b] m	16α′	28.2	1.39,[a] m
16β		1.72,[a] m	16β′		1.76,[a] m
17	57.5	1.22,[a] m	17′	55.2	1.00,[b] m
18	12.9	0.73, s	18′	12.3	0.71, s
19	15.8	1.07, s	19′	15.1	1.05, s
20	35.8	2.45, m (10.8, 6.6)	20′	38.6	1.84,[a] m
21	21.6	1.00, d (6.6)	21′	13.1	1.00, d (6.6)
22	140.1	5.29, dd (10.8, 10.8)	22′	82.4	4.16,[a] m
23	126.7	5.02, dd (10.8, 10.8)	23α′	30.1	1.84,[a] m
			23β′		1.47,[a] m
24	56.6	2.39, m (10.8, 10.1)	24′	56.4	2.24, ddd (10.1, 10.1, 3.3)
25	46.4		25′	91.0	
26	29.3	0.94, s	26′	26.2	1.31, s
27	23.2	0.88, s	27α′	56.2	1.66, d (13.9)
			27β′		1.88, d (13.9)

[a]Signals overlapped. [b]Signal buried under overlapping methyl signal.

between H-22′ and H-23a/b′ and between H-23a/b′ and H-24′ supported all connectivities between C-20′ and C-27′. HMBC correlations from Me-26 and Me-27 suggested that C-25 and C-27′ were connected, which was further supported by HMBC correlations between H-27a/b′ and C-25, C-26, C-27, C-25′, and C-26′. COSY correlations between H-24 and H-24′ and HMBC correlations from H-24 to C-24′ and H-24′ to C-24 supported a connection between C-24 and C-24′ to form the cyclopentane ring in **3**. The molecular formula for fibrosterol sulfate C (**3**) required 11 degrees of unsaturation, suggesting that the final degree of unsaturation was an ether linkage between C-22′ and C-25′ to give the oxabicyclo-[3.3.0]octane seen in **3**. The unusual quaternary ether carbon ^{13}C chemical shift (δ_C 91.0, C-25′) was compared to similar ring systems seen in ibhayinol (δ_C 91.4)[24] and kuhistaferone (δ_C 100.4)[25] and was consistent with the oxabicylo[3.3.0]-octane for **3** as drawn.

Fibrosterol sulfate C (**3**) exhibited ROESY data identical to those for **1** for the ABCD and A′B′C′D′ rings, supporting a 2*S*,3*R*,6*S*,2′*S*,3′*S*,6′*S* configuration for **3**. ROESY data and molecular modeling were utilized to determine the relative configuration of the oxabicyclo[3.3.0]octane in **3**

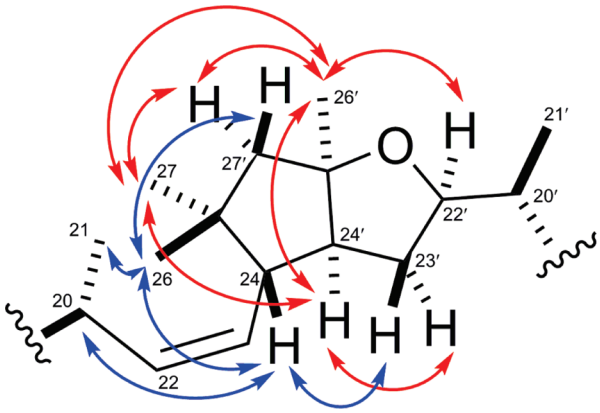

FIGURE 2. Key ROE correlations supporting the relative configuration of the bicyclo[3.3.0]octane in the side chain of fibrosterol sulfate C (**3**).

(Figure 2 and Supporting Information). ROEs between Me-26′ and H-24′ supported a *cis* ring juncture, with both Me-26′ and H-24′ being in the α configuration. ROEs between Me-26′ and H-22′ indicated that H-22′ was also in the α configuration. ROEs between H-24′ and Me-27 established that Me-27 was in the α configuration, while ROEs between Me-26 and H-24 suggested that H-24 was in the β configuration. The large coupling constant observed between H-24 and H-24′ (J = 10.1 Hz) also supported a *trans*

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relationship between H-24 and H-24′. Based on these data, fibrosterol sulfate C (**3**) was assigned as 2*S*,3*R*,5*S*,6*S*,8*S*,9*S*,10*R*,13*S*,14*S*,17*R*,20*R*,22*Z*,24*S**,2′*S*,3′*S*,5′*S*,6′*S*,8′*S*,9′*S*,-10′*R*,13′*S*,14′*S*,17′*R*,20′*R*,22′*R**,24′*S**,25′*R**. The relative configuration between C-24 and C-22′ could not be relayed through C-20′ due to overlapping signals; H-20′ and H-23α chemical shifts are identical (δ_H1.84), as well as H-21′ and H-17′ (δ_H 1.00).

Fibrosterol sulfates A (**1**) and B (**2**) inhibited PKCζ with IC_{50} values of 16.4, and 5.6 μM, respectively. Fibrosterol sulfate C (**3**) was not tested for biological activity due to the limited amount of material isolated. The risk of a false positive was eliminated by employing a counter screen that ensured **1** and **2** were not interfering with the signal detection. Compounds **1** and **2** were incubated with the phosphorylated U*Light*-PKC peptide and the antibody, and the TR-FRET signals were measured at 665 nm. The TR-FRET signals remained constant when **1** and **2** were incubated with the phosphorylated U*Light*-PKC peptide and the antibody, thereby eliminating the possibility of false-positive inhibition by this mechanism. Light-scattering measurements also indicated that fibrosterol sulfates A (**1**) and B (**2**) were soluble at PKCζ assay concentrations, ruling out the possibility that **1** and **2** were false positives due to aggregate formation.

The spheciosterol sulfates A−C, isolated from a *Spheciospongia* sp., were recently shown to inhibit PKCζ and NF-κB activation.[6] It was shown that the sterol side chain factors into their PKCζ activity; the longer side chain seen in spheciosterol sulfate C is 5-fold more active than spheciosterol sulfate B and 10-fold more active than the shorter side chains seen in spheciosterol sulfate A and topsentiasterol sulfate E.[6] The number of sulfates also appears to be important for PKCζ activity, as fibrosterol sulfate B (**2**) is 3-fold more active than fibrosterol sulfate A (**1**). Interestingly, the spheciosterol sulfates and the fibrosterol sulfates share a similar oxygenation pattern in the steroid rings, suggesting that the steroid oxygenation pattern may also be important for PKCζ inhibition.

Fibrosterol sulfates A−C (**1**−**3**) appear to be composed of two cholestene monomers, with differing configuration at C-3 and oxygenation at C-22 in only one monomer. These molecules would be excellent candidates for future biosynthetic studies.

Previous investigations of sponges from the genus *Lissodendoryx* have yielded steroids,[26,27] pyrrololactams,[28] cembranes,[29] and polyether macrolides.[30,31] Only a few steroid dimers have been isolated from marine organisms such as cephalostatins,[32−39] crellastatins,[40−42] ritterazines,[43−46] hamigerols,[47] bistheonellasterone,[48] and amaroxocanes.[49] The cephalostatins,[32−39] ritterazines[43−46] and bistheonellasterone[48] are all fused between the A and A′ rings, while the crellastains,[40−42] hamigerols,[48] and amaroxocane B[49] are fused through the side chains to form a dioxabicyclononane ring system. Amaroxocane A[49] and crellastatin M[42] contain a carbacylic ring fusion similar to the cyclopentane seen in fibrosterol sulfates A and B. Fibrosterol sulfate C (**3**) is unique in that there has never been a report of the oxabicyclo[3.3.0]octane in a dimeric sterol.

Experimental Section

Biological Material. *L.* (*A.*) *fibrosa* (Lévi, 1961) (Coelosphaeridae), sample PC00-04-56, was collected by SCUBA from Coron Island (11° 57.833′ N, 120° 06.311′ E), northern Palawan, Philippines; a voucher specimen is maintained at the University of Utah.

Extraction and Isolation. The *L.* (*A.*) *fibrosa* specimen (PC00-04-56) was exhaustively extracted with MeOH to yield 2.60 g of crude extract. The crude extract was separated on HP20SS resin using a step gradient of H_2O to IPA in 25% steps, and a final wash of 100% MeOH, to yield five fractions. The second fraction (0.46 g; 75/25 H_2O/IPA) was chromatographed on C_{18} (32 × 10 cm) using a step gradient of 100% 0.2 M aq NaCl to CH_3CN in 10% steps and a final wash of 100% MeOH to yield 12 fractions (175A−175L). The third HP20SS fraction (50/50 H_2O/IPA) was also chromatographed on C_{18} (32 × 10 cm) using the same gradient to yield 12 fractions (185A−185L). Fractions 175D (20.4 mg; 30% CH_3CN/70% 0.2 M NaCl in H_2O), 175E (7.2 mg; 40% CH_3CN/60% 0.2 M NaCl in H_2O), 185E (27.4 mg; 40% CH_3CN/60% 0.2 M NaCl in H_2O), and 185F (15.4 mg; 50% CH_3CN/50% 0.2 M NaCl in H_2O) were combined. The combined fractions were purified by HPLC using a C_{18} column (250 × 10 mm) employing a gradient of 2% CH_3CN/98% 0.2 M NaCl in H_2O to 30% CH_3CN/70% 0.2 M NaCl in H_2O over 5 min, followed by a gradient of 30% CH_3CN/70% 0.2 M NaCl in H_2O to 50% CH_3CN/50% 0.2 M NaCl in H_2O at 4.5 mL/min over 32 min to yield fibrosterol sulfate A (**1**, 6.4 mg) eluting at 18.6 min and fibrosterol sulfate B (**2**, 8.0 mg) eluting at 16.1 min.

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An aliquot of the third HP20SS fraction (186 mg; 50/50 H_2O/IPA) was chromatographed on LH20 (24 × 2.5 cm) using MeOH as eluant to yield 67 fractions (138.1−138.67). Fraction 138.4 was purified by HPLC using a C_8 column employing a gradient of 10% CH_3CN/H_2O to 35% CH_3CN/H_2O at 4 mL/min over 33 min to yield 9 fractions (159A-159I). Fraction 159I was further purified by HPLC using a C_{18} column (250 × 10 mm) employing a gradient of 2% CH_3CN/98% 0.2 M NaCl in H_2O to 30% CH_3CN/70% 0.2 M NaCl in H_2O over 5 min, followed by a gradient of 30% CH_3CN/70% 0.2 M NaCl in H_2O to 50% CH_3CN/50% 0.2 M NaCl in H_2O at 4.5 mL/min over 32 min to yield fibrosterol sulfate C (**3**, 0.3 mg) eluting at 28.4 min.

Desalting of the flash column and HPLC fractions was achieved by filtering the samples through C_{18} Sep-Pak cartridges; salts were removed by washing with 100% H_2O, and individual fractions or compounds were eluted with 100% MeOH.

Fibrosterol sulfate A (1): amorphous white solid; $[\alpha]^{22}_D$ +16.2 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.70) nm; IR (film, NaCl) ν_{max} 3377 (br), 1660, 1641, 1444, 1221, 1063, 966, 708 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 605.1459 $[M - 2Na]^{2-}$ (calcd for $C_{54}H_{84}O_{19}S_4Na_2$, 605.1480).

Fibrosterol Sulfate B (2): amorphous white solid; $[\alpha]^{22}_D$ +19.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.91) nm; IR (film, NaCl) ν_{max} 3224 (br), 1662, 1639, 1444, 1221, 968, 714 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 656.18432 $[M - 2Na]^{2-}$ (calcd for $C_{54}H_{83}O_{22}S_5Na_3$, 656.18418).

Fibrosterol Sulfate C (3): amorphous white solid; $[\alpha]^{20}_D$ +29.2 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.04) nm; IR (film, NaCl) ν_{max} 3346 (br), 2953, 1662, 1641, 1446, 1219, 1063, 962, 708 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 1233.4167 $[M - Na]^-$ (calcd for $C_{54}H_{84}O_{19}S_4Na_3$, 1233.4183).

PKCζ Assay. IC_{50} values were determined in a homogeneous TR-FRET-based PKCζ kinase activity assay (LANCE-Ultra, Perkin-Elmer). Compounds **1** and **2**, at various concentrations, were incubated with the U*Light*-PKC peptide substrate (50 nM), ATP (2 μM), and PKCζ (25 pM). The reaction was stopped with EDTA (15 mM) after 60 min, Eu-labeled anti-phospho-PKC peptide antibody was added, and the extent of phosphopeptide product formation was determined through the measurement of the TR-FRET signals at 615 and 665 nm wavelengths upon excitation at 340 nm. A decrease in the TR-FRET signal ratio was observed as a function of increasing compound concentration, and the IC_{50} for each compound was determined accordingly.

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Supporting Information Available: ^1H spectra of **1**−**3**, ^{13}C spectra of **1** and **2**, HSQC spectrum of **3**, methods attempted to determine the configuration of C-22 and the cyclopentane ring in **1**, a table of interatomic distances for **3** from molecular modeling, and a simplified 3D representation of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.